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Mohammad Abu Jafar Mazumder
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Functional Biopolymers

With 221 Figures and 19 Tables

Springer
Biopolymers are polymeric biomolecules usually obtained from living organisms or produced from renewable sources that might require polymerization and indicated to be as biodegradable polymer. When functionalized (naturally or synthetically), the biopolymer contains monomeric units that covalently bonded to form larger structures, show uniformly distributed set of molecular mass, and appear with chemically bound functional groups, which can be utilized as reagents, catalysts, reservoirs, and carriers. Functionalized polymer plays an essential role in the preservation and transmittance of genetic information and cellular construction in nature and function like storage of energy, micelle, hydrogel, and drug delivery carrier. Based on their well-marked chemical structures, biopolymers are classified in terms of their monomeric structure, polymer backbone, viability, degradability, and application. Even though many of these polymers were in use from the prehistoric time, but with the development of human technologies, designed biopolymeric systems have received increasing utilization in medical, pharmaceutical, food, and electronic industry, for applications such as encapsulation and delivery systems or to modulate the physicochemical and sensory properties of high-performance engineering polymeric materials. Biopolymers are currently used as a substitute of conventional synthetic polymeric materials due to their sustainable, renewable, and, more importantly, eco-friendly nature. The major advantages of the functional biopolymers have attracted huge attentions due to their ease of functional modifications either by chemical reactions on pendant groups, side chains, or by changing the physicochemical properties of the polymers so that they can show the required functional attributes such as high thermal stability, mechanical and chemical resistance, biodegradation, and oxygen barrier for the subsequent applications. However, it is very difficult to achieve all these properties within a single polymer molecule; therefore, there is an increasing demand for the preparation of composites along with biopolymers for improving the properties of the materials.

This reference book covers major areas of functional biopolymers that offer a comprehensive overview of the synthesis, properties, process, and biomedical applications of functional biopolymers as innovative sustainable materials. The primary focus of this book is to review the theoretical advances as well as experimental results and open up new windows for researchers in the field of polymers and sustainable materials. We believe this book will not only be useful for senior
researchers but also help senior undergraduate- and graduate-level students as a reference book. Chapters received from expert contributors cover various topics such as synthetic biopolymers, polymer micelles, blood-compatible polymers, and stimuli-responsive polymers. An up-to-date review of cell encapsulation strategies and cell surface and tissue engineering is also included in this work. The readers will discover more about hydrogels and polymers from renewable resources and specialty applications of functional biopolymers.

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We are thankful to all contributing authors and coauthors for their valued contribution to this book. The project would never have been possible without their sincere supports and contributions. We would also like to express our gratitude to the publisher, authors, and others for granting us the copyright permission to use their illustrations. Although sincere efforts were made to obtain the copyright permissions from the respective owners to include the citation with the reproduced materials, we would like to offer our sincere apologies to any copyright holder if unknowingly their right is being infringed. Among the editors, Dr. Mohammad A. Jafar Mazumder would like to take this opportunity to express his sincere thanks to Drs. Abdulaziz A. Al-Saadi (Dean, College of Sciences, KFUPM) and Khalid R. Alhooshani (Chairman, Chemistry Department, KFUPM) and also to his colleagues at the King Fahd University of Petroleum and Minerals (KFUPM), Saudi Arabia. Dr. Amir Al-Ahmed would like to take this opportunity to express his sincere thanks to Dr. Fahad Al-Sulaiman (Director CORE-RE, KFUPM) and also to his colleagues at the King Fahd University of Petroleum and Minerals, Saudi Arabia. Without their continuous encouragement, this book would have not been brought into its final form. We would also like to acknowledge the sincere efforts of the Springer team, especially Dr. Sylvia Blago, Dr. Sofia Costa, and Dr. Judith Hinterberg and others who were always so helpful and provided us their assistance in evolving this book into its final shape. We are most grateful to all of them.
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Dr. Mohammad Abu Jafar Mazumder is an Associate Professor of Chemistry at King Fahd University of Petroleum and Minerals (KFUPM), Saudi Arabia, who has a wide experience in design, synthesis, modification, and characterization of various organic compounds and ionic and thermo-responsive polymers using various spectroscopic and chromatographic techniques. Dr. Jafar Mazumder earned his Ph.D. in Chemistry (2009) from McMaster University, Canada, followed by 2 years MITACS Postdoctoral Fellowship in Chemical and Biomedical Engineering at McMaster University, Canada.

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Synthetic Biopolymers

Mahbuba Rahman and Mohammad Rubayet Hasan

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Abstract
Synthetic biopolymers are polymers that are modified from natural polymers or chemically synthesized from synthetic monomers in such a way that they can undergo natural degradation, without leaving any residues that are harmful to the living and natural environments. Over the last few years, synthetic biopolymers have attracted much attention, because of their distinct advantages over natural polymers in terms of stability and flexibility to suit a variety of applications. On the other hand, synthetic biopolymers are favored over synthetic polymers because of their biodegradable properties and their innocence to the environment. Thanks to the advancements made in new molecular designing tools and polymer chemistry, the synthesis of synthetic biopolymers can now be tailored to fit their specific applications. Synthetic biopolymers have found one of its most important applications in the medical field because of some of their unique properties such as stability, controlled release, nonimmunogenicity, and clearance from the body, which suits their application in human bodies. The current chapter reviews the synthesis, biodegradation, application, and commercial production of synthetic biopolymers, based on most recent literature, with a special focus on biomedical applications.

1 Evolution of Synthetic Biopolymers

Synthetic polymers have become part of our everyday life some 50 years ago. They are durable, light weight, and inexpensive, which made them an attractive choice for diverse and numerous applications, with products ranging from packaging materials, household goods, and biomedical supplies to even construction materials. However, synthetic polymers are mainly synthesized from petroleum based resources, e.g., oil and gas, which are nonrenewable. In a world with growing demand for energy, the use of fossil fuels for synthetic polymers is not sustainable. Moreover, the disposal of synthetic polymers is problematic because of their accumulative properties and adverse effects to the environment. Synthetic polymers are not biodegradable and recycling is expensive. As a result, their disposal is often dependent on incineration or combustion. The end products of these materials are toxic and hazardous to soil, water, and air [1]. Conversely, synthetic biopolymers are synthesized from renewable, bio-based source materials and are biodegradable. Apparently, the use of biopolymers by human beings is very primitive, compared to that of synthetic polymers. For example, chitin, which is a natural polysaccharide produced by brown algae, has been used as food additives long time ago [2].
The definition of “biopolymer” is broad. Biochemists or cell biologists often use the term “biomolecules” to refer to biopolymers that are synthesized as part of the growth phase or cell survival in organisms or plants. From this point of view, nucleic acids, proteins, amino acids, fatty acids, and polysaccharides are considered as biopolymers and these go through the process of natural degradation extracellularly or intracellularly. However, according to the polymer chemists, biopolymers are those that can be derived either from living organisms or renewable resources and their degradability can be influenced by the chemical and physical microstructure of polymers. Within this concept of biopolymer, synthetic biopolymers can be defined as those biopolymers that can be synthesized naturally or by classical chemical synthesis process where the molecules undergo complete or partial degradation, leaving zero or nontoxic residues inside the body or in the environment (Fig. 1) [3].

For the last two decades, commercial production of biopolymers is gaining momentum over synthetic polymers because of environmental concerns. In 2011, the worldwide production of biopolymers, consisting of natural derivatives and synthetic biopolymers (Fig. 2), was approximately 1.4 mio/t, and by 2015, this production is expected to be doubled to 2.8 mio/t. Again, the total production of biopolymer by different countries of Europe, Asia, North America, South America, and Australia have been recorded to be 37%, 29%, 18%, 15%, and 1%, respectively [4].

Synthetic biopolymers are now favored as a source material for medical equipment and supplies because of their higher stability compared to natural polymers during formulation, transportation, and use. In addition, they are sterilizable and resistant to heat and hydrolysis. Due to these advantages, synthetic biopolymers are now widely used in tissue engineering and regenerative medicine, gene therapy, novel drug delivery systems, implantable devices, and in many other products [5–7].

Fig. 1 Origin, synthesis, and degradation of synthetic biopolymers
In this chapter, we provide brief notes on different classes of synthetic biopolymers including their physicochemical properties, synthesis mechanisms, applications, mechanisms of biodegradation, and their limitations. Synthetic biopolymer products that are already in the market, as well as products that are expected to appear in the market in near future, are both included in the discussions, with more emphasis on medical and surgical products.

2 Synthesis of Synthetic Biopolymers

Synthetic biopolymers can be bio-based polymers or chemically synthesized polymers (Fig. 2). However, even the bio-based polymers need to go through the chemical synthesis process for functionalization. The chemical synthesis process, in general, is known as polymerization. Polymerization is of different types and they are classified based on the types of reactions involved in the synthesis process [8]. Some of these are discussed below:

2.1 Addition Polymerization

In “addition polymerization,” all of the atoms of the monomer molecules become part of the polymer. The repeating units or monomers have same composition. This type of polymerization is also known as chain-growth polymerization because the polymer growth proceeds by successive addition of hundreds or thousands of monomer units in the presence of reactive intermediates. Initially, an initiator species, R, with a reactive center is produced, which is typically a free radical, a cation, or an anion. Then propagation of the reactive center proceeds through successive addition of large number of monomer molecules to the reactive center.
Chain termination occurs when the reactive center is destroyed by one or more possible termination reaction. In “addition polymerization,” most of the monomers taking part in the reaction process have double bonds (e.g., polyacrylonitrile is produced from acrylonitrile monomers $n\text{CH}_2 = \text{CHCN} \rightarrow [-\text{CH}_2\text{CHCN} -]^n$) where cross-linking can be achieved using these monomers \[9\].

### 2.2 Condensation Polymerization

In “condensation polymerization,” some of the atoms of the monomers are split off in the reaction in the form of water, alcohol, ammonia, carbon-di-oxide, etc. The monomers that participate in these reactions have functional groups like hydroxyl group (–OH), amide group (–NH$_2$), or carboxyl group (–COOH) and each monomer has at least two reactive sites. An example of condensation polymerization is the synthesis of polyesters through ester linkages between monomers with functional groups like carboxyl and hydroxyl groups.

Other than synthetic polymers, several naturally occurring polymers exist such as cellulose, starch, wool, and silk. Cellulose, for example, is considered a condensed polymer as it releases water when converted to glucose.

Condensation polymerization is also known as “step-polymerization” which proceeds through stepwise reaction between the functional groups of reactants, and the size of the polymer molecules increases at a relatively slow pace until a large-sized polymer molecule is formed.

Some polymers can be formed by both polymerization processes. One such example is polyethylene glycol, which can be formed either by condensation polymerization involving dehydration of 1,2 ethanediol (ethylene glycol) or by addition polymerization of oxacyclopropane (ethylene oxide) \[9\].

### 2.3 Metathesis Polymerization

There are two different types of metathesis polymerization, (a) acyclic-diene metathesis (ADMET) polymerization and (b) ring-opening metathesis polymerization (ROMP). In ADMET polymerization, the reaction starts with an acyclic diene and ends up in a polymer with a double bond in the backbone with ethylene as a by-product.

In ROMP, a cyclic olefin that does not have a cyclic structure in its backbone is synthesized. ROMP is used to make big things in one piece or to form cross-linked thermostat materials \[9\].

### 2.4 Modification in Structure/Linking

The characteristics of biopolymers are defined by their applications, and the ultimate use of products made out of biopolymers. The inherent properties of the polymer such as chemical structure, molecular weight, solubility can affect the stability,
strength, biocompatibility, preparation, and processing methods as well as biodegradability of the polymer. Therefore, modifications are made at the structural level in order to attain product associated attributes.

The polymer should be designed to contain bond-cleavage segments in their molecular structure. The segments should contain bonds such as esters, anhydrides, carbonates, amides, orthoesters, urethanes, urea units (Fig. 3). These functional groups render the material susceptible to hydrolysis and thus make it easily biodegradable. Of the mentioned units, anhydride and orthoesters are most easily hydrolysable than ester and amide units. Other than the biodegradable units, factors such as molecular structure, compositions, cross-linking density, crystallinity, molecular weight, hydrophilicity and hydrophobicity, sample shape and morphology, ambient temperature, and pH values need to be considered. Polymers with low cross-linking density, low molecular weight, strong hydrophilicity, low crystallinity, thinner section, and porous structure are easily biodegradable [10, 11].

2.5 Cross-linking

Biodegradable polymers are prepared both by refining and modifying natural polymers such as polysaccharides, cellulose, and proteins or by chemically synthesizing routes which is mainly polycondensation, ring-opening polymerization (ROP), enzyme-catalyzed polymerization, thermally and photo-initiated free-radical polymerization, etc. Whatever the preparation method is, cross-linking structures with three-dimensional networks should be formed. In addition, the biodegradable segments mentioned earlier (amides, esters, etc.) should be incorporated in the molecular chains because these will endow the material with flexibility, high resilience, and good biodegradation. Introduction of cross-linking to a polymer affects the crystallinity, melting point, glass transition temperature, and solubility of the polymer. Mechanical properties of the polymer including tensile strength, impact strength, and modulus of the polymer are modified after the polymer is cross-linked [10, 12].

2.6 Surface Modification

Polymers to be used for nanoparticle designing require surface modification to achieve the long circulation of the therapeutic agent inside the body and targeting the specific site of action. The active agent is entrapped inside the synthetic
nanoparticle, which is diffused through the polymer matrix at a sustained rate at their site of action. The active agent can also be released through biodegradation of the polymer, and the rate of degradation can be made variable by changing the composition and molecular weight of copolymers. Surface modification can be done by surface coating with hydrophilic polymers or surfactants and through development of biodegradable copolymers with hydrophilic segments. The ultimate goal is to improve mucosal absorption of nanoparticles either by modifying the surface property or coupling a targeting molecule at the surface [13, 14].

Along with other desirable attributes of biopolymers, safety is also an important factor for certain applications. Many synthetic biopolymer products are used as implants inside the body. Therefore, these should not cause thrombosis, cell injuries, plasma and protein degeneration, enzyme inactivation, electrolyte disturbances/imbalances, inflammation, allergic reactions, toxification, and carcinogenesis. To meet these requirements, both degradable and nondegradable polymers should be of high purity and possess optimal physicochemical properties when they are applied in biomedical fields. To obtain this, the monomers should be inexpensive, nontoxic, and easy to use. In addition, various additives used during preparation and processing, such as initiators, catalysts, and cross-linking agents, should be nontoxic or removed completely from the final products. Also, when used for tissue engineering or drug delivery, their degradation rate should match with the rate of tissue regeneration and drug release [10, 15, 16].

3 Degradation of Synthetic Biopolymers

Biodegradability is one of the most important factors that make synthetic biopolymers preferable to synthetic polymers for a wide range of applications. In particular, synthetic biopolymers are of great demand in the biomedical field because of their biocompatibility and biodegradability. Degradation of polymers depends on several factors. These include physical and chemical properties of the molecule such as (a) availability of functional groups that increase hydrophilicity of the molecule, (b) size, molecular weight, and density of the polymer, (c) amount of crystalline and amorphous regions, (d) structural complexity, e.g., linear or branched, (e) presence of easily breakable bonds, e.g., ester or amide bonds, (f) molecular composition, and the (g) nature or physical form of the molecule (e.g., films, pellets or powder). Degradation also depends on environmental conditions. The presence of moisture, air, temperature, high energy radiation (UV, γ-radiation), light (photo-degradation), pH, and even the microorganisms (bacteria, fungi, etc.) affect the biodegradation of the molecule. Considering these factors, polymers are grouped into two major classes: (i) hydrolytically degradable polymers and (ii) enzymatically degradable polymers. Naturally occurring polymers undergo enzymatic degradation, whereas synthetic polymers undergo hydrolytic or combination of enzymatic and hydrolytic degradation depending on the monomers, chemical properties, and other factors mentioned above [17–20].
Degradation occurs in several steps. Initially, the polymer is fragmented into low molecular mass species either by abiotic reactions (e.g., oxidation, photodegradation or hydrolysis) or by biotic means (e.g., degradation by microorganisms). Depending on the end product and site of degradation (e.g., in vitro or in vivo), polymer fragments are bio-assimilated and mineralized by microorganisms or, excreted from the body once it undergoes complete digestion by the metabolic pathways (e.g., citric acid cycle, urea cycle). Polymers with hydrophilic property facilitate microbial attachment and degradation. However, polymers like polyethylene (PE) or polypropylenes contain hydrophobic groups –CH₂. These undergo fragmentation by abiotic reaction followed by insertion of hydrophilic groups on the polymer surface to enhance biodegradability. When a polymer is completely biodegraded, the final product produced is water and carbon-di-oxide. Under conditions of partial biodegradation, less toxic or environmentally friendly products are produced [21–24].

4 Common Synthetic Biopolymers and their Applications

4.1 Poly(α-Hydroxy Esters)

This is the most studied class of biopolymers. Various monomers can be used for their synthesis. Synthesis mechanisms are diverse and include naturally occurring polymers and chemically synthesized polymers. The later can be obtained in two principal ways: poly-condensation polymerization and ring-opening polymerization (ROP). Polycondensation involves direct esterification of hydroxy acids or diacids and diols, which yields low molecular weight polymers. The monomers should be highly pure; otherwise it will affect the molecular weight of the final product. On the other hand, ring opening polymerization (ROP) of cyclic esters proceeds under milder condition and is preferred for the synthesis of high molecular weight polymers. The advantages of ROP over the polycondensation route of synthesis include (i) shorter reaction time, (ii) absence of reaction by-products, and (iii) the ability of using six or seven membered lactones. Stannous octoate and 2-ethylhexanoic acids are examples of biocompatible catalysts that are used to control the rate of polymerization. To improve the biocompatibility of polymers, several routes of polymerization have been developed that do not require solvents. The polymers contain hydrolytically labile ester linkages and are susceptible to biodegradation [19, 24–27].

4.1.1 Poly(Glycolic Acid) (PGA)

This is the simplest and linear aliphatic polyester (Fig. 4). The monomer unit is a cyclic lactone called glycolide. The polymer is synthesized by ring opening polymerization (ROP). PGA is highly crystalline (45–55% crystallinity) and therefore exhibits a high tensile modulus with low solubility in most organic solvents. Melting point of PGA is 220–225 °C and glass transition temperature is 35–40 °C [19, 24, 25, 28].
Despite the fact that PGA is poorly soluble, through the application of techniques such as extrusion, injection, compression molding, particulate leaching, and solvent casting, PGA has been modified to take variety of forms and structures. PGA has excellent fiber forming ability. Therefore, it is used for developing biodegradable synthetic sutures called DEXON\textsuperscript{®}. The nonwoven PGA is extensively used as scaffolding matrices for tissue regeneration due to its excellent degradation property. Another nonwoven, polyglycolide-based, fabric-fibrin glue composite matrix is undergoing clinical trial due to its excellent skin closing ability without requiring sutures and its ability to help regenerate tissues. PGA also has excellent crystalline property. Therefore, it has been investigated as internal fixation devices for bones (Biofix\textsuperscript{®})\cite{19, 24, 25, 28}.

PGA is degraded by nonspecific scission of the ester backbone. Inside the body, polyglycolide is degraded into glycine, which is further converted into carbon dioxide and water via the citric acid cycle or excreted in the urine.

Despite its excellent mechanical properties exhibited by high crystallinity, low solubility and high rate of degradation limit the application of PGA. For biomedical applications, glycolide was combined with caprolactone, lactide, or trimethylene carbonate to form copolymers\cite{19, 24, 25}.

### 4.1.2 Poly(Lactic Acid) (PLA)

PLA is obtained from polycondensation of D- or L-lactic acids or from ring opening polymerization of lactides (Fig. 5). Lactide is a chiral molecule and exists in two optical forms. The active forms are L-lactide, which is the natural isomer and D-lactide, which is a synthetic blend. Poly(L-lactide) (PLLA) has a glass transition temperature of 60–65 °C and a melting temperature of approximately 175 °C. Like PGA, PLLA has high crystallinity (approximately 37%). The degree of crystallinity depends on the molecular weight and polymer processing parameters\cite{19, 24, 25, 29}.

High molecular weight PLAs have better mechanical property and these are obtained through ring opening polymerization. This route of polymerization enables the control of final properties of PLA by adjusting the proportion of enantiomers. PLA can also be polymerized by melt/solid state polymerization, solution
polymerization, or chain extension reaction. Commercialized PLA have various ratios of D/L lactide [29].

The trade name and suppliers of different types of PLA are listed in Table 1. PLLA has god tensile strength, low extension, and high modulus (approximately 4.8GPa). Due to these good mechanical properties, the polymer is applied in orthopedic fixation devices such as soft tissue fixation screws, suture anchors and interference screws [19]. PLLA also forms high strength fibers. Due to this property, PLLA has been investigated as scaffolding material for developing ligament replacement or augmentation devices to replace the nondegradable fibers. Some PLLA fiber-based materials are currently under investigation to be used as long-term bloods vessel conduits. An injectable form of PLLA (Sculptra®) is under clinical trial for the restoration or correction of facial fat loss or lipoatrophy [25].

PLA is used to prepare packaging material such as lawn waste bags, cups, cutlery, plates, bottles, films. The advantage of using PLA for these products is its availability and lower price, compared to other biodegradable polyesters. In automotive, PLA is mixed with fibers of kenaf to replace the panels of car doors and dashboards. In electronic applications, compact discs based on PLA and computer cases made of PLA is already in market [19, 24, 30]. In the construction industries PLA fibers are used in padding and paving stones of carpet. Due to its less inflammability, it is safer than any other synthetic fibers. PLA fibers are less allergic and they are resistant to UV radiation. PLA fibers are used in combination with natural fibers for making sport clothes. PLA and its copolymers are also used in diapers, cotton stalk, and sanitary products.

Poly(DL-lactide) (PDLLA) is an amorphous polymer with glass transition temperature of 55–60 °C. Due to the amorphous nature, it has much lower strength compared to PLLA and is degraded at a rate, which is much faster than PLLA. It is considered a

<table>
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highly preferable material for drug delivery and also a preferable scaffolding material for tissue regeneration [19, 24, 30]. Poly(L-lactide) (PLLA) is a hydrophobic polymer. The presence of the methyl group (–CH₃) is responsible for this property. It is therefore more resistant to hydrolysis due to this side group. The rate of degradation however depends on the degree of polymer crystallinity and the porosity of the matrix. Biodegradability of PLA can be achieved by employing hydroxy acid comonomer components or by racemization of D-L- isomers. Polylactides undergo hydrolytic degradation by random scission of the ester backbone. The degraded product lactic acid is a by-product metabolite in human and undergoes final degradation into water and carbon-di-oxide by citric acid cycle [19, 24, 30].

Major disadvantages of PLA is its brittleness and poor thermal stability. It can be plasticized to improve the chain mobility and crystallization. Plasticization can be done using oligomeric acid, citrate ester, or low molecular weight polyethylene glycol [19, 24, 30, 31].

4.1.3 Poly(lactide-co-glycolide) (PLGA)
L-lactide and DL-lactide are used as copolymers with glycolic acid monomers (Fig. 6). The polymer is synthesized by solvent evaporation method. Different ratios of poly(lactide-co-glycolide) are commercially developed, of which Lactel® (Durect Corporation, USA) is mentionable. Purasorb®PLG is a semi-crystalline bio-resorbable co-polymer with L-lactide and glycolide ratio of 80 L:20G. Another co-polymer with 90% glycolic acid (GA) and 10% L-lactic acid (LA) was used for the development of multifilament suture Vicryl®. Generally, the amorphous polymers contain a ratio of 25 L:75G monomers. On the other hand, copolymer with the ratio of 80 L:20G is semi crystalline. The higher the ratio of the monomers L/G, the lower is the degradation rate [19].

PLGA is applied in the form of meshes (Vicryl Mesh®), suture reinforcements, skin replacement materials, and dura mater substitutes [19, 24, 30].

PLGA undergoes bulk erosion through hydrolysis of the ester bonds. The rate of degradation depends on several parameters including the ratio of LA/GA, molecular weight, and shape and structure of the matrix. This biocompatible co-polymer has been approved by FDA for human use due to its controllable degradation rate and good processibility, which enables fabrication of a variety of structures and forms.

PLGA has good cell adhesion and proliferative properties, making it a potential candidate for tissue engineering applications. Applying micro- and nano-fabrication techniques, three dimensional scaffolds based on PLGA have been developed [19, 24, 30].

PLGA is also used in guided tissue regeneration by providing a permeable material for space preservation (CYTOPLAST Resorb®). Another application of PLGA is as drug delivery vehicle (LUPRON DEPOT®) for the controlled release of gonadotropin hormone in prostate cancer and endometriosis [19, 24, 30].

PLGA is also used as drug delivery vehicles such as microspheres, microcapsules, nanospheres, and nanofibers for the controlled release of drugs or proteins (Fig. 7). However, due to the bulk degradation of the polymer or release of acidic biodegradation products there is a risk of protein denaturation [13, 32].
4.2 Poly(p-dioxanone) (PPDO/PDS)

Poly(p-dioxanone) is a colorless, semi-crystalline aliphatic polyester. The polymer is synthesized by the ring opening polymerization of \( p \)-dioxanone (Fig. 8). The polymer has low glass transition temperature ranging from \(-10^\circ C\) to \(0^\circ C\) [33].

PDS was the first commercially developed suture (PDS\(^\text{®}\)), which was also investigated for orthopedic applications such as fixation screws for small bone and osteochondral fragments (Orthosorb Absorbable Pins\(^\text{®}\)) [19, 34].

The polymer undergoes degradation by the nonspecific scission of the ester backbone. Inside the body, it is broken into glyoxylate and excreted in the urine or converted into glycine which subsequently degrades into carbon di-oxide and water [19, 33].

4.3 Poly(\(\varepsilon\)-caprolactone) (PCL)

Poly(\(\varepsilon\)-caprolactone) is a semi-crystalline polyester, synthesized by the ring-opening polymerization (ROP) of a relatively cheap monomer, \(\varepsilon\)-caprolactone (Fig. 9). PCL is soluble in wide ranges of solvents, which makes it highly processable. Its glass transition temperature is \(-60^\circ C\) and has low melting point (60–65 °C). PCL has the ability to form miscible blends with a wide range of polymers [19]. Some commercially available PCL are listed in Table 2.
The polymer undergoes hydrolytic degradation due to the presence of the labile aliphatic ester linkages, which slows down its degradation rate (2–3 years). Therefore, PCL was investigated for use as delivery vehicle for long-term drugs and vaccines. Capronor® is a long term contraceptive device composed of PCL [19, 35].

PCL also has low tensile strength (23 MPa) and extremely high elongation breakage (>700%). It also has excellent biocompatibility. It is therefore considered as a material for the development of various micro and nano-sized drug delivery vehicles and as scaffolds for tissue engineering. Composites of PCL with calcium phosphate-based ceramics are also investigated for suitable scaffolds for bone tissue engineering [19, 28, 36].

Since PCL is slowly degradable, copolymers with lactide or glycolide have been prepared to improve the biodegradation. Copolymerization of ε-caprolactone with DL-lactide yielded materials that are more rapidly degradable than the native polymer and resulted in fibers that are less stiff than those made of polyglycolide. MONACRYL® is a copolymer of ε-caprolactone and DL-lactide, which is commercially available. Copolymers of ε-caprolactone, glycolide, lactide, and poly(ethylene glycol) units have been developed to be used as drug delivery vehicle for small to medium sized biologically active molecules (SynBiosys®). Other than medical applications, PCL is used in shift compostable packaging [19, 37, 38].

Like PLA, PCL and its derivatives are used to prepare disposable cutleries, plates, etc.; they are also used in diapers, cotton stalk and sanitary products [4].
4.4 Poly(Trimethylene Carbonate) (PTMC)

These are obtained by the ring opening polymerization (ROP) of trimethylene carbonate. This is an elastomeric aliphatic polyester with excellent flexibility and poor mechanical strength. The high molecular weight PTMC is investigated as candidate implant material for soft tissue regeneration. The low molecular weight PTMC are investigated as suitable material for developing drug delivery vehicles [19, 39].

Unlike the other esters, PTMC undergoes surface degradation with the rate of in vivo degradation faster than the in vitro degradation [2].

Due to low mechanical performance of PTMC, several co-polymers were developed with other cyclic lactones. Copolymers with glycolide and dioxanone show improved mechanical properties. For example, poly(propylene carbonate) (PPC) is synthesized via copolymerization of propylene oxide and carbon dioxide. Although it has good compatibility, the thermal stability and biodegradation is improved by blending it with other polymers. Commercially available copolyester carbonate (PEC) has Tm of about 100–110 °C. The content of carbonate can be changed. Mitsubishi Gas Chemical Co. (Japan) commercializes poly[oligo(tetramethylene succinate)-co(tetramethylene carbonate)] (PTMS). The introduction of carbonate into PTMS lowered the Tm and increased its susceptibility to enzymatic and microbial attacks [19, 40].

Examples of commercially available products are flexible suture materials (Maxon®, BioSyn®) and orthopedic tacks and screws (Acufex®) [19].

4.5 Poly(alkenedicarboxylate)

Poly(butylene succinate) (PBS) (Fig. 10) and its copolymers are among the most common materials under this group. PBS is synthesized by polycondensation reactions of glycols (e.g., ethylene glycol and 1,4-butanediol) with aliphatic dicarboxylic acids (e.g., succinic acid and adipic acid) [2, 41].

The molecular weight of different copolymers ranges from tens to several hundreds of thousands. The use of small amount of coupling agents as chain extenders increases molecular weight of the polymers. Some important polymers of this class are PBS, PES (polyethylene succinate), PBSA (polybutylene succinate co-adipate), etc. Commercially available polymers of this class are Bionolle®, Enpol®, Skygreen®, Lunare SE®, etc. The nature of the diacids and diols used in these polymers influences the physical properties and biodegradation rates [2, 42, 43].

The copolyester PBSA has reduced tensile strength due to the incorporation of adipic acid. While PBS is the polyester with highest tensile strength, the copolymer PBSA shows improved elongation [2].

PBS is a white crystalline thermoplastic, with melting temperature around 90–120°C and glass transition temperature about −45 °C to −10 °C. The mechanical properties of PBS resemble those of polyethylene and polypropylene. Biodegradation of PBS depends on the physical environment. Due to insufficient
biocompatibility and bioactivity, surface modification of PBS by means of plasma treatment was used for medical application [2, 44, 45].

In the agricultural field, PBS is used in the fabrication of mulching films or slowly released materials for pesticide and fertilizer.

PBS and its copolymers are used for disposable cutleries, plates, etc. They are also used in diapers, cotton stalk, and sanitary products.

4.6 Fumarate-Based Polymers

Poly(propylene fumarate) PPF is a fumarate based, linear, unsaturated, hydrophobic polyester synthesized by several ways including the transesterification of fumaric diester. The molecule contains unsaturated fumarate group on the polymer backbone which can be used to further cross link the polymer to improve its material properties (Fig. 11). It is highly viscous at room temperature and soluble in wide variety of organic solvents [2, 46].

Several cross-linked or composites of PPF have been developed for medical applications. Cross-linked PPF is developed by co-polymerizing with acrylic monomers (e.g., methyl methacrylate or N-vinyl pyrrolidone) using different initiators. Again, composites of PPF with ceramics (e.g., tricalcium phosphate or calcium sulfate) were created, with high tensile matrices, suitable for orthopedic applications. The cross-linked PPF supports cell viability and functions as growth factor delivery system, which makes them promising candidates for bone tissue engineering applications [2, 15, 47].

PPF undergoes bulk erosion via hydrolysis of the ester bonds. Its degradation depends on several parameters including the molecular weight, types of cross-linkers, and cross-linking density. The degradation products of PPF are fumaric acid and 1,2-propane diol.

![Fig. 10 Poly(butylene succinate)](image)

![Fig. 11 Poly(propylene fumarate)](image)
Oligo(poly(ethylene glycol) (OPF) is another fumarate-based polymer, composed of alternating polyethylene glycol (PEG) and fumarate moieties. OPF is highly hydrophilic and cross-linking occurs through the fumarate groups. The mechanical property of OPF is controlled by the ratio of fumarate to PEG and the molecular weight of PEG. PEG blocks give hydrophilic property of the copolymer. With the increase in the molecular weight of the PEG, the swelling property of the hydrogel increases. This hydrophilic property enabled OPF hydrogels to be used to encapsulate mesenchymal stem cells for bone engineering applications. Like PPF, degradation of OPF is mediated by hydrolysis of the ester bonds [5, 48, 49].

4.7 Polyhydroxyalkanoates (PHA)

These are natural polyesters. There are more than 100 types of PHAs which are synthesized by more than 200 different species of bacteria as carbon storage material. Of the different types of PHAs, polyhydroxybutyrate (PHB) is the most studied one (Fig. 12). PHB is highly crystalline (above 50%). Its glass transition temperature (Tg) is 55 °C and melting temperature (Tm) is 180 °C. In addition to the bacterial synthesis pathways, PHB is also synthesized by chemical modifications involving ring opening polymerization of β-butyrolactone [2, 50, 51].

Some of the mechanical properties of the polymer are comparable to synthetic degradable polyester, PLA. However, the polymer is brittle and therefore it is plasticized with citrate ester.

PHB is biodegradable by different microorganisms. The polymer is modified by using grafted monomers such as styrene, sodium-p-styrene sulfonate, methyl acrylate, and acrylic acid [50, 52, 53].

Poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) is a copolymer of hydroxybutyrate and hydroxyvalerate (Fig. 13). It was first synthesized by Imperial Chemical Industries (ICI) in 1983 by adding propionic acid to bacterial feedstock. PHBV is highly crystalline with Tg in the range −5 °C to 20 °C and the Tm 108 °C. The pure copolymer is brittle like PHB. Its mechanical properties are modified by increasing the content of hydroxyvalerate. Other approaches include mixing with other polymers or additives. However, due to chemical incompatibility, additives are used, such as saccharin as nucleating agents, glycerol as plasticizers, triacetin or tributyrin and glycerol monomer tristearate as processing lubricants [54–56].
Commercially available PHB (Table 3) is used in small disposable products and in packaging materials. PHBV has piezoelectric property and is used in electric simulation applications. PHB is degraded into D-3-hydroxybutyrate, which is a natural constituent of human blood. Due to this property, PHB is suitable for applications as drug carriers and tissue engineering [57, 58].

### 4.8 Aromatic Co-polyesters

This group includes a wide range of polyesters or copolyesters with aliphatic monomeric units of different sizes. Aromatic polyesters are insensitive to hydrolytic and microbial degradation. To increase their biodegradability, aliphatic-aromatic copolyesters are made. Commercially available copolyester poly(butylene adipate-co-terephthalate) (PBAT) is obtained by polycondensation between 1,4-butanediol and a mixture of adipic acid and terephthalic acid. The polymer shows good mechanical and thermal properties when the concentration of terephthalic acid ranges from 35% to 55%. Introduction of lactic acid, glycolic acid, or succinic acid is currently used to prepare novel biodegradable polymers by melt reaction. Commercially available PBAT (Ecoflex®, Easter Bio®, and Origo-Bi®) is used in tyres [2, 19, 59, 60].

### 4.9 Polyamides and Poly(ester-amide)

Polyamides contain amide bonds as in the polypeptides (Fig. 14). They have high crystallinity and strong chain interactions. Polyamides are degraded by enzymatic and microbial attacks. Biodegradation can be increased by the introduction of different side groups such as benzyl, hydroxyl, and methyl groups through copolymerization [2, 19].
Copolymers with amide and ester groups are easily degradable. The higher the ester content, the greater is the rate of degradation. Aliphatic poly(ester-amide)s are synthesized from 1,6-hexanediol, glycine, and diacids with various methylene groups varying from 2 to 8. These copolymers are highly crystalline [2, 61].

Copolymers with 1,2-ethanediol, adipic acid, and amino acids, including glycine and phenylalanine, show susceptibility to enzymatic degradation. The degradation rate is controlled by modifying the phenylalanine and glycine ratios. CAMEO is a poly(ester-amide) blend based on leucine or phenylalanine. It is used for site-specific delivery of small hydrophobic drugs and peptides. Another commercially available polyamide is BAK 2195®. It is used for injection molding. Other commercially available amides are listed in Table 4. They are applied in broad areas such as in electrical and electronic devices, automotive, mechanical engineering, sport and leisure goods, blow molded tubes, pharmaceutical vials or tubes, and in heat and UV resistance and extrusion applications. [2, 19, 62, 63].

The presence of the ester bonds imparts biodegradation of amides. Their degradation takes place by hydrolytic cleavage of the ester bonds [2, 19].

4.10 Polyurethanes (PUs)

These polymers have a wide range of physical and chemical properties. They have been tailored for use in coating, adhesives, fibers, foams, and thermoplastic elastomers. PU is prepared from three constituents, which are a disocyanate, a chain extender, and a polyol (Fig. 15). These react to form segmented polymer with alternating hard segment and soft segment. The soft segments are derived from polyols (e.g., polyester polyols and polyether polyols), whereas
the hard segments are formed from the diisocyanate and the chain extender. However, most common isocyanates are toxic. Therefore, aliphatic, biocompatible diisocyanates have been used. Poly(ester urethane)s are prepared by reaction between lysine diisocyanate and polyester diols based on lactide or \( \varepsilon \)-caprolactone. Another biocompatible diisocyanate is 1,4-diisocyanobutane. Biodegradation of these polymers depends on the chemical nature of the segments, especially the soft segments. Recently, by introducing chain extenders with hydrolysable ester linkage in the hard segment has been shown to increase its degradability [64–69].

PUs with polyether base are resistant to biodegradation. However, if the polyol is a polyester (e.g., PCL, PLA, and PGA), these are readily degradable. Novel biodegradable poly(ester urethane) (Degrapol\(^\text{\textregistered}\)) consisting of poly(L-lactic acid) (PLLA) and poly(butylene succinate) (PBS) blocks has been synthesized. It is synthesized via a chain extension reaction of dihydroxyl terminated PLLA and PBS pre-polymers. Toluene-2,4-diisocyanate is used as chain extender and the crystallization of the polymer is caused by the PBS segment. PBS segments also improved the extensibility of PLLA [2, 70].

Another novel polymer is based on chitin/1,4-butandiol blends. At first, a prepolymer is synthesized from poly(e-caprolactone) and 4,4-diphenylmethane diisocyanate. The prepolymer is extended with chitin and 1,4-butane diol at different ratios. At high chitin content, the mechanical and also the biodegradability of the

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polymer are increased. Polymers of this type have attractive features to be applied in the medical field [71, 72].

Another development in this field is the formulation of waterborne polyurethane or poly(urethane-urea). These have the advantage of low viscosity at high molecular weight, nontoxicity, and good applicability. In addition, they are more environment-friendly and easily biodegradable. A novel waterborne PU using rapeseed oil-based polyol in the soft segment was synthesized which has very good mechanical properties. These are used to modify plasticized starch with higher performances and better biodegradability [2, 73–77].

Commercially available PUs (Table 5) are used in medical and orthopedic applications. Degrapol® has been used to develop highly porous scaffold for tissue engineering (Fig. 16). PolyNova® is developed for orthopedic applications [19].

Table 5  Trade names and suppliers of polyurethanes and their copolymers

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In vitro organ formation

Direct implantation of polymer scaffolds
- Polyanhydrides
- Polyurethane
- Polyelectroactive materials
- Polylphosphoester
- Poly(propylene fumarate)
- Polyesterurathane

In vivo implantation
- Bone tissue
- Vascular and bone tissues
- Nerve tissue
- Bone tissue
- Genitourinary tissues

Fig. 16  Tissue engineering using polymer scaffolds
4.11 Poly(Ortho Esters)

Four different classes of polyorthoesters have been developed: POEI, POEII, POEIII, and POE IV (Fig. 17). Among these, POEI is synthesized by transesterification between a diol and diethoxytetrahydrofuran. POEII is synthesized by the reactions between diols with diketene acetal 3,9-bis(ethyledene 2,4,8,10-tetraoxaspiro[5,5]undecane), POEIII is synthesized by direct polymerization of a triol with an orthoester, and POE IV is developed by incorporating short segments based on lactic or glycolic acid into POEII polymer backbone. Of these four polymers, γ-hydroxybutyrate is a hydrolytic product of POEI and has autocatalytic effect to further the degradation of the material. POEII is therefore synthesized with control of the degradation rate using itaconic and adipic acids. POEIII forms viscous gel-like material at room temperature. This property allows it for the incorporation of therapeutic agents into the polymer matrix without the need for solvents. The release of 5-fluorouracil (5FU), a cancer chemotherapy drug, from POEII has been investigated. However, the gel-like consistency inhibits the scaling up of the synthetic procedure. Therefore, POE IV is developed. Once the polymer is exposed in the aqueous environment, the polymer undergoes hydrolysis and the lactic or glycolic acid liberated, catalyzes further polymer hydrolysis [19, 78–80].

Of the four POEs, POE IV has been extensively studied due to its biocompatible nature, scalable synthetic procedure, and controlled release profiles, making it compatible to be used with a wide range of pharmaceutical agents.

4.12 Polyanhydrides

These polymers (Fig. 18) contain two hydrolysable sites in the repeating unit, which make them susceptible to biodegradation. Both aromatic and aliphatic polyanhydrides have been studied. Aromatic polyanhydrides degrade slowly and
may take a long period, whereas aliphatic polyanhydrides can degrade in a few days [2, 5, 81, 82].

Polyanhydrides are synthesized by a number of polymerization processes. These include melt condensation of diacids/diacid esters, ROP of anhydrides, interfacial condensation, dehydrochlorination of diacids and diacid chlorides, or the reaction of diacyl chlorides with coupling agents such as diphosgene. Both homo and co-polyanhydrides with different properties have been developed [5, 81].

Aliphatic homo-polyanhydrides, like poly(sebacic anhydride) (PSA), have limited applications due to their high crystallinity and fast degradation rate. Copolymers of anhydride with imide were synthesized to improve its mechanical property for specific medical applications. Polyanhydrides with different linkages such as ether, ester, and urethane have been developed too. Several copolymers of sebacic anhydride and hydrophobic aromatic co-monomers have been developed, with controllable degradation rate. The degradation rate is controlled by adjusting the hydrophobic and hydrophilic components of the copolymer. By increasing the hydrophobicity of the building blocks of the polymer, their degradation rate was lowered. On the other hand, incorporation of acrylic functional groups in the monomeric unit produces photocrosslinkable polyanhydrides [19, 82–84].

Aliphatic polyanhydrides were developed as a fiber forming polymers for textile applications. In addition, due to their hydrolytic instability and surface eroding nature, aliphatic polyanhydrides are used as drug delivery vehicle. Their degradation products are nontoxic and biocompatible. The most extensively studied polyanhydride, poly[(carboxy phenoxy propane)-(sebacic acid)] (PCPP-SA) has been approved by FDA to be used as localized delivery vehicle for the controlled delivery of chemotherapeutic agent, e.g., 5-fluorouracil (5FU), in brain cancer. Another co-polymer of 1:1 sebacic acid and erucic acid dimer has been used as potential delivery vehicle for gentamicin in the treatment of osteomyelitis [19, 82, 85–87].

4.13 Pseudo Poly(Amino Acids)

These are (Fig. 19) naturally occurring biodegradable polymers. However, their application is limited because of their water insolubility, lack of functional groups,
and immunogenic properties. To overcome these limitations, pseudo amino acids have been developed where the amino acids are linked to nonamide bonds such as esters, imino carbonates, and carbonates. Tyrosine-derived poly(amo acid)s using desaminotyrosyl-tyrosine esters as the building blocks have been the most investigated polymer [19].

The presence of the aromatic backbone renders the molecule with good mechanically competent polymer for biomedical applications.

Of the different pseudo polyamino acids, tyrosine-derived polycarbonates are a versatile polymer class. The glass transition temperature ranges between 50 °C and 90 °C and their mechanical properties can be easily tailored by varying the pendant alkyl chain. The polymer is amorphous, hydrophobic in nature and undergoes slow hydrolytic degradation. The degradation products are desaminotyrosyl-tyrosine and alcohol in in vitro condition and desaminotyrosine and tyrosine in vivo. The good in vitro and in vivo osteocompatibility and mechanical properties make the polymers suitable candidate for developing orthopedic implants, fracture fixation pins, etc. [19, 88].

**4.14 Poly(Alkyl Cyanoacrylates)**

PCA are the major class of biodegradable acrylate polymers. These are neutral polymers and prepared by the anionic polymerization of alkyl cyanoacrylic monomers with a trace amount of moisture as the initiator. The polymer contains carbon-carbon sigma bonds on polymer backbone (Fig. 20). The degradation rate of the polymers depends on the length of the alkyl side groups: lower alkyl derivatives and higher alkyl derivatives. The lower alkyl derivatives produce toxic products such as cyanoacetic acid and formaldehyde. Therefore, higher alkyl derivatives are preferred [19].

PCA has been shown to be an excellent material as synthetic surgical glue, skin adhesive (Dermabond®), and an embolic material. PCA has been studied for their suitability to be used for nanoparticles based drug delivery vehicles, too. It has several advantages over other polymeric materials due to its easy preparation methods, high utility size ranges, absence of solvent residues, ability to form stealth nanoparticles, and ability to absorb or encapsulate a wide range of drugs. Several PCA-based nanoparticles are currently under late stage clinical trials for cancer therapy [19, 89].
4.15 Polyphosphazenes

Polyphosphazenes are hybrid polymers of inorganic-organic constituents. The polymer backbone has alternating phosphorus and nitrogen atoms containing two organic side groups attached to each phosphorus atom (Fig. 21). The R group represents a variety of organic and organometallic side groups. The phosphorus nitrogen backbone is hydrolytically stable. However, incorporation of certain groups such as amino acid esters, glucosyl, glyceryl, glycolate, lactate, and imidazole sensitizes the polymer backbone [19, 90].

The most extensively investigated route for polymer synthesis involves two-step preparative protocol. In the first step, a reactive macromolecular intermediate, poly(dichlorophosphazene) is formed by ring opening polymerization (ROP) of a cyclic trimer, and in the second step, chlorine side units are replaced by any broad range of organic side groups [19, 90].

The presence of the phosphorus-nitrogen backbone gives unusual flexibility of the polymer. Therefore, the side groups play crucial role in determining the properties of these polymers and allows possibility of designing polymers with highly controlled properties of crystallinity, solubility, appropriate thermal transitions, and hydrophobicity/hydrophilicity [19, 35, 90].

Among the different classes of polyphosphazenes, poly[(amino acid ester) phosphazenes] are the most studied ones. And among these, polyphosphazenes substituted with glycine ether esters show fastest degradation. The in vivo and in vitro degradation of the amino acid ester polyphosphazenes and their biocompatibility have been well studied. These polymers elicited minimal to mild tissue responses when implanted subcutaneously in rat models. However, many of these polymers showed excellent osteocompatibility and have been investigated as matrices for bone tissue engineering [19, 86].
4.16 Polyphosphoester

These are another class of phosphorus containing polymers developed as biomaterials. The unique property of these polymers is their good biocompatibility and similarity to the biomacromolecule, nucleic acid.

Polyphosphoesters can be developed by several ways including ring opening polymerization, polycondensation, and poly addition reactions. The general structure of polyphosphoester is shown in Fig. 22, where R and R’ can be varied to develop polymers with wide ranges of physicochemical properties. R = H produces polyphosphites, R’ = alkyl/aryl group produces polyphosphonates, and R’ = arylxy/alkosy group produces polyphosphates [19, 91].

Polyphosphoesters undergo both hydrolytic and enzymatic cleavage. The breakdown products are phosphate, alcohol, and diols. Varying these groups can readily alter the physico-chemical properties of the polymers. The pentavalency of the phosphorus atoms allows for chemical linkages to be made between drugs or proteins and the polymer backbones, enabling the development of novel polymer pro-drugs. Most of the polymers show good in vitro cytocompatibility and good in vivo tissue compatibility [19, 92].

The synthetic flexibility of polyphosphoesters has allowed for the development of co-polymers with other monomers, such as DL-lactide to form poly(lactide-co-ethyl phosphate). This polymer has been investigated as a potential candidate for drug delivery applications of the chemotherapeutic drug paclitaxel. PACLIMER® microparticle contains 10% (w/w) paclitaxel and has undergone phase I human clinical trials for the treatment of ovarian and lung cancer. The drug delivery system showed delivery of paclitaxel in a sustained manner while maintaining appropriate biodegradation and biocompatibility. Poly(lactide-co-ethyl phosphate) is also under investigation as a scaffold for tissue engineering [19, 92].

Polyphosphoester polymers undergo degradation at 37 °C due to the hydrolytic cleavage of the phosphoester bonds in the polymer backbone. Therefore, polyphosphoesters and other cationic polymers (e.g., chitosan, polyamidoamine poly-L-lysine, polyarginine, polyornithine, polyethyleneimine) are used as nonviral gene delivery system in vitro (e.g., in tissue culture) and in vivo (e.g., mice tumor xenografts). These polymers bind plasmid DNA, form complexes called polyplexes, and are taken up by the cells via endocytosis. These polyplexes are relatively stable and protect the DNA from nuclease degradation, facilitating DNA escape from endosomes and finally releasing into the nucleus where transgene expression takes place.

Fig. 22 Polyphosphoester
place (Fig. 23). Transfection efficiency can be enhanced by conjugation of specific ligands, endolysolytic elements, or nuclear localization signals to the polymer [93].

### 4.17 Polyvinyl Alcohol

Polyvinyl alcohol (PVA) is a water-soluble synthetic polymer with a hydroxyl group in the structure (Fig. 24). The polymer is synthesized by the polymerization of vinyl acetate to polyvinyl acetate which is then hydrolyzed to PVA. The extent of hydrolysis and content of acetate group affect the crystallinity and solubility of the polymer. The melting point for fully hydrolyzed PVA is 230 °C and 180–190 °C for partial hydrolyses. PVA is soluble in highly polar and hydrophilic solvents like water, dimethyl sulfoxide (DMSO), ethylene glycol (EG), and N-methyl pyrrolidone (NMP). Its water solubility depends on three factors: the degree of polymerization, hydrolysis, and solution temperature. Although PVA is slowly biodegraded, it decomposes rapidly above 200 °C. It is nontoxic in the environment at a concentration of 5%. Commercially available polyvinyl alcohol is incorporated with photosensitive group like ketones. Ketones yield poly(enol-ketone). These are easily hydrolyzed and biodegraded than polyvinyl alcohol. Some commercially available PVAs are listed in Table 6 [94].
PV A has excellent film forming property. It also forms hydrogels when cross-linked in presence of difunctional cross linking agents such as glutaraldehyde, acetaldehyde, formaldehyde, and other monoa1dehydes and in the presence of sulfuric acid, acetic acid, or methanol. Acetal bridges are formed between the pendant hydroxyl groups of the PVA chains.

PV A has wide applications in paper making, textiles, adhesives, construction industry, food packaging and barrier films, cosmetics, ceramic industry, thermoplastic processing, plasticizers, fishing rods, and bait-bags, etc. In addition, PVA hydrogels are used for various biomedical and pharmaceutical applications. PVA hydrogels are nontoxic, noncarcinogenic, and bioadhesive in nature. They also show high degree of swelling in water or biological fluids, have a rubbery and elastic nature, and stimulate natural tissues so that they can be readily accepted into the body. Due to these properties, PVA gels are used for contact lenses, the lining for artificial hearts, and drug-delivery applications. It is also used as a stabilizer in emulsions, in topical pharmaceuticals (lotions), and in ophthalmic formulations [14, 95].

### 4.18 Polyolefin

Polyolefin is a synthetic polymer derived from petroleum sources. The monomer unit is olefin or alkene. Polyolefin is also known as thermoplastic material. Some commercially available polyolefins are polyethylene and polypropylene. Generally, polyolefins are resistant to bio-degradation due to the presence of photoinitiators and stabilizers. Therefore, to facilitate its degradation, antioxidant additives are added. Additives based on metal combinations, such as Mn$^{2+}$/Mn$^{3+}$, can be used to initiate oxidative degradation. The polyolefins are then degraded by a free radical chain reaction where hydroperoxide are produced initially. They are then thermolyzed or pyrolyzed to give chain scission, yielding low molecular mass oxidation products with hydrophilic properties. These are further biodegraded by microbial genera such as *Pseudomonas* sp., *Bacillus* sp. Polyolefins are used to make blown films, heat shrink electrical insulation sleeves for crimped wire terminals, rash guards for undergarments and wetsuits, footwears, seat cushions, spa pillows, for HEPA filter certification, etc. [19].

### 4.19 Proteins

Proteins are synthesized naturally and exist in the form of structural or functional proteins. They are the major structural component of many tissues. Chemically,
proteins are thermoplastic heteropolymers constituted by both polar and nonpolar α-amino acids. Amino acids are able to form a variety of intermolecular linkages resulting from different interactions. These offer a wide range of chemical and functional properties in protein molecules. Exploiting these properties, proteins and other amino acid-derived polymers are used as biomaterials for sutures, hemostatic agents, scaffolds for tissue engineering, and drug delivery vehicles [96].

Under natural condition, proteins are synthesized in four major stages. At first, linear sequences of various amino acids are formed where the amino acids are held together by peptide bonds. The constituent amino acids then participate to form secondary structure by hydrogen bonding. This primary structure then arranges in the most stable structures of α-helices and or β-sheets. Finally the secondary structures join together to form three-dimensional tertiary structures which in turn join with other protein chains to form three dimensional quaternary structures [19, 97]. The following proteins are important as biomaterials:

4.19.1 Collagen
Collagen is a natural polymer and the primary protein content of animal connective tissues consisting of different polypeptides. It is a rod-type polymer nearly 300 nm long with a molecular weight of 300,000. There are more than 22 different types of collagens identified. Of these, Type I-IV are the most common types. Type I collagen is the most abundant protein in mammals and is composed of three polypeptide subunits with similar amino acid composition. Collagen mostly consists of glycine, proline, and hydroxyproline. The flexibility of collagen is obtained by increasing the content of glycine [19, 98, 99].

Collagen undergoes enzymatic degradation within the body via the enzymes collagenases and metalloproteinases yielding the corresponding amino acids. Collagen is mostly soluble in acidic aqueous solutions. Therefore, it can be processed into different forms such as sheets, tubes, sponges, foams, nanofibrous matrices, powders, fleeces, injectable viscous solutions, and dispersions. Degradation properties of collagen can be altered by enzymatic pretreatment or cross-linking using various cross-linking agents [19].

Collagen is one of the primary initiators of the coagulation cascades. Its high thrombogenic property has led to its application as hemostatic agent. Sulzer-Spine® Tech consisting of bovine collagen and bovine thrombin is used for cardiovascular and spinal surgical procedures. Other mentionable hemostats are CoStasis® Surgical Hemostat and Floseal® [19].

Collagen forms the major component of the extracellular matrix and serves as a substrate for cell attachment, proliferation, and differentiation. Therefore, collagen is used as matrix for tissue engineering and wound dressing. Integra® Dermal Regeneration Template is the US FDA approved bilayer skin substitute for full thickness or deep partial thickness thermal injury. Promogran® is a novel spongy collagen matrix used for treating diabetic and ulcer wounds. Other FDA approved collagen-based wound dressings are Biobrane®, Alloderm®, TransCyte®, Orcel®, and Apligraf® [19, 100, 101].
Collagen has been used for the localized delivery of low molecular weight drugs, including antibiotics. Sulmycin®-Implant, Collatamp®-G, and Septocoll® are collagen-based gentamicin delivery vehicles [19].

Cross-linked absorbable collagen sponges are used as protein carrier vehicles. Bioactive proteins such as recombinant human bone morphogenic protein (rh BMP-2) incorporated in collagen matrices in conjunction with a titanium interbody spine fusion cage for anterior lumbar spinal fusion have been approved by the US FDA (InFUSE® Bone graft/LT-CAGE® Lumbar Tapered Fusion Device) and in Europe (InductOs®) for the treatment of acute tibia fracture in adults [19].

Collagen has also been used for gene and plasmid DNA delivery. Absorbable collagen sponges have been investigated as scaffolding material for accelerated tissue regeneration. Duragen® is used as collagen matrix graft developed for spinal dural repair and regeneration. Collagraft® is the US FDA approved composite of fibrillar collagen used as biodegradable synthetic bone graft substitute [19, 102, 103].

Although collagen has excellent biocompatibility, biodegradability, and porous structure, their applications are limited due to their mild immunogenicity. Other concern is that the cost of pure collagen is very high [96].

4.19.2 Gelatin
Gelatin is a degradation product of collagen. Gelatin consists of 19 amino acids. It is water soluble and has good film forming abilities. The mechanical properties of the films depend on the physical and chemical composition of the amino acids and their molecular weight. The physical properties of gelatin films are improved by combining gelatin with other biopolymers such as soy protein, oils, fatty acids, or polysaccharides. The mechanical properties of the films can be modified by using different plasticizers and grafting. Methyl methacrylate and poly(ethyl acrylate) are used as grafting material. Gelatin is degraded by hydrolysis of the amides in the polymer [2, 19, 104].

4.19.3 Elastin
It is a major protein component of the vascular and lung tissue. Elastin is a highly cross-linked polymer composed of a number of insoluble polymers covalently bonded to tropoelastin molecules. Tropoelastins are produced intracellularly by smooth muscle cells and fibroblasts and are cross-linked extracellularly to form secondary structures. It is composed of several repeating sequences of the pentapeptide VPGVG, the hexapeptide APGVGV, the nanopeptide VPGFGVGAG, and the tetrapeptide VPGG [19, 105].

In vivo biocompatibility studies have shown that elastin elicits immune response, to some extent. This limits its application in biomedical sciences. In addition, the polymer is insoluble in nature and shows minimal interaction with platelets. To overcome these limitations, synthetic elastin has been developed from human tropoelastin. The synthetic form can be transferred to appropriate molds at 37 °C. The matrice was found to have good mechanical and biological properties making them promising elastic biomaterials for appropriate applications. In addition,
tropoelastin undergoes folding when the temperature is above 25 °C, a property called inverse temperature transition (ITT). These properties of elastin have been extensively investigated to be used as smart, injectable drug delivery system [19, 105].

4.19.4 Elastin-Like Peptides (ELP)
These are artificial polypeptides composed of the pentapeptide repeats of human tropoelastin VPGXG except for the fourth amino acid. The X in ELP can be any amino acid except proline.

ELPS have excellent biocompatibility, nonimmunogenic properties, and the degradation products are composed of natural amino acids and nontoxic products. Like elastin, ELPs also exhibit a reversible ITT property [19].

ELPs are investigated as drug carrier vehicle and as potential biomaterial for cartilage tissue engineering. In addition, due to the synthetic versatility of ELP, tailored substrates can be developed for engineering various tissues by incorporating specific cell binding epitopes in ELP [19, 106].

4.19.5 Albumin
It is the most abundant protein in human blood plasma. Albumin is a water-soluble protein. Its primary function is to carry hydrophobic fatty acid molecules around the blood stream and maintain blood pH. Albumin is composed of low content of tryptophan and methionine, high content of cystine and charged amino acids (e.g., aspartic acid and glutamic acid), and lysine and arginine [19].

Albumin is degraded in all tissues of human and is therefore a highly preferred degradable polymer for medical applications. Due to its solubility and presence of functional groups along the polymer chain, albumin can be easily processed into various shapes and forms of membranes, microspheres, nanofibers, and nanospheres. In addition, because of its excellent biocompatibility, albumin is now being investigated as a carrier vehicle for intravenous drug/gene delivery. Albumin based adhesives (CryoLife Inc.) have been approved by the FDA for reapproximating the layers of large vessels, such as aorta, femoral, and carotid arteries [19, 107].

4.19.6 Fibrin
Fibrin is derived from fibrinogen and composed of three pairs of polypeptide chains. Like collagen, fibrin also takes part in blood clotting process. The fibrin clot undergoes degradation by a process called fibrinolysis by a complex cascade of enzymatic reactions present in the body [108].

Fibrin has excellent biocompatible, biodegradable, and injectable properties, and in the presence of several extracellular matrix proteins (e.g., fibronectin), it favorably affects cell adhesion and proliferation. Fibrin sealant products are used clinically for homeostasis and tissue sealing applications in various surgical procedures. Bioseed® is a fibrin-based product used to treat chronic wounds. Its biodegradability and injectability have been investigated for application as a carrier vehicle for bioactive molecules [19].
4.19.7 Gluten
It is of plant origin. Wheat gluten contains the main group of proteins known as gliadin and glutenin. Gluten films are brittle and completely biodegradable and require plasticizers to modify its biodegradation properties. By plasticizing gluten with glycerol, a malleable phase can be obtained [2, 19, 109].

4.19.8 Soy protein
Soy protein is another vegetable source polymer. It has long been used for emulsification and texturizing. Soy proteins with different percentage of the protein content are used as texturizers. Soy protein films are hydrophilic in nature and used to produce flexible and edible films [19, 110].

4.19.9 Poly(Amino Acids)
This includes natural poly(amino) acids and synthetic poly(amino) acids.

**Natural poly(amino acids)** These are biodegradable ionic polymers which exhibit polydispersity in addition to α-amide linkages and other amide linkages that involve β- and γ-carboxylic groups as well as ε-amino groups. Cyanophycin, poly(ε-L-lysine), and poly-γ-glutamic acid are examples of this class of amino acids. Among them, poly-γ-glutamic acid (γ-PGA) is the most studied amino acid [111].

Poly-γ-glutamic acid (γ-PGA) is an anionic, water-soluble biodegradable homopolyamide and is composed of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups. It is produced by microbial fermentation. However, several modified forms of the amino acids have been developed to be used as drug delivery vehicles, tissue engineering scaffolds, and thermosensitive polymers. Poly(-γ-glutamic acid) benzyl ester (γ-PBG) was used as a carrier vehicle for the chemotherapeutic agent 5-flourouracil. Poly(-γ-glutamic acid)-sulfonate (γ-PGA-S) polymer has been investigated to be used as scaffolds for tissue engineering applications, and poly(-γ-glutamic acid) with controlled propylation has been used as thermosensitive polymer. γ-PGA has limited application due to its limited availability [19, 112].

Poly (L-lysine) is another amino acid of bacterial origin. It has antibacterial, antiviral, and antifungal activity. The polymer has also been investigated as a potential candidate for developing drug carrier vehicles as it forms biocompatible hydrogels. However, its application is limited due to its cytotoxicity resulting from very high positive charges [19, 113].

**Synthetic poly(amino acids)** The following are mentionable in this group:

Poly (L-glutamic acid) (L-PGA) is composed of naturally occurring L-glutamic acid residues linked together through amide bonds (Fig. 25). It is synthesized by several routes. The amino acid is highly susceptible to degradation by lysosomal enzyme yielding monomeric L-glutamic acid [19].

Poly L-glutamic acid is highly charged at physiological pH which makes it an attractive candidate as gene/plasmid delivery vehicle. In addition, the α-L-carboxylate side chains of the molecule are highly reactive and can be chemically modified to introduce various bioactive ligands or modulate the physical property of the polymer. L-PGA conjugated anticancer drugs are highly soluble in aqueous phase.
This property is also used to develop biodegradable MRI contrast agents. L-PGA has also been investigated to be used as biodegradable biological adhesive and hemostat, by cross-linking it with gelatin. L-PGA-based adhesives demonstrated better soft tissue binding and hemostatic properties than fibrin based glue [19, 114, 115].

**Poly(aspartic acid)** (PAA) is chemically synthesized from aspartic acid by thermal polymerization. It is a highly water-soluble ionic polymer with carboxylate content much higher than that of poly(glutamic acid). Like L-PGA, PAA undergoes lysosomal biodegradation, too. PAA is highly functional and therefore has been investigated as a plasma extender [19, 116].

### 4.20 Polysaccharides

Polysaccharides are macromolecules formed from many monosaccharide units linked by glycosidic bonds. Although polysaccharides are synthesized by all living organisms, new synthetic routes are currently available to modify the polysaccharides to be used for biomedical applications.

#### 4.20.1 Hyaluronic Acid

Hyaluronic acid (HA) are present in every tissues in vertebrates and are a member of the glycosaminoglycan family, consisting of linear polysaccharides of alternating units of N-acetyl-D-glucosamine and glucuronic acid (Fig. 26). Unlike other glycosaminoglycans (e.g., chondroitin sulfate, dermatan sulfate, keratin sulfate and heparin sulfate) present in the body, HA is not covalently linked to proteins [19].

HA is water soluble and forms highly viscous solutions. HA present in synovial fluid and vitreous humor significantly contributes to the viscoelastic properties of these tissues.

HA is extracted from rooster combs and bovine vitreous humor. However, several bacterial fermentation methods are under study to scale up its production.

HYAFF® is a modified HA and has been used for wound dressing applications. It is a benzyl ester and undergoes hydrolytic degradation via ester bond cleavage. HA also plays important role in tissue repair by promoting mesenchymal and epithelial cell migration and differentiation, which in turn enhances collagen deposition and angiogenesis. OSSIGEL® containing a viscous formulation of HA containing fibroblast growth factor is under clinical trial as a synthetic bone graft to accelerate bone
fracture healing. HYAFF®11 is another vehicle and currently being used as a carrier vehicle containing a variety of growth factor and morphogenes and bone marrow stromal cells. HA-based materials are also used as injectable soft tissue fillers. AMVISC® and AMVISC® PLUS are used as vitreous humor substitute as well as to protect the sensitive eye tissue during cataract extraction, corneal transplantation, and glaucoma surgery. SYNVISC® and ORTHOVISC® are clinically used as synovial fluid substitute to relieve pain and improve joint mobility in osteoarthritis patients [19, 117, 118].

In the body, HA is degraded by free radicals, such as nitric oxide and metalloproteinases (MMPs) found in the extracellular matrix, followed by endocytosis. HA also undergoes lysosomal digestion, yielding mono- and disaccharides, followed by further degradation into ammonia, carbon dioxide, and water by the citric acid cycle. HYAFF®, which is the benzyl ester, undergoes hydrolytic degradation due to the presence of ester bond in the polymer. The degradation takes place in the absence of enzymatic activity and takes 1–2 weeks or 2–3 months depending on the degree of esterification [19].

4.20.2 Chitin and Chitosan
Chitin is the second most abundant natural biopolymer consisting of co-polymers of N-acetyl-glucosamine and N-glucosamine with β-1,4 linkage (Fig. 27). Chitosan is derived from chitin by partial alkaline deacetylation (Fig. 28). Chitosan is soluble in weak acid solutions and forms cationic polymer with a high charge density, which allows it to form polyelectrolyte complexes with wide range of anionic polymers [19].

Chitosan is nontoxic after oral administration and an FDA approved food additive. The presence of highly reactive amino groups along the polymer backbone makes chitosan highly susceptible to chemical or biological functionalization. Both chitin and chitosan have shown stimulatory properties on macrophages and chemoattractive properties on neutrophils. These properties along with its antibacterial and hemostatic properties give chitosan enormous potential for wound healing applications and wound healing accelerator [19, 119].

Several chitosan-based bioadhesive drugs/vaccine are currently under development. Chitosan forms micro-/nanosphere formulations without the use of organic
solvents and maintains immunogenicity of the antigen, therefore has been used as immunoadjuvants. Chitosan has been used to develop several chitosan-drug conjugates for cancer therapy. Chitosan, in the presence of certain phosphate salts, undergoes temperature controlled phase transition. Due to this mild gelling condition, the hydrogel can be used as a potential delivery vehicle for growth factors, small molecular weight drugs, and cells for localized therapy. Chitosan also exhibits anti-oxidative and radical scavenging properties. Chitin and its derivatives are used as anticholesterolemic agents and blood anticoagulants. Both chitin and chitosan have been modified to N-carboxymethylchitosan or N-carboxyethylchitosan. These are used in cosmetics and wound treatment [120].

Chitosanase, lysozyme and papain degrade chitosan in vitro. The in vivo degradation of chitosan is caused by hydrolysis of the acetylated residues by the lysosomal enzymes [120, 121].

4.20.3 Alginic Acid
Alginic acid is a polysaccharide present in brown algae. It is a nonbranched, binary copolymer composed of β-D-mannonic acid monomer linked to α-L-glucuronic acid monomer through a 1,4-glycoside linkage.

Alginic acid forms gels in the presence of counter-ions (e.g., Ca$^{2+}$). The pH, type of counter-ions, and the functional charge density of the polymer affect the degree of cross-linking and the gelling properties and are used for the encapsulation of various components.

Alginate gels are used as encapsulating agent for controlled release of drug delivery systems. Alginate encapsulated herbicides, microorganisms, and cells are in use [122].

4.20.4 Starch
It is a well-known hydrocolloid biopolymer and one of the cheapest biodegradable polymer. Starch is commercially available (Table 7) in the form of granules and
mainly extracted from potatoes, corn, wheat, and rice. Starch is composed of amylose (poly-\(\alpha\)-1,4-D glucopyranoside) and amylopectin (poly-\(\alpha\)-1,4-D-glucopyranoside and \(\alpha\)-1,6-D glucopyranoside). The former one is a linear and crystalline polymer, whereas the later one is a branched, amorphous polymer. The relative amounts of these copolymers vary with the sources and yield materials with different mechanical and biodegradable properties. The higher the amylose content, the greater is the elongation and strength of the material [123].

Thermoplastic starch or plasticized starch offers an alternative for synthetic polymer (e.g., starch-based composites). Starch is used as a biodegradable composite. However, starch-based products are brittle and therefore chemical modifications are performed. Starch has two functional groups. These are hydroxyl group and ether bonds. The hydroxyl group has nucleophilic character and the mechanical properties of starch can be modified by acetylation. For example, starch acetate is prepared by acetylation of starch with a mixture of pyridine and acetic acid. The starch acetate has a high content of linear amylose and therefore it is more hydrophobic than starch. Another approach to improve the mechanical properties of starch is blending. Blends also offer advantages to modify the composition of the material properties, low cost of development compared to the synthetic

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<td>Starch Tech Inc.</td>
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<td>Terraloy® BP</td>
<td>Teknor Apex Company</td>
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materials, and they are partially biodegradable than traditional synthetic plastics. Some of the starch blends include starch-poly(ethylene-co-vinyl alcohol) (EVOH), starch polyvinyl alcohol, starch PLA, starch PCL (this is commercially available), starch PBS, starch-PHB, etc. [2, 19].

Starch-based polymers are mostly used in agriculture. For example, plastic films are used for greenhouse coverings. Young plants are susceptible to frost and therefore must be covered. Biodegradable polymers used in the coverings conserve moisture reduce weeds and increase soil temperature. These properties improve the rate of growth of the young plants. In addition to these properties, biodegradable polymers undergo biodegradation at the end of the season. Another application is that these polymers can be used on the production of bands of sowing allowing regular seed distribution along with nutrients. Biopolymers are also used for filtration and drainage and can be used as geogrids [2, 19].

Starch-based films are also used in mulching and low-tunnel cultivation system. Starch films undergo biodegradation in contact with soil microorganisms and the degraded product is nontoxic. Other advantage is that water and high temperature does not affect the mechanical behaviors of the films.

In automotive application, starch-based polymers are used as additives in the manufacturing of tires as this reduces the resistance to the movement and the consumption of fuels [2, 19].

### 4.20.5 Cellulose

Cellulose is another polysaccharide, produced by plants. It is a linear polymer with a very long repeating unit of cellobiose. Cellulose is crystalline and insoluble in all organic solvents. To improve its process-ability, cellulose is transformed to derivatives by reaction of one or more hydroxyl groups present in the repeating unit. Cellulose ether, esters, and acetals are the main derivatives and are commercially available. Cellulose undergoes enzymatic biodegradation by fungi and bacteria. The mechanical properties of cellulose esters and their biodegradation can be decreased or modified by increase in the degree of substitution. Cellulose acetate (CA) is another commercially available derivative of cellulose. Commercially available cellulose derivatives are mentioned in Table 8. CA has high Tg, which limits its

<table>
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<th>Trade/product name</th>
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<td>Kareline PLM S5050 etc.</td>
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<td>Cellophane™</td>
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thermal processing. Therefore, these are either plasticized or blended with flexible polymers. The CA derivatives are used in fiber or film applications [124].

5 Conclusions

Synthetic polymers or plastics constitute a significant part of the world around us today, providing convenience and comfort in our daily lives. However, a multitude of problems are associated with the use of synthetic polymers because of their negative impact to the environment and health. While natural polymers failed to provide the durability, flexibility, and cost-effectiveness that is provided by synthetic polymers, synthetic biopolymers have offered a solution to the growing problem of accumulating plastics in the world today. Worldwide production of synthetic biopolymers has recently been boosted by advancement in polymer chemistry and our enhanced knowledge on the relationship between the molecular structure of polymers and their biodegradable properties. Being originated from natural, renewable, or nonrenewable resources or chemically synthesized materials, the synthesis of biodegradable polymers proceeds through a polymerization process, followed by modification in their structure to render the material susceptible to biodegradation, and cross-linking, which characterizes the mechanical strength of the polymer. In some cases, surface modification is also made in order for their suitability to be applied in vivo. The safety of biopolymer-based, medical devices is also ensured through further purification and optimization of physicochemical properties of the polymeric materials used as raw material. Degradation of synthetic biopolymers occurs by means of hydrolytic or enzymatic reactions or a combination of both and therefore depends on various structural and physicochemical properties of the polymer. It is important that the polymer is fragmented through abiotic means first, to facilitate subsequent bio-assimilation and mineralization by microorganisms or complete digestion by the metabolic pathways in human bodies.

Application of synthetic biopolymers as biomaterials requires biocompatibility, bioabsorbility, and mechanical resistance. Common synthetic biopolymers include aliphatic polyesters, mostly derived by poly-condensation or ring-opening polymerization process. Along with simpler polyesters such as PGA and PLA, co-polymers are available such as PLGA, PBSA. A variety of forms and structures are made for use as biodegradable, synthetic sutures, scaffolding matrices for tissue regeneration and ligament replacement, skin closing adhesives, bloods vessel conduits, and as internal fixation devices for bones, etc. Polyhydroxyalkanoates, such as PHBs and their co-polymers, are synthesized by bacteria, considered suitable as drug carriers. Polymides and co-polymers of amide and ester groups are other types of synthetic biopolymers also used as drug delivery vehicles. Polyurethanes were made biodegradable through chemical modification and are used in medical and orthopedic applications. Other synthetic polymers with application in the medical field include poly(ortho esters), polyanhydrides, pseudo poly(amino acids), poly(alkyl cyanoacrylates), polyphosphazenes, polyphosphoester, polyvinyl alcohol, polyolefin, etc. Natural polymers, which are enzymatically degradable, such as proteins or
polysaccharides have long been used in biomedical applications, such as gelatin in encapsulating drugs and preparing hydrogels, chitin in drug delivery, chitosans in anti-oxidative and radical scavenging activities, collagen and poly-L-leucine to prepare skin substitutes or wound dressing, etc.

While synthetic bio-polymers have immense potential to be applied in a wide range of product areas, the global production of these polymers is only a tiny fraction of that of synthetic polymers, representing a huge disparity in the market situation of these products. A great deal of research and efforts are still necessary for efficient and inexpensive, industrial scale production of synthetic bio-polymers. For products that are potentially bio-degradable, further evidence is required to ensure their compliance to the environmental safety requirements. Also, for a sustainable and viable market, the production of synthetic bio-polymers must rely on renewable resources instead of petrochemicals. The production process must be optimized for efficiency, and the prices of products made out of synthetic bio-polymers need be competitive to those of synthetic polymers. With increasing public awareness about the detrimental effects of synthetic polymer-based products to human health and environment, and with the introduction of novel biotechnological principles and techniques to polymer chemistry, it is likely that synthetic bio-polymers will be made more useful to the society.

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Polymers from Renewable Resources

Heather Goldsborough and Victoria V. Volkis

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Abstract
Plastics are an indispensable part of our daily life but also have many applications in electronics, medicine, and environmental protection. Traditional plastic industry consumes about 7% of the global production of fossil fuel. As the non-renewable fossil fuel will exhaust within the next century, the development of green polymers from renewable natural resources will play an ever-increasing role for future generations toward a sustainable society. Some of natural polymers such as cellulose, for example, have a very long history of use without major modifications. Others are newer. This chapter provides a brief review of alternative plastic materials from renewable sources, such as polysaccharides, lignin, biomass and bio-oils, tannin, cellulose, and many others. The application of those

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materials is getting wider and wider, and they can replace many of traditional plastics. In addition, the use of much “greener” plastics from renewable resources contributes to reducing the environmental impact of fuels and other petrochemical products and traditional plastics, which are responsible for atmospheric pollution and for the increasing level of greenhouse gases that are the main reasons for global warming.

1 Polymers from Renewable Resources

Today, plastics are an indispensable part of our daily life – they appear in almost everything we use, including clothing, furniture, packaging, and transportation. The use of these plastic materials consumes about 7% of the global production of fossil fuel. As the nonrenewable fossil fuel will exhaust within the next century, the development of green polymers from renewable natural resources will play an ever-increasing role for future generations toward a sustainable society [1]. Equally alarming problems are related to the environmental impact of fuels and other petrochemical products (organic chemicals, plastics) which are responsible for atmospheric pollution and for the increasing level of greenhouse gases that perturb the Earth’s climate [2]. Due to these concerns, there is significant research going on with the aim of developing synthetic polymeric materials from renewable sources based on sustainability.

Some of natural polymers have a very long history of use without major modifications. Cellulose, paper, cotton, and wood are just some of examples. Some others are results of targeted search to replace expensive natural materials by synthetic ones made from renewable resources. Thus, progressive decline of fossil resources, together with the ongoing increases in oil prices, has initiated an increase in the search for alternatives based on renewable resources for the production of energy. The prevalence of petroleum-and carbon-based chemistry for the production of organic chemical goods has generated a variety of initiatives aimed at replacing fossil sources with renewable counterparts. In particular, major efforts are being conducted in polymer science and technology to prepare macromolecular materials based on renewable resources. Also gaining momentum is the utilization of vegetable biomass either by the separation of its components and their development or after suitable chemical modification [3, 4]. The concept of the biorefinery in which renewable resources (biomass) are used arises from the need to overcome environmental challenges and simultaneously find an alternative to limited fossil resources.

Bio-based polymers have been used for food, furniture, and clothing for thousands of years. The emergence of polymer chemistry hails during the second half of the nineteenth century: the discovery of the vulcanization process of natural rubber by Charles Goodyear in 1839 determined the development in the next years of the rubber industry; the first plastic material obtained from cellulose by chemical modification named celluloid (cellulose nitrate blended with camphor) was successfully commercialized thanks to the discovery of Alexander Parkes. The
petrochemical revolution after the Second World War determined a shift toward the use of fossil resources, and within a few decades, huge quantities of plastics were produced [5].

Biomass production by nature is estimated at 170 trillion tones per year, only 3.5% of which are used by humans. Most of these compounds are used for food; 33% are used for energy, paper, furniture, and construction; and only 5% (300 million tons) are used for other nonfood applications such as chemicals and clothing [6]. There are multiple natural resources that can be used to produce polymeric materials and/or modify naturally occurring polymers. Examples of such resources are:

- Vegetable oils,
- Glycerin and derivatives
- Polysaccharides (sorbitol, chitin, chitosan)
- Vegetable proteins (like gluten)
- Tannins and lignin
- Natural fibers: hemp, flax, bagasse
  . . . And many others. [7]

Figure 1 presents some examples of polymeric materials produced from harmful monomers and how these materials can be replaced by biopolymers produced from harmless and bio-based building blocks.

Polymeric materials from renewable resources found applications in medical implants, health products, building industry, packaging (especially biodegradable packaging), and electronics.

Polymers from renewable sources, also named bio-based polymers, can be produced:

- By direct extraction and separation from biomass (e.g., starch, cellulose, alginates)
- By classical chemical synthesis from bio-based building blocks produced via biotechnological routes (e.g., polylactic acid, polyamide 11)
- By fermentation processes using microorganisms (e.g., polyhydroxyalkanoates)

**Fig. 1** Examples of polymeric materials produced from harmful monomers and their possible replacement by biopolymers produced from harmless and bio-based building blocks.
Renewable resources, in turn, can be divided to following big groups:

- **Oils and derivatives** can be a source of triglycerides, fatty acids, and glycerol carbonate, which may be used as building blocks.
- **Saccharides** are represented by chitin, chitosan, cellulose, and other materials.
- **Polyphenols** are important antioxidants mainly represented by flavonoids, tannins, and anthocyanins.
- **Other sources** include gluten, lignin, natural fibers, and more.

Polymers from renewable resources may be produced using biotechnology involving microbes (e.g., polyhydroxyalkanoates) or chemically synthesized using bio-based monomers, like in a case of polyamides. Bio-based polymers can be produced by conventional chemical routes from building blocks synthesized using renewable sources. Such polymers are 100% bio-based if their monomer/s totally derive from renewable sources; whereas they are partially bio-based if both bio- and fossil-derived monomers are used in the polymerization to produce the polymer.

However, the use of bio-based plastics has some significant limitations, such as the relatively high production cost and the often poor performance of some bio-based plastics. These limitations influence both the market growth and the scope of potential applications for these polymers. In order to extend the large-scale applicability of bio-based polymers, it is necessary (i) to decrease the chemical/biotechnological processes costs by increasing scientific and technologic understanding of biomass conversion into plastics, (ii) to improve properties of some bio-based polymers in order to fit requirements of industrial applications through a deeper knowledge of structure-properties relations, and (iii) to create innovative bio-based plastics allowing new competitiveness. Indeed, in addition to economic and environmental advantages, the production of bio-based polymers also represents an occasion for the scientific and industrial innovation in the polymer area.

### 2 Polysaccharides and Their Derivatives

The most abundant polysaccharides in nature are cellulose, chitin, and the two constituents of starches – amylose and amylopectin. Their structures are presented on Fig. 2.

Thermoplastic starch (TPS) is a biodegradable polymer made by mechanically disrupting starch granules in an aqueous solution of plasticizer, such as glycerol [9, 10]. While it is made from a renewable resource, low in cost, and compatible with existent thermoplastic manufacturing technology, TPS quickly loses integrity when moisture is present. This drawback limits its usage to dry environments, such as some food packaging, and applications in which dissolution is desirable, such as detergent pods. Potential remedies to the hydrophilicity of TPS include chemical surface modification, blending with hydrophobic polymers, the addition of fillers, and dipping of final products in solutions of more hydrophobic compounds [11]. Starches can also be mixed with synthetic polymers for inclusion in foam packing materials [12].
Fig. 2  Structures of cellulose (1), chitin (2), amylose (3), and amylopectin (4)
Bulk cellulose has been the major component of paper products for years, but many other applications for various forms of this abundant polymer exist. Cellulose nanofibers between 2 and 15 nm in diameter and up to 1 μm long have been isolated from the “whiskers” of sea squirts and from vegetable cellulose. Their strength makes them promising as reinforcement for composite materials and has already proved effective in the manufacture of higher tensile strength paper. There is interest in replacing glass fibers used to reinforce thermoplastics with modified cellulose nanofibers [4, 13]. This substitution potentially decreases cost and density, makes the materials burnable and recyclable, and lowers their abrasivity (see Sect. 12 for more information). The hydroxyl groups of starch and cellulose have made many chemical modifications including esterification, etherification, and urethane formation possible [9, 14, 15] allowing for various groups including fatty acids, silyls, siloxanes, alkyl boranes, and alkyl aluminums to be appended to the surface [16–20].

Modification of polar hydroxyl groups not only helps to lower hydrophobicity but also improves adhesion between the fibers and the nonpolar plastics in which they are included. If reactive groups are appended, covalent bonds may be created between the fibers and the matrix through copolymerization, creating maximum continuity and adhesion between the two [9]. In a case where polymerization occurs via condensation, rather than by a standard radical mechanism, a complementary group to that of the polymer is appended rather than an alkenyl group. For instance, polyesterification can be performed between a polymer and a carboxylic anhydride appended fiber. Another useful chemical modification of cellulose, trifluoroacetylation, yields a compound that is both hydrophobic and lipophobic but reverts back to its original state through hydrolysis when exposed to water overtime. This cellulose derivative is applicable to certain packaging and agricultural settings. If modified with 3,3,3-trifluoropropionic or pentafluorobenzoic acid, the resulting cellulose has the same properties but only reverts back in an environment that is both basic and has a high pH. Such compounds offer more permanence while still maintaining recyclability.

Admicellar polymerization is another means of incorporating cellulose fibers into polymers [21]. It involves the suspension of fibers in water. Addition of anionic surfactant forms a bilayer or admicelle around the fibers which has a hydrophobic center. Water-soluble monomer is added to the mixture, where it inserts itself between the bilayers. Polymerization of the monomers is induced, creating a polymer-encased cellulose fiber with a surfactant interlayer that can now be incorporated into polymer matrices.

Oxypropylation of cellulose and starch outer surfaces has been used to create materials with outer thermoplastic matrices and inner fibers [22, 23]. These composites can be heat pressed into reinforced sheets (Fig. 3).

3 Polymers Based on Lignin and Suberin

The second most abundant compound in both wood and other plants is lignin. It has a highly branched and variable structure, consisting generally of methoxylated phenolic groups connected by three carbon aliphatic chains. Alkene and hydroxyl
groups are also present [25]. The exact substituents of lignin differ by plant species.

When isolating cellulose pulp for papermaking, cleaved lignin fragments are produced as a major by-product [26]. These fragments have diverse structures. Since mixtures are difficult to use as feed streams for chemical processes, lignin fragments are generally diverted to combustion systems for energy production. Some components of these mixtures, however, may have applications in chemical synthesis once isolated. The abundant hydroxyl groups of lignin oligomers make them easily modifiable and subsequently polymerizable. Combined with poly(ethylene oxide) blends, lignins have been used to make carbon fibers [27, 28].
Breaking lignin oligomers down to monoaromatic units, containing only one ring each, either by chemical degradation or enzymatic hydrolysis, would produce unsaturated compounds or compounds with multiple condensation groups, respectively [20]. These monomers could then be polymerized by chain reactions or condensation reactions as appropriate to form aromatic polyethers, polyester, polyurethanes, and others.

Suberin is an aromatic-aliphatic polymer found mostly in the outer bark of higher-order plants but also in some roots. Its hydrophobic character protects trees from water loss as well and makes them waterproof. One of the most concentrated sources of suberin is cork wood.

Hydrolysis and methanolysis have been used to free suberin monomers, [29] which were consequently polymerized by polycondensation into polyesters with hydrophobic properties (Figs. 4, 5, and 6).

4 Polymers from Vegetable Oils

Vegetable oils contain varied triglycerides that have historically been used as thinning agents, siccative agents, and macromonomers for alkyd resin production in the ink and paint industries. Triglycerides contain both ester groups and other...
functional groups along some chains, such as hydroxyl groups and unsaturated bonds that offer opportunities for modification. Such modifications can be performed to improve polymerization potential. Breakage of the ester bonds produces hydrocarbon chains with terminal reactive sites. Unsaturated bonds can be converted to hydroxyl groups, which then, along with those already present, can be converted to styrenic or acrylic groups if desired. Cargill Company has already used this process to synthesize polyols from soybean oil suitable for polyurethane production. Grenoble has engineered a monomer with up to eight acrylic substitutions that is UV sensitive enough to replace petroleum-based fast-coating varnishes [30].

5 Polymers from Tannins

Tannins are polyphenol oligomers found mostly in the bark and sometimes the wood of trees, particularly those within the southern hemisphere (Fig. 7).
Traditionally used in leather tanning, tannins are also used to make wood adhesives that do not require formaldehyde [31]. Some formulations now include starch as well, leading to novel properties [32].
Sugars are used as precursors for monomers such as furans and lactic acid (Fig. 8). One of polymers, shown on the Fig. 9, is produced from a furan derivative that has properties similar to Kevlar [33]. Another is photo- and electroluminescent that can be doped to conduct electricity and has promise as a cross-linking dimer – Fig. 10.

**6 Polymerization of Monomers Derived from Sugars**

Sugars are used as precursors for monomers such as furans and lactic acid (Fig. 8). One of polymers, shown on the Fig. 9, is produced from a furan derivative that has properties similar to Kevlar [33]. Another is photo- and electroluminescent that can be doped to conduct electricity and has promise as a cross-linking dimer – Fig. 10.
A third type is analogous to PET (Fig. 11). Furans are also able to form macromolecules with dienophiles through Diels-Alder reactions. These reactions are reversible between 60 °C and 100 °C, giving the compounds thermal reversibility (Fig. 12).

7 Polylactic Acid and Other Polymers from Biomass

Polylactic acid (PLA) a thermoplastic aliphatic polyester, and highly versatile polymer, is an example of 100% bio-based polymer since it is produced by polymerization of lactic acid (2-hydroxypropionic acid) which is produced via bacterial fermentation of carbohydrates from different renewable sources like corn starch, sugarcane, or molasses. PLA is produced either by polycondensation of lactic acid or by ring-opening polymerization (ROP) of lactide, the cyclic dimer of lactic acid, as it is shown on Fig. 13.

As shown on Fig. 13a, two equilibria exist in the dehydrative polycondensation of l-lactic acid: one is hydration/dehydration equilibrium of carboxyl and hydroxyl terminals, and the other is ring/chain equilibrium between l-lactide and PLLA [34]. The ROP of lactide can be induced by the catalysis of strong organic acids. In the ordinary cationic ROP, strong organic acids, such as super acids, are utilized as the catalysts as shown in Fig. 13b. In the initiation step, the monomer activated by the protonation is ring-opened by the carbonyl attack of an alcohol initiator to form the lactyl alcohol with recovery of acidic proton. Successive
attack of this lactyl alcohol on the protonated monomers propagates the polymer chain. Namely, the terminal hydroxyl group acts as the propagating species reacting with the protonated monomer, which corresponds to the monomer activation mechanism. Since the acid catalyst is free from the propagating polymer, it is readily removed, and less than one catalyst per monomer chain is needed. This feature is an advantage of the cationic catalysts over the metal alkoxides, which ought to remain attached to the propagating chains as described above. In the acid-catalyzed ROP, however, the propagation is likely contaminated by chain termination or transfer reactions that may be related to the reactivity of the protonated monomer.

Lactic acid is often derived by fermenting simple sugars obtained from the breakdown of starch but can also be synthesized from glycerol. It is then converted to lactide, an anhydrous ring forming dimer. Ring-opening melt polymerization is performed to form polylactic acid (PLA), a polymer widely used in packaging and fiber technologies, with some applications in the chemicals products. Examples of products containing PLA include carpet, clothing, and plastic bottles. Corn is one of the most viable and affordable feedstocks for this process (Fig. 14).

While lactic acid can be synthesized chemically, production from sugar has the added benefits of being environmentally friendly and producing a chiral molecule.

Fig. 11 Synthesis of the novel polyester simulating the structure and properties of PET

Fig. 12 Diels-Alder equilibrium between a monosubstituted furan derivative and an N-substituted maleimide
Processing converts some lactic acid to its other chiral form. Further modification leads to three different lactides for polymerization (Fig. 15).

The agricultural, forestry, fishery, and food and beverage industries produce multiple inedible by-products that might become more useful if oxypropylated [35]. Solid sugar beet pulp, for example, can be converted to a viscous polylol for production of polyurethanes and polyesters [36]. The addition of hydroxyl groups to many biomass materials has been shown to produce similar results.

8 Bacterial Cellulose

Bacterial cellulose has a unique bundled structure that many believe would prove useful in many applications from electronics [37] to nanocomposites [38]. Unfortunately, it is prohibitively expensive to source, relegating it to high-tech niche markets needing only small quantities.

![Diagram of lactic acid and lactide conversion](image-url)

**Fig. 13** (a) Polycondensation of lactic acid; (b) ring-opening polymerization (ROP) of lactide
Fig. 14  The processing route to PLA combines bioprocessing and chemical processing

Fig. 15  The optical isomers of lactic acid
9 Terpenes and Rosin

Terpenes, terpenoids, and rosin are a class of hydrocarbon-rich biomass derived from plants and trees with great abundance and low cost, holding much potential for utilization as organic feedstocks for green plastics and composites. Together, these natural resources amount to more than 1.5 million tons per year in the world— their use could have a very positive impact on the industry of engineering polymers and rubbers [39] (Fig. 16).

Terpene polymerization is difficult to control.

10 Glycerol

Glycerol is an abundant by-product of the biofuels industry, being obtained by hydrolysis of vegetable oils. Its availability has sparked a recent surge in investigations regarding possible uses. DuPont’s poly(trimethylene terephthalate) utilizes 1,3-propane diol synthesized from glycerol as one of its major starting materials [3].

11 Chitin and Chitosan

Chitin nanofibers are obtainable from crustacean exoskeletons [4]. Because chitin is insoluble in most liquids and difficult to modify chemically, the derivative molecule chitosan has received far more attention. Chitosan is soluble in acidic, water-based solutions and far easier to modify than chitin [40]. It is derived by the deacetylation of amide functional groups to amino groups. The degree to which this reaction has occurred is one means by which chitosans are classified. Chitosans can be precipitated from solution, spun into fiber, or made into film. They are used in the pharmaceutical, food processing, agricultural, metal sequestration, and biomaterials industries. The addition of chitosan films to paper improves many of its properties.
including optics, printability, manipulability, and permeability [41]. Chitosan is similar to cellulose in polarity and reactivity.

Chitin is a high molecular weight polysaccharide composed of $N$-acetyl glucosamine monomers. This polysaccharide is the main component of the exoskeletons of most arthropods and the organic matrix of mollusk shells and is found in the cell walls of fungi [42]. Pure chitin can be separated from these biological structures through a standard procedure consisting of decalcination and deproteinization and easy conversion of the extracted material to chitosan (deacetylated chitin) by an additional step [43] (Fig. 17).

Earlier tests by Volkis et al. revealed that chitosan, the conversion product of chitin, is a successful carbon capture material which meets all of the characteristics listed above including, most importantly, reversible sorption.

The standard process of chitosan extraction from shellfish is a three-step process outlined by George Roberts, consisting of demineralization, deproteinization, and deacetylation. The raw crustacean shells are converted to a powdered form through milling. This powder is composed of a blend of raw proteins, calcium carbonate complexes, and chitin polymer. The first stage is the removal of calcium from the network through the use of a strong inorganic acid. Roberts recommends an HCl bath (concentration 0.25 M; ratio 40:1) for 15 min at room temperature [43].

Fig. 17 Chitin and chitosan oligomers
The next step is deproteinization to remove the proteins present in the network. This is performed with a NaOH bath (concentration 1 M; ratio 15:1) at 70 °C for 24 h. These two processes resulted in chitin with 0.01% residual calcium and 0.6% residual protein. The next step in the process is deacetylation of the chitin; this is simply the removal of the acetyl groups from the polysaccharide and is carried out in a variety of conditions including low temperature, reduced alkali, and enzymatic methods.

Low temperature processing refers to the use of NaOH at temperatures ranging from 30 °C to 80 °C. At lower temperatures, a working ratio of 56:1 and concentrations of 33 wt% are used. Another option is the reduced alkali process; this involves using reduced amounts of NaOH to lessen costs and reduce the negative effects of disposal of the waste solution that would be present on an industrial scale. This process uses one part chitin to two to five parts NaOH in a heat bath. Acetone can also be used to homogeneously distribute the NaOH throughout the mixture. One successful method to increase solubility is to swell the chitin in NaOH for 72 h at temperatures below 30 °C before initiating the deacetylation process. Chitosan samples prepared from this general method resulted in a material that captured carbon dioxide efficiently [44]. Although this is the established method of preparing chitosan from the shells of marine organisms, we propose that a shortened method, in which we simply remove the calcium complexes from the shells leaving only the chitin and associated protein structures, will more efficiently yield a carbon capture material that is just as or more successful than the standard samples as prepared above with reduced swelling.

Chitosan as a natural resource possesses biologically reproducible, biodegradable, environmentally nonpolluting, biocompatible, nontoxic, and biologically functional properties which allows to act as a multipurpose material for various biological and biomedical applications [45]. With that being said, the degree of antimicrobial activity of a particular substance often correlates with its applicability. Many polymers that exhibit antimicrobial properties are able to form thin film layers that is used for food packaging. Over the past decade, antimicrobial packaging has proven to be one of the most promising active packaging systems due to its high efficiency in terminating and inhibiting spoilage of pathogenic microorganisms that contaminate food [46].

Disparities pertaining to chitosan’s bactericidal efficiency can be attributed to many factors. Within each specific role that is played, each of them can be classified into four categories:

1. Microbial factors that are related species and cell age
2. Intrinsic factors of chitosan that include positive charge, density, molecular weight, concentration, hydrophilic and hydrophobic characteristics, and chelating capacity
3. Physical state factors including water solubility and solidity of chitosan
4. Environmental factors which include ionic strength, pH, temperature, and reaction time

Chitosan acts as a model biopolymer for the forming antimicrobial films mainly due to its nontoxicity. The natural antibacterial/antifungal properties along with its
ability to form antimicrobial films allow chitosan to be used extensively as both a biodegradable and antimicrobial packaging material.

Chitosan as previously mentioned possesses several biological applications. Moreover, chitosan has also been recently proven to significantly inhibit tumor growth within cancer cells [47]. Intratumoral administration of various chitosan compounds alone has been shown to successfully promote antitumoral effects within a metastatic breast cancer model [48]. In addition, chitosan was also recently found to activate macrophages into cytotoxic macrophages which act to suppress Meth-A-tumor growth in Balb/c mice [49]. Following that, the wide array of various tissue engineering applications has been found to impair the need for biodegradable materials. Chitosan as a natural polymer is a great biocompatible and biodegradable material, and through recent discovery, various natural polymer composite materials have been able to yield promising scaffolds for bone tissue engineering applications [50]. Through the next generation of biomaterials, scientists will seek to combine both bioactive and bioreabsorbable materials that will act to mimic the various functions of bone and will thereby activate within the vivo mechanisms of bone regeneration [51]. Also, through extensive research and experimentation, it has been determined that composite materials, which include the combination of varying biodegradable polymers and bioactive ceramics, will be highly suitable for bone regeneration [52].

Other important biomedical application of both chitin and chitosan includes both tissue engineering and drug delivery systems [53]. Chitosan is often useful for many biomaterial and biomedical applications due to its unique ability to be modified into many forms such as beads, film, fibers, and scaffolds [54]. The intricate dimensional structures of scaffolds are what allows it to simulate the extracellular matrices found within the human body [55]. The structure itself consists of a large surface area that enables cells to be seeded and penetrate into pores [56]. Next, chitosan has also been used expansively as an excipient within oral formulations and vehicles for parenteral drug delivery systems [57]. In addition, chitosan has also been further used for manufacturing sustained release systems that are deliverable by other routes such as the nasal and ophthalmic pathway [58]. Following that, chitosan also possesses the ability to form colloidal particles which entrap bioactive molecules through cross-linking, ionic cross-linking, and ionic complex formation, allowing for the association of bioactive molecules to polymers and controlling drug delivery [59]. With that being said, both chitin and chitosan controlled delivery systems are currently under a developmental stage but are still being used for a variety of reagents within several environments [60, 61].

The complex structures present within artificial kidney systems have allowed for the constant hemodialysis within chronic kidney failure patients. As a result, chitosan membranes have been recently suggested to act as an artificial kidney membrane due to their permeability and high tensile strength [62, 63]. The semi-permeable membrane of artificial kidneys has played an integral role in helping to treat many chronic kidney failure patients, and until recently these membranes were made exclusively from cellulose and cuprophane. However, because the primary function of cellulose can be compared to that of a filter, there is little selectivity in the separation of two related molecules [64].
Chitosan has been recently declared as a potential candidate for both burn and artificial skin treatment. This conclusion has been drawn due to chitosan’s ability to form strong, water-absorbent, biocompatible films. These strong films can develop directly on the site of the burn through the use of an aqueous solution of chitosan acetate. In addition, this particular form of chitosan treatment allows for oxygen permeability, which is necessary in preventing oxygen deprivation in tissues [65]. Also, chitosan has been found to have an enhanced effect on both wound healing and wound dressing processes [66]. Through experimental studies, it has been shown that chitin fibers, non-woven mats, sponges, and films exhibit an increase in wound healing processes by 30%. Chitin has also been discovered to be an effective coating for various biomedical materials [67].

Aside from the many biomedical applications that both chitin and chitosan possess, chitin and chitosan fibers have been shown to also have potential applications in wastewater treatment, where over the years the removal of heavy metal ions by chitosan through chelation has garnered a lot of attention [68]. Its use within the apparel industry could also potentially be a long-term possibility [69]. Chitin derivatives have also been produced and further used to establish antistatic soil repellent to textiles within the textile industry. Additionally, chitin can also be for printing and completing preparations while chitosan is able to effectively remove dyes from processing effluents [70].

Chitosan use within the food industry has also drawn considerably attention due to the fact that it is considered nontoxic for warm-blooded animals. A compound known as microcrystalline chitin (MCC) has been shown to possess great emulsifying properties and also a gelling agent that is primarily used to stabilize food. As a result, the use of MCC has aided in the solution of problems related specifically to flavor, color, and shelf life posed by various sources of fiber [71].

Chitin and chitosan can also be used for a variety of applications for chromatographic separations. The presence of free amino groups and hydroxyl and secondary hydroxyl groups enables chitosan to function as a good chromatographic support structure. More specifically, the use of chitosan in thin layer chromatography for the separation of nucleic acids has also been reported [72]. Both chitin and chitosan have been used as a sorbent material for solid-phase extraction of phenols and chlorophenols through the use of high liquid chromatography [73].

Within color photography, chitosan has been used primarily as a fixing agent for acid dyes in gelatin while also improving diffusion, important in developing photographs [74, 75]. Through extensive studies, it has been determined that both chitin and chitosan can be readily derivatized by exploiting the reactivity of its primary amino and secondary hydroxyl group in order to find potential applications within varied areas.

12 Polymeric Blends

Polymer blends are made either by melt processing or in aqueous conditions. Melt processing is applied when working with water-sensitive compounds such as cellulose [76] and starch [77] or compounds like natural rubber [78] that are not very
water soluble. Aqueous conditions are used when a component of the blend degrades at melting temperatures or as a means to promote homogeneity. Components of polymers made in such a manner include starches such as chitin/chitosan, proteins such as collagen, and drugs.

PLA, a transparent polyester made from lactic acid, is biodegradable, generally low in toxicity, and durable under mechanical stress but has a poor thermal properties. Blending is a means to deal with this shortcoming. Simply blending the L(PLLA) and D(PDLA) isomers (enantiomer polymer blending) of PLA improves thermal stability by 50 °C over the pure polymers [79]. It also reduces its tendency to hydrolyze [80] and is useful in controlling degradation time [81]. Blending PLA with poly(aspartic acid-co-lactide) (PAL) or poly(sodium aspartate-colactide) (PALNa) increased hydrophilicity, improved compostability, and may help reduce static buildup [82]. Various types of polyhydroxybutyrate (PHB) have also been blended with PLA in an attempt to widen the range of physical properties available and improve the polymer’s processability [83]. Blends of PLLA and 4,40-thiodiphenol (TDP), a compound with hydroxyl groups, produced changes in both thermal and dynamic mechanical properties as well as created a eutectic phase behavior [84]. Because starch is a far cheaper material than PLA but PLA has far superior mechanical properties, blends of the two have been widely studied in an attempt to produce a more cost-effective, biodegradable plastic with similar properties [85, 86]. The hydrophilic character of starch and hydrophobic character of PLA mean that mixing is incomplete, uneven, and thermodynamically unfavored. Various additives have been tested to increase adhesion between the two components, including methylenediphenyl diisocyanate (MDI), [87] dioctyl maleate (DOM), [88] poly (vinyl alcohol) (PVOH), [89] and poly(hydroxyl ester ether) PHEE [90].

Reactive blending/extrusion has also proved effective at improving adhesion. Either maleic anhydride was attached to PLA by radical reaction or a copolymer of poly(caprolactone) (PCL) with a polysaccharide backbone was added to the mixture.

PHAs are polymers produced in the cytoplasm of prokaryotes grown during fermentation. Bacterial poly(hydroxybutyrate) (PHB) is a specific form of PHA. While a renewable resource, PHB is unfortunately brittle and thermally unstable. Various blends have been created to improve these characteristics. Added components that lead to improved characteristics include cornstarch [91], grafted copolymers of starch and glycidyl methacrylate (starch-g-PGMA), [92] and cellulose propionate (CP) [93].

Multilayer composites of natural products, specifically TPS, and a water-resistant biodegradable polymer, such as a polyester, may prove a solution to the issue of water sensitivity described in Sect. 2 [94]. These multilayer composites are produced by multilayer co-extrusion and may have uses in food packaging, disposable containers, and controlled drug/pesticide release.

When extra strength, stiffness, or sound attenuation is desired from a polymer without too much added weight, natural fiber composites are often the answer as opposed to traditional glass fiber inclusion. Fibers in use or investigated thus far include jute, oil palm, ramie, flax, and regenerated cellulose [95]. Cellulose reinforced starch in particular has been the subject of extensive research because it is cheap to
produce, biodegradable, renewable, and made of easily sourced materials. Hot press molding, [96] foaming, [97] extrusion, [98] and injection molding [99] have all been used to produce such a composite with desirable properties including increased water resistance. Natural fibers have also been used to reinforce PHB [100].

Starch/clay, starch/polyester/clay, and starch/PCL/clay nanocomposites have been shown to improve the properties of starch-based products including their ability to act as a barrier [101–103]. Similar work has also been done with PLA/clay nanocomposites [104].

13 Conclusions

Polymers from renewable resources find more and more use in the production of industrial, consumer, and medical products. The renewable polymer industry is growing rapidly. Using such polymers instead of traditional ones helps resolve multiple issues such as:

- Reduce carbon emissions
- Reduce landfill due to biodegradability and combustion
- Lessen dependence on oil

This is a relatively new and rapidly growing field of science with more applications in such important fields as biomedical science, military science, electronics, and food science are under investigation and soon to be added to the list.

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Polymeric Micelles

Iliyas Khan, Avinash Gothwal, Gaurav Mishra, and Umesh Gupta

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Abstract

Polymeric micelles (PM) are means of novel drug carriers for poorly soluble hydrophobic drugs. Outer shell of polymeric micelles is hydrophilic in nature which further led these carriers to stay longer in blood and can accumulate in tumor-specific region due to their smaller size through enhanced permeation and retention (EPR) effect. The polymeric micelles can also be modified through different ligand to achieve active targeting of drugs. Polymeric micelles can be synthesized and prepared through different self-assembly methods. These can be used to improve solubility, residence time of drug in blood, and inhibition of efflux pump, to enhance pharmacokinetic parameters, and to achieve sustained release of drugs at target site without any side effects in an efficient manner. These types of novel drug delivery systems are aimed to enhance the efficacy and reduce the side effects of anticancer drugs in an efficient way. The present chapter highlights the structure, methods of preparation, the micellar architecture, and the role of these carriers in the anticancer drugs.

Keywords
Polymeric micelles · Solubility · Hydrophobic · Hydrophilic · Anticancer drug delivery

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>mPEG-b-p(HPMAm-lactate)</td>
<td>(Poly(ethyleneglycol)-b-poly N-(2-hydroxypropyl) methacrylamide-lactate)</td>
</tr>
<tr>
<td>(PEG-b-PPhe)</td>
<td>poly(ethylene glycol)-block-poly(phenylalanine)</td>
</tr>
<tr>
<td>(Vitamin E TPGS2k)</td>
<td>D-α-Tocopheryl polyethylene glycol succinate 2000</td>
</tr>
<tr>
<td>ABC</td>
<td>Accelerated blood clearance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BCS</td>
<td>Biopharmaceutics classification system</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DOX</td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetric</td>
</tr>
<tr>
<td>EPR</td>
<td>Enhanced permeability and retention</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastro-intestinal tract</td>
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From ancient time there was a big problem of protecting the drug from degradation and excretion and to avoid adverse effects of lethal drugs. The traditional way of drug delivery is continuously changing and getting advanced day by day. The introduction of nanocarrier has changed the entire scenario of drug delivery research. Current chemotherapy has a lack of selectivity toward neoplastic cells. Resistant types of tumor required high dose of chemotherapy instead of normal dose, resultant in toxicity to normal cell and enhance the toxicity of treatment. Hence to keep in mind to reduce the toxicity and targeted delivery of chemotherapeutic drugs, novel nanocarriers are used [1]. The use of different nanocarriers such as nanoparticles, liposomes, dendrimers, carbon nanotubes[5], etc. has resulted in enhanced effectiveness and reduced side effects. Each of nanocarrier has its own pros and cons. They slowly release drugs at targeted site and slowly degrade in in vivo, stimuli reactive such as temperature or pH sensitive and long circulation time in the body [2, 3]. Hence, nanocarriers were discovered and have used frequently deliver drugs through different routes for the reason of protecting the drug against degradation and/or excretion to prevent adverse effects of toxic drugs and targeted drug delivery. In these, polymeric micelles have been widely used nanocarrier for anticancer drug delivery. Micelles are colloidal dispersion size range between 5 to 100 nm mainly. Nanometric size leads to a very cosmic chance to develop the formulation into the injectable dosage form. Cancer has a leaky vasculature characteristic, and the nanosized formulations are penetrated easily to the tumor site due to enhanced permeability and retention (EPR) phenomenon [4]. They are made up of amphiphilic colloids. At certain concentration and temperature, these colloids are formed through amphiphilic or surface active agent [5]. These amphiphilic molecules exist separately at low temperature in aqueous medium, but at higher concentration, aggregation takes place. These aggregates are called micelles. The concentration at which the formation of micelles starts is called critical micelles concentration (CMC) [6] and the temperature lower where amphiphilic molecules represent as
unimers and beyond exist as aggregates, called critical micellization temperature (CMT) [7]. If, CMC value is lower; resulting into stable micelles formation [8]. Conventional surfactant micelles have less thermodynamic stability in physiological solution. So due to this disadvantage, new micelles have been discovered for drug delivery which is made up from block copolymeric chain of hydrophilic and amphiphilic monomer unit. They have less CMC value which makes polymeric micelles (PM) more stable as compared to conventional micelles [9]. PM were first proposed by Ringsdorf et al. in 1984. PM displayed a class of micelles which is made up from block copolymer entailing hydrophobic and hydrophilic monomer unit (Fig. 1). Amphiphilic block and AB-type graft copolymers with increasing the chain length of hydrophilic block beyond the range of hydrophobic part, resulting in spherical micelles in aqueous environments [10]. The release pattern of drug from polymeric micelles from its hydrophobic core is in a sustained manner, and it depends on the length of the hydrophobic blocks and monomer species. Scientists developed many types of the polymeric micelles with new monomer units, and they have vast groups concerning moieties and targeting ligands, but only a few were reported successfully for the delivery of drugs. Due to longer circulation time within, blood system indicates to improve congregation at tissue sites because of EPR effect (Fig. 2). The above specific characteristic makes available one of the solidest influences in the use of polymeric micelles for delivering of anticancer drugs, maximum of which have very low solubility in aqueous medium [11]. The ground rule of polymeric micellar drug delivery system is revised with an attention on the application of delivery of oral drugs and anticancer therapy, the most widely inspected applications for polymeric drug delivery. Flexibility in monomer classes, surface modification, and block polymer length ratio offer polymeric micelles having multifunctionality. But simultaneously polymeric micelles have mainly two weaknesses: one is the low payload of drugs and second is less stability in aqueous medium. This part pledges with the drug loading property of polymeric micelles [12]. The present chapter is all about the applicability and properties of the polymeric micelles.
micelles used in the drug delivery with special emphasis on anticancer drugs. The previous reported study revealed that the polymeric micelles can target very effectively the solid tumors which is a convincing approach for the passive targeting [13, 14]. The cellular uptake of the drug is enhanced due to the polymeric carrier to the cancerous cells and believed that increases in endocytotic transportation and also sidestepping multidrug resistance [15].

1 Architecture of Polymeric Micelles

Amphiphilic polymers are self-assembled in aqueous environments, to form the core-shell structure, either fluid or solid core. If the solid core is formed, then it’s called nanospheres; and when the fluid core is formed, then it is called PM. The core of PM is a dense region consisting of the hydrophobic part. The core encapsulates the low water-soluble drugs due to hydrophobic interactions. The outer surface of PM made up from hydrophilic part of an amphiphilic polymer (Fig. 1) [16]. Polymeric micelles have specific roles in drug delivery which is described below.

1.1 Core (Internal Case)

Hydrophobic core is the main component which determines the PM capacity to solubilize the less water soluble drugs. Capability of the hydrophobic core to encapsulate the drugs or compounds mainly depends on compatibility between the drug molecules and hydrophobic internal core [17]. Compatibility between less water soluble drugs and hydrophobic internal core of PM can be assessed by comparing polarity. To quantify this interaction, Flory-Huggins parameters used to estimate the congeniality using polarity.
\[ \chi_{sp} = (\delta_s - \delta_p)^2 \nu_s / \kappa T \]  

Here,
\begin{align*}
\chi_{sp} &= \text{Solubility parameters for drugs and hydrophobic core} \\
\nu &= \text{Molar volume of drugs} \\
\kappa &= \text{Boltzmann constant} \\
T &= \text{Temperature in Kelvin}
\end{align*}

Hypothetically, if \( \chi_{sp} \) is decreased or minimized, it leads to better compatibility and more effective encapsulation of less soluble drug [18].

Core-forming components cover a broad array of structural assortment and polarity for solubilizing the less soluble drugs. Hydrophobic interactions are responsible for the stability between drugs and the hydrophobic core, due to its thermodynamically stable formed complex [19].

### 1.2 Outer Shell (Corona)

The outer shell (corona) of PM is made up from hydrophilic part of the amphiphilic polymer. Corona should possess the effective stealth property for the PM. It should contain sufficient hydrophilicity, surface charge, block copolymer chain length, and reactive group for attachment of targeting moieties [20–24]. Corona is responsible for the protection of drug from aqueous environment and also enables it for the stability and stealthy recognition in vivo through the reticuloendothelial system (RES) and also enhanced its vascular permeability [25, 26]. Owing to these properties, PMs possess effective pharmacokinetics property, biocompatibility, and better biodistribution of drug incorporated into it. Therefore in vivo behavior of the drug may be controlled independently through the outer shell of the inner core [27]. Various types of hydrophilic block of polymer are used in PM, which generally possesses molecular weight between 1 to 15 kDa. Generally, solubilization can increase PM size due to enlargement of hydrophobic core, and other factors which can affect drug loading are core and corona size. If the hydrophobic blocks are larger than the core size, increase the encapsulation efficiency. If the hydrophilic block chain length increases, then the CMC value also increases [2]. Corona also acts for the interaction with a biological active component like protein within circulating blood [28].

### 2 Merits and Demerits

One of the major pros of PM is good edifice stability in comparison to the micelles containing lower molecular mass, so it is the promising carrier for in vivo delivery. The second major advantage of the PM is increasing water solubility, and it integrates a large amount of hydrophobic drugs [29]. Initially it was a very inordinate problem with polymer-drug conjugate system, and due to this, loss of
solubility was observed due to the incorporation of hydrophobic drugs. Many of
the researchers reported this problem with the synthesis \([30–33]\). Further, this
problem was shorted out with the incorporation of hydrophobic drug in large
amount within the inner core of the micelles \([34, 35]\). Concurrently the PMs can
endure the solubility of the drugs by hindering intermicellar aggregation of the
hydrophobic core through the use of hydrophilic part on the outer surface of
the shell, and it acts as a boundary for the intermicellar aggregation. Lower
toxicity is another characteristic which leads to the polymeric micelles. It is
generally seen that lower polymeric surfactants are behaving as a less toxic
material. So it is more used as a carrier system for drug delivery. It is also
advantageous because it can be filtered through renal and the molecular mass of
the constituting micelles that is lesser than the critical molar mass of the renal
filtration. It is seen that PMs have two phases: the first one is inner core and
the second phase is an outer shell. This geometrical behavior of the PMs is utilized
for many purposes in the drug delivery process. The inner core of the micelles is
responsible for drug loading and the pharmacological activity, whereas the outer
surface is playing the role of interaction with the bio-components like cell
and proteins. This one leads to the determination of pharmacokinetics profile
of biodistribution of the drug. So, in vivo release of the drug is maintained
by outer shells of polymeric micelles \([34]\). This unique feature is favored nowa-
days at a very rousing rate because of the biphasic nature of the PMs which
are dependent on the types of the chain forming the polymeric micelles through
block copolymer.

Within this series there is one more advantage attached with the PMs, i.e., its
particle size, which is very small, and diameter, which mainly lies between 5 and
100 nm with very narrow distribution. Due to this smaller size range, it is very easy
to attain stability and longer extent of movement in the blood stream. Owing to its
smaller size, it is not easily captured by reticuloendothelial cells, and it is also
rapidly excreted through the kidney \([31]\). One of the major drawbacks is stated
here, that it cannot be easily recognized in scientific papers that relatively high
levels of the chemistry of polymer are desirable in the polymeric micelles studies.
Another drawback is the method of incorporation; if it is not satisfactory, then it
can create a problem. Yokoyama et al. reported that physical combination ef-
fi-
ciencies were reliant on approaches of incorporation \([35]\). Through trial and error,
researchers needed to search an appropriate incorporation method for every
drug separately. Many of the incorporation methods are appreciable at laboratory
scale, but it fails at industrial scale due to the physical factors such as solvent
exchange rate and diffusion process so that it is needed to develop a substantial
development of technology of incorporation of drug molecule into the polymeric
micelles \([36]\). Similarly, it is valuable citing that the regulator of micelles disso-
ciation and drug release rate is crucial for drug aiming and it rheostat of these
problems are occasionally technically problematic to improve for the targeting.
One line statement for the pros of polymeric micelles is smaller diameter with
the lean distribution, high stability in structure, good water solubility, and
low toxicity.
3 Methods of Preparation

There are various methods that encompass the preparation of polymeric micelles such as o/w emulsion, dialysis, solid dispersion, and microphase separation method. All the above different process covers in two steps: synthesis of amphiphilic block co-polymer and then transformation of it toward critical micelle concentration (CMC) [37]. It is observed that at CMC, both the interface and the bulk turn out to be saturated form with the monomers. Under CMC, the quantity of the amphiphilic polymer at the air/water interface upsurges with increasing concentration; for both surfactant and high-molecular-weight block copolymer, removal of ordered water molecules into the bulk aqueous phase carries association of micelles, that is, in stable form [38]. Polymeric micelles consist of highly regulated block copolymers with distinct core-shell structure. Functional groups, such as amines and carboxylic acids in the core-forming segments, are useful for introducing drugs into the micelle core [39]. Another groundbreaking, single-step process of formation of PMs comprises the solution mixture of drug and polymer in a water-tert-butanol system by the help of lyophilization. Reconstruction of this freeze-dried cake of drug-polymer mixture with injectable vehicle catalyzes the impulsive formation of PMs [40]. Other different methods are direct dissolution, [41] complexation [42], chemical conjugation [43], and several solvent evaporation methods. After polymer synthesis, polymers are permissible to self-assemble in biphasic systems followed by exhaustive modification. PMs having cross-linking in the core are produced after quickly heating aqueous media of block copolymer to above their CMC, followed by illumination in the presence of a photoinitiator [44]; this cross-linking is responsible for stabilization of morphology of micelles [20].

3.1 Solid Dispersion Method

In solid dispersion method, polymer and drug are dissolved into a suitable organic solvent system. The organic solvent was evaporated under reduced pressure to form a polymeric matrix. Further, water was added into preheated polymeric matrix, to form drug-loaded polymeric micelles [45, 46]. Lei and co-workers prepared curcumin-loaded polymeric micelles using single- or one-step solid dispersion method. In this method they dissolved curcumin and MPEG-PCL copolymer in dehydrated alcohol. Further, the solution was dried using rotary evaporator, and afterward it was dissolved into NS to self-assembled micelles [47].

3.2 Dialysis Method

As the name suggested, in this technique dialysis bag is used. Briefly, addition of fewer amounts of water to the solution of polymer and drug in a water-miscible organic solvent such as dimethylformamide (DMF) with continuous stirring which is then dialyzed against excess amount water [48]. In this process, water replaces the
organic solvent gradually so that process of self-association is fast and entrapment of drug is also desirable rate in assembled structural format (Fig. 3). The micelles remain in dialysis bag with the help of semipermeable membrane, and only unloaded drug is removed from micelles during the dialysis [49]. The method described here is well suited to the laboratory scale rather than feasible to the large scale industry. One of the major drawbacks is noted in this method, i.e., the removal of the complete unbounded drug from the formulation [50]. Sung and co-workers prepared micelles of poly(ethylene oxide)–poly(β-benzyl L-aspartate) (PEO-PBLA) copolymer, and they used different organic solvents such as DMF, acetonitrile, THF, DMSO, ethyl alcohol, N,N-dimethylacetamide (DMAc), etc. which are dialyzed against water using dialysis tube. They found that the highest yield of micelles was obtained with DMAc which was 87% [51].

![Fig. 3](image-url) Schematic graphical preparation of polymeric micelles through dialysis method

### 3.3 Oil-in-Water Emulsion Solvent Evaporation Method

In this method, the formation of emulsion was reported with continuous aqueous phase and internal organic phase. Formation of emulsion is done by dissolution of the polymer and drug simultaneously in water-immiscible organic solvent such as tetrahydrofuran, acetone, chloroform, or mixture of ethanol and chloroform [52, 53]. Further, solution is slowly added to distilled water by instant shaking and stirring, and many times polyvinyl alcohol (PVA) is added into water, and resulted into formation of emulsion rearranged to form expected polymer (Fig. 4). This emulsion is then preserved in the air with stirring so as to remove all the organic solvent [54]. This method is beyond the limit for the addition of the polymer which can be added either in an aqueous or organic phase and exemplified by the reported literature [55–57] that solubilization of doxorubicin (DOX) and indomethacin in poly(ethylene oxide)-b-poly(β-benzyl-L-aspartate) (PEO-b-PBLA) forms a micelle through the o/w emulsion method.
3.4 Microphase Separation Method

Polymeric micelles are molded impulsively and drugs are entrapped in the inner part of the micelles. In this method, polymer and drug were added into organic solvents such as tetrahydrofuran (THF). The drug and polymer solution were added into aqueous solution dropwise under continuous stirring. Further, the organic solvents were removed under reduced pressure, and a blue color micelles solution is formed [58].

3.5 Freeze-Drying Method

The freeze-drying method (Fig. 5) used an organic solvent like t-butanol which can be freeze-dried for purpose of dissolution of drug and polymer. After that this solution is added with water and then freeze dried. Further it was reconstituted in aqueous isotonic media [59]. This method is pharmaceutically feasible and can be utilized in large-scale production within industry. Freeze-drying was used for the encapsulation of PTX with its derivatives in poly(N-vinylpyrrolidone)-b-PDLLA (PVP-b-PDLLA) [59]. Another side, one thing should always keep in mind is that the freeze-drying method may not be used with the PEO-containing block copolymer due to insolubility of PEO in t-butanol [60].

4 Characterization

The main purpose of characterization of the PMs is because it is being used for the delivery of drug, and for this it should be stable for a sufficient amount of time in blood stream so that it should reach the target site.
4.1 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is used frequently to disclose the structure and composition of polymers synthesized to produce PMs. It expresses number-average molecular weight of the polymer in which first calculated the molar composition of the polymer from the integral proton intensity at different parts per million (ppm), then the data observed is calculated by molar ratio of the recovered polymer from NMR [61], and then the average molecular weight was calculated from that data based on the assumption that the weight of initial polymer is taken.

4.2 Morphological Characterization

For determination of surface morphology of prepared micelles, atomic force microscopy (AFM) is used to characterize the particle size. A probe tip with atomic-scale sharpness restored on the sample a data generated on the basis of force operated between surface and tip of the probe. For the observation of three-dimensional microscopy of the colloidal particle, confocal microscopy can be performed with very high precisions rate with time rate at large limit by avoidance of the scattered light outside the plane of interest [48]. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide the way to observe the surface of
4.3 CH50 Test (Complement Activation)

By the help of this test, the amount of human serum protein (complement protein) adsorbed on the PMs is calculated. The activation of the complement by opsonization though which the immune system eliminate the foreign particles. Butsele et al. measured complement activation by measuring the lytic capacity of a normal human serum toward antibody-sensitized sheep erythrocytes afterward introduction to the micelles. The test is done by the incubation of the aliquots of the human serum through increasing concentration of the micelles [63]. The total amount of serum needed to hemolyze 50% of the sheep erythrocytes to expose in micelles was calculated, and the released amount of the hemoglobin was applied for the dye in colorimetric titration. Small amount of the adsorbed protein on micelle gives large amount of the lysis of erythrocyte.

4.4 Determination of CMC of Polymeric Micelles Through Dye Solubilization Method

In this method, fluorescent probes are used which are nonpolar in nature such as 1,6-diphenyl-1,3,5-hexatrine or pyrene [64, 65]. Pyrene is a polyaromatic particle, especially partitions to the hydrophobic core of the micelles, with a synchronous alteration in property of fluorescent such as vibrational change in the spectrum, and in excitation spectrum, there should be red shift. The CMC is defined as the point where cross-linking of the extrapolation in the absorbance for a large range of the concentration of polymer occurs [66, 67]. There are many more methods employed to determine the CMC value, and earlier the widely used technique was the light scattering for the observation of aggregation number and the molecular weight. In this the inception of micellization may be only detected when the CMC lie only within the range of the scattering sensitivity, but it is very rare for block copolymer in water [68]. There are several copolymers on which the hydrophobic chain length decides occurrence of the micellization and the hydrophilic chain length is less considered [69].

4.5 Differential Scanning Calorimetric (DSC) Method

Nature and crystal space within the PM are measured by the glass, melting point temperature, and enthalpy in DSC. Owing to this various states of polymeric core and the interaction between the polymer and drug are observed through DSC [61].
4.6 Electrophoretic Technique

This method is utilized to measure the nature of surface coverage with respect to PMs. It gives information about the biodistribution and in vivo clearance. This technique utilized the separation on the basis of size and charge. The charged molecule passes across the gel when they are situated in the electric field, and the gel filters the molecule according to the charge and size of the molecule. For this technique isoelectric focusing and two-dimensional polyacrylamide gel electrophoresis are used as an analytical tool [70].

4.7 Flow Cytometry Method

This method mainly involves for the counting of the microscopic particle in which a beam of light is focused on the liquid or fluid. The detector is attached where the stream of single wavelength of light is passed for detection [71]. In the case of PM which is ligand coupled, observed for the capability of binding of micelles to the receptor [48].

4.8 Measurement of Optical Transmittance and Lower Critical Solution Temperature (LCST)

The LCST is a temperature below which the mixture is miscible in all ratio, the optical transmittance measurement method it generally used to determine polymer’s LCST. The determination of the LCST lies on the degree of polymerization of polymer, branching, and polydispersity when it is polymer mixture. To know the effect of the temperature in case of thermosensitive polymer, optical transmittance is measured by varying temperature of aqueous polymer at specific wavelength under ultraviolet light [48]. It is useful for the determination of the thermoresponsive micelles size. Diameter of these polymers was rapidly increased with the temperature above the LCST [72].

5 Drug Targeting Strategies

Major hurdles in treating cancer are delivery of anti-neoplastic agents to the cancerous cells and cytotoxicity of the anti-neoplastic agents to the normal cells and moreover, increasing resistance in the cancer needs to the increased dose which increases toxicity and put the patient with more incompliance [48]. Surgery is the choice of treatment, but it can’t be good for all patients because of factors such as tumor size, site, and metastasis, and the second choice is systemic chemotherapy, but the dark side of it is systemic toxicity. Hence, polymeric micelles are the best option for the targeted delivery and to reduce the toxicity as well; additionally, polymeric micelles enhance bioavailability because of stealth nature and targeted delivery
through enhanced permeability and retention (EPR); and hydrophobic drugs can be administered intravenously (I.V.). There are two possible well-defined mechanisms for targeted delivery: active targeting and passive targeting. In this section we will discuss about polymeric micelle-based targeting of bioactive agents.

5.1 Passive Targeting

In starting it was thought that there was only one targeting strategy for anticancer drugs, that is, receptor-mediated targeting to therapeutic sites; thus, several reports were documented of engineered polymeric micelle in which various ligands have been attached to polymeric micelles [10, 73]. Plethora of literature available is related to enhanced bioavailability by passive targeting in polymer-conjugated and drug-encapsulated nanocarriers [74]. The best possible mechanism for passive targeting of polymeric micelles in solid tumors is enhanced permeability and retention (EPR) (Fig. 2), which was first acknowledged by Maeda et al. 2001 [75, 76], as the histopathological and pharmacological studies reveal that solid tumor possesses characteristics such as highly unorganized, incomplete, hyper-vasculature, secretion of vascular permeability factors stimulating extravasation, and immature lymphatic capillaries [77]. Studies have shown that EPR leads to passive accumulation of macromolecules and nanoparticulate in solid tumor, resulting decreased side effects and enhanced therapeutics. Most of the human solid tumors have the effective pore size of 200–600 nm in diameter which allows for passive targeting to tumors [78]. Secretion of various factors such as nitric oxide, prostaglandins, bradykinin, basic fibroblast growth factor in tumor tissues, and overexpression of genes such as vascular permeability factor or vascular endothelial growth factor these factors cause enhanced permeability of tumor microvasculature.

5.2 Active Targeting

The principle of active targeting is receptor-mediated endocytosis using ligand as receptor agonist. As the micelles are made up of polymers and can be decorated with ligands, ligands play the critical role in the targeting which leads to increase the selectivity for tumor cells and reduces systemic toxicity and advesiative effects compared to untargeted chemotherapy [79]. When the ligands bind to their specific receptors on the cell membrane, the micelles are taken on by endocytosis [80]. Active targeting felicitates higher intracellular drug concentrations in the tumor. Ligands for active targeting can be any agent which has a higher binding affinity toward the receptors on the cell membrane, like monoclonal antibodies (mAbs) or their Fab fragments, oligosaccharides, or peptides [79]. Kabanov et al. were one of the first to report micelle-based active targeting by using murine polyclonal antibodies against α2-glycoprotein to deliver the haloperidol to the brain [22]. Similarly, Vega et al. reported mAb-based targeting of micelles in antineoplastic therapy [81]. Solid tumors were also targeted with the mAb C225 which has binding affinity with
epidermal growth factor receptor (EGFR) [82]. Other than monoclonal antibodies, folate is also a vital ligand for active targeting of cancer cells as folate is an essential vitamin for the biosynthesis of nucleotide bases and consumed cancerous cell so it can be used as a ligand [79, 83, 84]. The folate receptor is overexpressed in most of the cancers, including the ovary, brain, kidney, breast, myeloid cells, and lung, so it can be used in all the aforementioned malignancies as targeting ligand. Similarly angiogenesis regulators can be used as a targeting ligand based on ligand-receptor interactions [85]. When tumor cells cluster and reach a size of around 2–3 mm, diffusion of oxygen and nutrients to the tumor is repressed. This induces tumor angiogenesis, which allows tumors to grow beyond their diffusion limit [86]. Nasongkla et al. explored active targeting using αvβ3 integrin with the micelle-conjugated cyclic pentapeptide c(Arg-Gly-Asp-d-Phe-Lys) (cRGDfK) [87].

At present, polymeric micelles are being investigated in targeted anticancer therapy in vitro and in vivo due to promising characteristics and targeting efficiency. Polymeric micelles have higher cellular uptake and cytotoxicity, and tumor regression was demonstrated, making active targeting a significant added value to passively targeted polymeric micelles for anticancer therapy.

6 Anticancer Drug Delivery

Plethora of literature available regarding PMs based drug delivery for the effective and efficient treatment of cancer because of the promising nanocarrier for the delivery of hydrophobic anticancer drugs (Fig. 6 and Table 1).

6.1 Paclitaxel

Paclitaxel is obtained from bark of pacific yew tree Taxus brevifolia. It acts on tubulin and inhibits cellular division resulting in cell death [95]. It is hydrophobic in nature and needs to be delivered through nanocarrier such as PMs. Several strategies were reported regarding PMs for paclitaxel drug delivery. In this contrast, many authors prepared PMs using different block copolymer. Block copolymers are self-assembled into micellar form in aqueous media. Polymeric micelles are mostly used to solubilize the hydrophobic drugs. So due to this, increasing water solubility, better efficacy, and safety profile parameter were enhanced using polymeric micelles. Drug delivered through polymeric micelles was less toxic compared to naïve paclitaxel. Kim et al. (2001) reported paclitaxel drug delivery through PMs. The author prepared PMs using amphiphilic diblock copolymer (mPEG-PDLLA) monomethoxy poly(ethylene glycol)-block-poly (D,L-lactide) and evaluated safety profile and pharmacokinetic and tissue distribution of polymeric micelles compared to naïve paclitaxel. In vitro cell line studies were done using human ovarian cancer cell line OVCAR-3 and MCF-7 human breast cancer cell line. The obtained results concluded that the IC50 values of polymeric micelles in both of the cell lines was 0.002 μg/mL compared to naïve taxol which was 0.002 and 0.004 μg/mL for ovarian
and breast cancer cell lines. The toxicity was reduced and improved anticancer efficacy [88]. Similarly, solubility enhancement was reported by Cho et al. (2004). They reported that after using a hydrophobic agent, sodium salicylate, the solubility of paclitaxel was enhanced 100-fold in aqueous media without disrupting block copolymer of (PEG-b-PPhe) poly(ethylene glycol)-block-poly(phenylalanine). This is due to sodium salicylate, and it was a predictable tool for maintaining better sink condition for less soluble drugs such as paclitaxel [89]. Further, more exhaustive

**Table 1** Drug incorporated into polymeric micelles

<table>
<thead>
<tr>
<th>Block copolymer</th>
<th>Drug</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG-PDLLA</td>
<td>Paclitaxel</td>
<td>[88]</td>
</tr>
<tr>
<td>PEG-b-PPhe</td>
<td>Paclitaxel</td>
<td>[89]</td>
</tr>
<tr>
<td>MPEG-PLA</td>
<td>Docetaxel</td>
<td>[90]</td>
</tr>
<tr>
<td>Dextran-PLGA</td>
<td>Docetaxel</td>
<td>[91]</td>
</tr>
<tr>
<td>PLLA-mPEG</td>
<td>Docetaxel</td>
<td>[92]</td>
</tr>
<tr>
<td>PLGA-mPEG-folate</td>
<td>Doxorubicin</td>
<td>[93]</td>
</tr>
<tr>
<td>PEG-PLG</td>
<td>Cisplatin</td>
<td>[94]</td>
</tr>
</tbody>
</table>

*Fig. 6* Various types of anticancer drugs encapsulated in polymeric micelles
study was carried out by Soga et al. (2005), in which they explained the block copolymer pHPMAmDL-b-PEG (poly(N-(2-hydroxypropyl) methacrylamide lactate) and poly(ethylene glycol)). This block copolymer has thermo-sensitivity, which was one of the important advantages for selection. Using this block copolymer, the drug loading was up to 2 mg/mL in PMs [96]. For targeting delivery, Seow et al. (2007) reported cholesterol-grafted poly(N-isopropylacrylamide-co-N, N-dimethylacrylamide-co-undecenoic acid). In this, folate was attached into hydrophilic moiety of polymer for targeting into folate receptor on cancer cell lines. The overall conjugated micelles showed better solubility, enhanced targeting, and significant cytotoxicity [97]. After that a dual-targeting approach was established by Liu et al. (2011), in which they explained two targeted molecules such as folate and hyaluronic acid for polymeric micellar formation of paclitaxel. This dual-targeting moiety has an ability for high payload, sustained-release property, and effective and prominent cellular uptake [98]. Another research was carried out using triblock copolymer by Zhang et al. (2012). They investigated poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) copolymer for preparation of polymeric micelles. The results suggested that the overall improvement in pharmacokinetic property and in vivo efficacy of PMs compared to naïve drug [99].

6.2 Docetaxel

Docetaxel is an anticancer drug obtained from Taxus baccata. It acts on beta tubulin, stabilizing the microtubule formation and resulting in cell death [95]. The main drawback of this drug is its hydrophobic in nature and so some new carriers are needed for docetaxel delivery to the cancer cells. In this regard, Mu et al. (2010) reported effects of mixed MPEG-PLA and pluronic micelles for improved bioavailability and multidrug resistance of docetaxel. Mixed micelles shown improved and admirable solubility profile over naïve drug. Pluronic polymer displayed an effective and promising role in high payload, improved pharmacokinetic profile, and overcomes multidrug resistance in vivo [90]. Another research group, Mi et al. (2011), prepared targeted formulation for docetaxel using folic acid-conjugated D-α-tocopheryl polyethylene glycol succinate 2000 (vitamin E TPGS2k) micelles. TPGS2k formed micelles were used for delivery of hydrophobic drug which showed the more stability. Significantly synergistic effects were obtained using copolymeric micelles formulations. Folic acid was used as a targeted ligand for targeted drug delivery for better enhancement of cellular uptake [100]. Chen et al. (2012) reported Pluronic P105 and F127 copolymers using thin film hydration method. They evaluated that the prepared micellar formulation showed significantly improved pharmacokinetic as well as solubilization property compared to naïve docetaxel. The in vivo tumor efficacy was also impressive for effective tumor suppression than docetaxel alone [101]. Further new strategy was reported by Raza et al. (2016) using dextran-PLGA copolymer. They too reported that the micelles formation of copolymer showed effective pharmacokinetic profile and
biological half-life. Bioavailability was approximately 16 times enhanced than naïve drug [91].

### 6.3 Doxorubicin

Doxorubicin (DOX) is an anthracycline antibiotic isolated from fungus *Streptomyces peucetius*. It is a chemotherapeutic agent used against non-Hodgkin lymphomas, breast cancer, etc. It inhibits the synthesis of nucleic acid, resulting in cell death. Many researchers delivered doxorubicin through nanocarriers such as micelles, nanoparticles, liposomes, etc. [102]. Yoo et al. (2000) prepared PLGA-PEG copolymer-based micelles for DOX. They evaluated that the cytotoxicity of formed micelles was enhanced than free DOX due to endocytosis mechanism. Co-polymeric micelles sowed more intense fluorescent in the nucleus than DOX alone [103]. Similarly again the same research group, Yoo et al. (2002), prepared PLLA-mPEG copolymer-based micelles using self-assembled method. Encapsulated doxorubicin was released from micelles slowly up to 25 days as a sustained-release manner which exhibits its sustained-release behavior. Cytotoxicity was performed in lymphoblast cell line HSB-2. The observed cytotoxicity of micelles was 5 times more than naïve DOX. It was concluded that the micelles formation showed better and effective IC\textsubscript{50} value compared to DOX alone [92]. Further Yoo et al. (2004) also described the targeted strategy using ligand at the terminal of copolymer. They used folate as a ligand for targeting delivery. In this PLGA-mPEG-folate was used as long-chain molecules for attaching the DOX at the one terminal of PLGA. They used these molecules for self-assembled in to PMs. Folate act as targeted molecules in this formulation. Flow cytometry, confocal images, and cytotoxicity results showed that the folate ligand attached micelles have more cellular uptake and are more cytotoxic than without ligand attached micelles. Biodistribution and in vivo study also suggested that the folate ligand attached micelles had more availability in tumor region and had significantly decreased tumor volume than naïve DOX [93]. Other group of researchers, Talelli et al. (2005), reported core cross-linked PMs with sustained release of DOX. They used (poly(ethylene glycol)-b-poly[N-(2-hydroxypropyl) methacrylamide-lactate] (mPEG-b-p(HPMAm-Lactate) diblock polymer for micelles. They explained that the di-block co-polymeric micelles were more accumulated in tumor-specific region and prolonged circulation in blood stream due to EPR effect. Cytotoxicity and in vivo results were more comprehensive in co-polymeric micelles compared to DOX alone [104].

### 6.4 Cisplatin

Cisplatin is a metallic compound and a well-known anticancer drug used in many sarcomas such as bone, soft tissues, blood vessels, etc. It is also used in solid tumor. The main side effects of cisplatin are nephrotoxicity [105]. To overcome this side
effect and increasing efficacy, a new novel carrier is needed for delivery of cisplatin. In this point of view, Nishiyama et al. (2001) investigated cisplatin-loaded polymer metal complex micelles delivered to the lung cancer. They observed that the cisplatin-loaded micelles had more circulation in plasma and tumor accumulation in specific area due to micelles. They also evaluated that nephrotoxicity was reduced due to specific organ tumor accumulation of cisplatin-loaded micelles [106]. Further, other research group, such as Oberoi et al. (2012), explained cisplatin-loaded poly (ethylene glycol)-b-poly(methacrylic acid) cross-linked micelles for ovarian cancer. They also observed that the copolymeric micelle-loaded cisplatin showed more prolonged blood circulation and tumor accumulation. The main side effects of cisplatin were renal damage, and the results indicated that the overall exposure of cisplatin to kidneys was minimized with the use of polymeric micelles [107]. Subsequently in the same year, Wang et al. (2012) reported PEG-PLG-conjugated dithiodipropionic-Pt (IV) micelles for ovarian cancer cell line. They found that the consequential micelles show high payload capacity of cisplatin and improved in vitro cytotoxicity against ovarian cancer cells compared to naïve cisplatin [94].

7 Scientific Opinions

7.1 Yokoyama’s Report

Yokoyama et al. (2004), enumerated that the enumerated that various PMs were used as anticancer drug targeting systems since the late 1980s, and most of this research team’s report involved the elementary study stage mainly on three important explanations. The main point of attraction of this report was enhanced antitumor effect, biodistribution and pharmacokinetic profile, and also the physiochemical characteristic of the carrier system. It also explains the limits, the biological behavior, and the toxicity of the carrier which are explained below.

In the first report, the research team successfully prepared PM formulations with anticancer drug, i.e., DOX, and it was chemically conjugated to aspartic acid residues of the poly(ethylene glycol)-b-poly(aspartic acid) block copolymer through amide bond formation. Prepared micellar structure have amphiphilic characteristics Doxorubicin was further assimilated into the inner core by means of physical entrapment having π-π interaction with established chemical conjugation with the DOX molecule [35]. Thus PMs encompassing both the physically entrapped and chemically conjugated and DOX in the inner core remained with the PEG. Outer shell did not demonstrate any in vitro or in vivo actions. Cytotoxic activities were found with physically entrapped DOX. The inactivity of the conjugated DOX resulted from the fact that the DOX is directly conjugated without any spacer forming amide bond with the aspartic acid residue of the block co-polymer which is a very stable bond for the further cleavage. Further by more exploration, it shows the more providence of pharmacological active free DOX. Therefore for the purpose of the anticancer activity assay, the block co-polymer acts as the carrier molecule not as active ingredient [35].
In this study, they showed tumor-selective delivery and enhanced in vivo anti-tumor activity. Physically entrapped DOX in the polymeric micelles was showing nine times more activity than the naïve DOX, whereas the accumulation of the micellar DOX (physically entrapped) in normal organs and tissues was lesser than or the same as the accumulation of free DOX. This study stated that the increment in the pharmacokinetic behavior of the micelles with DOX. The point of notice is that after 1 h of intravenous administration, it was found that the amount of DOX-entrapped micelle was much more than the free DOX. The results showed the effectivity and importance of the EPR effect in drug targeting strategies [35].

The third and main study reported by this group was biological activity of polymeric micelle as a carrier regarding the accelerated blood clearance (ABC) phenomenon. ABC is a process where clearance rate of the carrier system is substantially increases with the rapid injection. It is very well studied with the liposome coated with PEG, having long circulating phenomenon at the first injection. PEG-coated liposome when injected in appropriate first dose and second dose of the same liposome at appropriate interval (5–7 days). This activity resulted in the immunological activity caused by the first dose and the clearance is considerable. They reported that ABC induction developed at mainly two organs such as the liver and spleen. Both organs are most relative organ to the MPS. So rheostat uptake at the MPS might be an important aspect of ABC induction [35]. It is also reported that the phenomenon of the ABC is blocked when the cytotoxic drug was carried by the PEG-coated liposomes trials on experimental animal model or the clinical trials on human. So cytotoxicity plays a greater role in the ABC phenomenon, and the use of less cytotoxic drug is a new direction of therapy suggested by this report.

### 7.2 Kabanov’s Report

Kabanov et al. (1997) reported their study with the unique polymer. In this system at specific site, at the place of carrier, the block co-polymer worked as a biologically active agent. A polymer chain was used, such as poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide); it is also known as ABA type of polymer. They reported that pluronic polymers explicitly inhibited the production of adenosine triphosphate (ATP) in mitochondria p-glycoprotein [108, 109], which showed a significant role in multidrug resistant cancer cells and the efflux action of anticancer drug in an ATP-dependent manner. Thus, the drug efflux action was inhibited by pluronic polymer over the inhibition of ATP production by the help of such block co-polymer successful avoidance of the multidrug resistance both in vitro and in vivo [108]. It was an unexpected result in cancer therapy by block co-polymer as pluronic polymers deficient with the charge containing the functional group, and it was assumed that strong interaction with the protein. They also reported that the appropriate chain lengths with good hydrophilic/hydrophobic balance with the pluronic polymers. Further many more researchers did not showed the inhibition of the P-glycoprotein with synthetic polymer. It was limited with the polymer, but the matter of concern was biological activity, mainly anticancer activity. Because the
inhibition of mitochondrion and P-glycoprotein by the carrier system was a major concern of toxicity, Kabanov et al. reported that the enhanced in vivo anticancer activity with animal model and the confirmation of this enhanced activity were noticed with the in vitro cytotoxicity study (with presence and absence of polymers). There was an advisable step to the researcher to examine and explore more P-glycoprotein in much more detail, if researchers observe that the anticancer drug in combination with the polymer showing more effectivity [108].

8 Applications

8.1 Pharmaceutical Application

Polymeric micelles are mainly planned to show fundamental parameters in drug depiction; the first one is solubilization of hydrophobic drugs, and the second one is the controlled or sustained release of a drug.

8.1.1 Solubilization of Hydrophobic Drug

It is expected that 90 percent of the chemical entity of the drug is hydrophobic in nature, and they mainly belong to the Biopharmaceutics Classification System (BCS) II and IV [110, 111]. The major problem with these classes of drugs is the very low bioavailability due to the low solubility profile, although it has potential pharmacodynamics profile. So they are not effective due to low bioavailability. Conventional way of increasing solubility by solubilizing agent shows the toxicity behavior as well [60]. In this regard, PMs showed very effective behavior toward the dissolution rate and the increasing of solubility profile. They provide the hydrophobic environment to the drug so that the drug can incorporate into micelles according to its favorable environment, ionic interaction, and chemical conjugation. Due to its solubility, it may increase from 10 to 8400 fold, that is, a very beneficial application of polymeric micelles [112].

8.1.2 Sustained Release of Drug

Polymeric micelles are found to be more stable profile with lower CMC when it is compared to low molecular weight surfactant micelles. They show good retention of drug for longer circulating time period within the systemic circulation, and their dissociation is also slow. Due to this characteristic feature, PMs can reach at tumor target site with higher accumulation in large amount [113]. For the sustained release of drug, there should be specificity into the micelles, i.e., it should be stable to dilution due to low CMC value, good viscosity, and chain mobility behavior that is lower at core [114]. The novel drug delivery system enhances the bioavailability shielding of the loaded drug from the cruel atmosphere of the gastrointestinal tract (GIT) [115], so that they provide the safe conveyance of drug and pH-sensitive micelles to specific target site of delivery. They have adhesive properties with mucous so the residence time in gut is increase. One of the important applications
of the PMs is increasing absorbance in GIT due to the smaller size and high membrane permeability [116].

8.1.3 Delivery of Genes

Recently, the progress in therapy is enhanced by the biological mechanism driving life process at molecular state, and it provokes the innovation in the novel nucleic acid-based therapies such as DNA and siRNA as modern medicines. But the clinical handling and application are hindered due to its long molecular weight, anionic nature, and lower stability on physiological conditions. There is one more hurdle associated with it, lower cellular uptake. If DNA or RNA is directly put into the blood stream, then it will be eliminated very quickly due to obstacle attack of the DNase and RNase. These problems may be eradicated by the incorporation of the DNA and RNA into the nanocarrier. For the intracellular delivery to the nucleus in gene therapy, accumulation at the specific site of tissue is needed, and it is achieved by the use of the vectors like retroviruses and adenoviruses which are very common carriers in the section of gene therapy in clinical trials [117, 118].

On the other section, it is seen that the non-viral vectors having the active polymer constituent are more effective, and they are very good and reliable alternative on the aspect of safety, are cheaper, and also may be prepared in larger amount [119–125]. Due to the advantages, polymeric micelles are prominent for the delivery of the nucleic acid by forming the polion complex with the anionic nature of the DNA and RNA. Block copolymer having cationic charge so the complex is found to be more stable and the plasmid DNA (pDNA) associated with a PEG-polycation such as PEG-poly(lysine) (PEG-PLys) giving polion complex micelle (PIC micelle or polyplex micelle) having very good size range of 100 nm. Therefore the zeta potential of the complex was natural. Further the complex having pDNA exhibits the better introduction of gene into the cultured cells. The more advanced achievement was that pDNA was degraded rapidly by the nuclease, but due to incorporation into the polymeric micelles, it was found effective up to 3 h; it is much more than the necked insertion of pDNA into the blood circulation [124].

8.2 Biomedical Application

Biomedical application of PMs is in photodynamic therapy for treatment of different disease mainly for macular degradation and tumors. It is also having active involvement in administration of photosensitizer [126]. They are observed as a means of transportation for photosensitizer to avoid side effect like hypersensitivity and work for the efficacy and selectivity of the photodynamic therapy. One of the study reported by the Guo et al. that, photosensitizer loaded PMs integrating cyanine dye for the accurate anatomical tumor localization through near-infrared fluorescent imaging and side by side cancer therapy through consecutive synergistic photothermal therapy. In this series PMs are also used for various types of medical imaging techniques such as magnetic resonance imaging (MRI), ultrasonography, and X-ray computed tomography and for visual optimization on delivery of drug
incorporated in micelles. In the above imaging technique, micelles have role as a contrast agent [127]. In recent years it is confirmed through many studies that micelles accomplished a good biodistribution with longer retention of carbocyanine dye within the tumor, and due to this retention, the increase in the near-infrared fluorescence is observed by long duration of imaging in both hypovascular tumor and hypervascular tumor [128]. Polymeric micelles are nowadays used in both of the purposes such as high-contrast cancer imaging and superior photothermal therapy. It also triggered photothermal damage to cancer cell by organelle destabilization and this causes tumor necrosis to photo-irradiation [129]. In X-ray computed tomography technique for high resolution, iodine is absorbed by X-ray as a contrast agent. Torchilin et al. reported that PLL-PEG polymeric micelles containing iodine have average diameter 80 nm and having iodine 34% w/w used for the computed tomography. In another extent in this series, the above compound was administered through tail vein in rat and enhancement in blood pool spleen and heart for 3 h, and this technique was used for computation of the biodistribution of DNA micellar nanoparticle and pharmacokinetic activity [130].

9 Conclusion and Future Prospects

Polymeric micelles are the novel and cogent carriers for anticancer drug delivery. This new and versatile approach allows encapsulation of hydrophobic drugs, sustained drug release, targeted tumor site action, enhancing pharmacokinetic properties, reducing side effects, and effective tumor accumulation. Polymeric micelles have demonstrated effective and versatile delivery carrier for anticancer drugs. This approaches for drug delivery is very prominent and successful. The new block copolymers and new core-forming blocks give the newer occasion to integrate new molecules with suitable compatibility and solubilization property. They can help to provide a new versatile carrier for least soluble drugs to more toxic drugs in a universal formulation which will be in lower risk factor such as toxicities and instability problem.

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Stimuli-Responsive Polymers

Emily T. Baldwin and Laura A. Wells

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Abstract
Stimuli-responsive polymers undergo a change in their physical properties in response to a given physical or biological stimulus. In biomedical applications, a physician could use an external stimulus, such as light, to promote a change in a device. Alternatively, the microenvironment surrounding an implant could provide a natural, internal stimulus, such as a change in pH or the increased concentration of an enzyme, to promote changes in a device. This chapter focuses on the biomedical applications of polymers that respond to the physical stimuli temperature, pH, and light and polymers that respond to the biological stimuli glucose, enzymes, and other proteins.
1 Applications of Stimuli-Responsive Polymers

After implantation, the biological microenvironment surrounding biomedical devices will change over time due to the healing response (host responses) surrounding the device. Changes will continue throughout the lifetime of implanted devices as the host ages or as diseases progress. To allow medical devices to adapt to these ongoing changes in vivo, materials may be designed to change in response to physical or biological stimuli allowing them to adapt in response to changes in their microenvironment or be changed externally by physicians. As outlined in Fig. 1, both physical and biological stimuli may be used to alter the properties of polymer devices. Polymers may be designed to change in response to many different physical and biological stimuli – this chapter will focus on temperature, pH, light, glucose, enzymes, and other proteins.

Stimuli may cause a variety of changes in polymer systems to promote gelation of injection solutions, to trigger drug release, or to be used as sensors, to name a few (Table 1). Polymers may respond directly to stimuli or are modified with functional groups that change in response to stimuli causing the bulk or surface properties of a polymer to change.

Fig. 1 Different stimuli may be used to cause changes in the chemical properties of polymers resulting in physical changes that may be harnessed for biomedical applications ranging from injectable scaffolds for tissue engineering to on-demand delivery of therapeutics.
Temperature, pH, and light are examples of physical stimuli that may be introduced during device implantation or after implantation. Temperature is typically changed during material/medical device implantation from cool temperatures to body temperature, pH varies from tissue to tissue and within cells, and light can be introduced externally by physicians during implantation or after implantation (through light-permeable tissues).

### Table 1  An overview of how physical changes in polymer systems are used in various biomedical applications

<table>
<thead>
<tr>
<th>Stimuli causes . .</th>
<th>Result</th>
<th>Example applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosslinking</td>
<td>De-crosslinking</td>
<td>Gelling, degradation, capsule</td>
</tr>
<tr>
<td></td>
<td></td>
<td>breaking, membrane dissolving</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerization</td>
<td>Depolymerization</td>
<td>Polymer formation, degradation of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>synthetic polymers into small</td>
</tr>
<tr>
<td></td>
<td></td>
<td>oligomers</td>
</tr>
<tr>
<td>Solubilization</td>
<td>Precipitation (de-solubilization)</td>
<td>Dissolution of polymers, such as</td>
</tr>
<tr>
<td></td>
<td></td>
<td>the shell of a microsphere</td>
</tr>
<tr>
<td>Changes in charge or</td>
<td>Hydrophilic/hydrophobic</td>
<td>Swelling, shrinking</td>
</tr>
<tr>
<td>arrangements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color change</td>
<td>No color change</td>
<td>Switch in macromolecule bound to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>polymer</td>
</tr>
</tbody>
</table>

### 2  Physical Stimuli

Temperature, pH, and light are examples of physical stimuli that may be introduced during device implantation or after implantation. Temperature is typically changed during material/medical device implantation from cool temperatures to body temperature, pH varies from tissue to tissue and within cells, and light can be introduced externally by physicians during implantation or after implantation (through light-permeable tissues).

#### 2.1  Temperature

One of the most common stimuli to promote changes in polymer properties is temperature. The temperature of polymer devices may be controlled during implantation or during the injection of liquid from a low or high temperature to body temperature, 37 °C. Commonly, temperature is used to allow for the injection of liquid polymer followed by its gelation in situ for applications varying from drug and cell delivery to noninvasive techniques to deliver scaffolds or cosmetic fillers.

Temperature-induced volume phase transitions of polymers allow liquids to precipitate into solids or gels due to changes in their hydrophobicity (triggered by changes in temperature), typically across a threshold that is dependent on their polymer composition. Polymers that have lower critical solution temperature (LCST) are a liquid below the LCST and precipitate above the LCST. Other polymers have an upper critical solution temperature (UCST) and will precipitate below...
the UCST. These volume phase transitions are due to changes in the hydration state of the polymer due to a shift in the hydrogen bonding of the polymer molecules and the dissolution versus the ordered state of water at the polymer \[1\]. Cooled polymers that gel above an LCST between 4 °C and 37 °C are popular in biomedical applications. Unfortunately, polymers heated to a UCST above 37 °C could cause damage to tissue before they cool to body temperature and are therefore not commonly used in biomedical applications.

### 2.1.1 Synthetic Thermoresponsive Polymers

Synthetic polymers give the opportunity to tailor their LCST according to monomer composition and can be composed of a variety of monomers, shown in Fig. 2. While there are many different thermoresponsive polymers, a select few will be focused on in this chapter. Poly(N-alkylacrylamide)s include the popular poly(N-isopropylacrylamide) (PNIPAM) as well as poly(N,N-diethylacrylamide) (PDEAM), which is less popular because its LCST is dependent on tacticity which adds complexities during synthesis \[2\]. PNIPAM is a very popular polymer for thermoresponsive biomedical applications because it is simple to synthesize through the free radical polymerization of isopropylacrylamide and because its LCST is 32 °C in aqueous solution and can be easily increased through the addition of hydrophilic monomers or decreased with hydrophobic monomers \[3\]. Furthermore, additional monomers can add functional groups for the immobilization of biomolecules.

A wide range of biomedical applications have been explored for PNIPAM, ranging from tissue engineering to controlled release. Cell sheet engineering is

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**Fig. 2** Select range of different thermosensitive polymers that have LCST. PNIPAM poly(N-isopropylacrylamide), PDEAM poly(N,N-diethylacrylamide), PMVE poly(methyl vinyl ether), PVCa poly(N-vinyl caprolactam), PETOx poly(N-ethyl oxazoline), P(GVGVP) pentapeptide GVGVP. (Reprinted from \[1\] with permission from Elsevier)
enabled by growing cells on coatings of PNIPAM and then using low temperature to release them (as sheets) from growth surfaces allowing for their transfer to in vivo systems [4, 5]. Alternatively, temperature can be used to form microgels to encapsulate drugs or insulin to protect and then release them in a controlled manner [6, 7]. PNIPAM can also be injected as a liquid (polymers) and gelled in situ due to the increase in temperature to 37 °C (above the LCST, for most animal and in vitro applications) which is advantageous for tissues and organs that are difficult to access. For example, PNIPAM-grafted collagen is a focus for injectable cell scaffolds to deliver retinal pigment epithelial cells to the retina in the posterior segment of the eye [8].

Introducing degradable crosslinks or monomers that solubilize gelled PNIPAM over short time frames is a strategy used to enable the degradation and clearance of PNIPAM used in in vivo biomedical applications. For example, the incorporation of hydrazone crosslinks [9] or caprolactone-based crosslinks [10] that undergo hydrolysis will enable the solubilization of PNIPAM networks over time. Chain solubility may be altered over time by copolymerizing with dimethyl-gamma-butyrolactone which undergoes lactone ring opening to promote the solubilization of a PNIPAM gel to enable clearance from implant sites [11].

Polyethylene glycol (PEG) is popular among biomedical applications for its low toxicity and ability to decrease non-specific protein adsorption when grafted to polymer surfaces. In addition to these interesting features, PEG methacrylate hydrogels have decreased swelling at lower temperatures. Increases in the molecular weight of the PEG chains result in a further decrease in the temperature at which swelling decreases [12].

Poloxamers, whose proprietary name is Pluronic® and are triblocks of poly(ethylene oxide) and poly(propylene oxide), are liquid polymers whose LCST varies according to the chain lengths of the blocks. For example, Poloxamer 407 (Pluronic F127) is a nontoxic liquid at low concentrations with an LCST at 4–5 °C and can form reversible micelles to entrap and release hydrophobic drugs or also be formulated to gel at 37 °C when at high concentrations of 18 w/v% [13, 14].

2.1.2 Natural Thermoresponsive Polymers

Naturally derived polymers can undergo a phase shift due to the presence of different ionic groups along their backbone. Many polysaccharides from both animals and plants exhibit a transition temperature that may be altered with chemical modification of the polysaccharide or by mixing the polysaccharide with a second polymer. Furthermore, many are biodegradable because of naturally occurring enzymes.

Cellulose derivatives have a higher LCST than PNIPAM, which may be adjusted by altering the chemistry of the cellulose. For example, solutions of methylcellulose undergo a phase transition between 40 °C and 50 °C, which can be lowered through modifications [15]. The flow properties of cellulose solutions are often improved through the addition of additives, such as surfactants, which can be toxic, or other polymers. For example, methylcellulose has been mixed with hyaluronic acid to develop an injectable material that gels at 37 °C. The resulting hydrogel have extended the delivery of proteins [16] and the delivery and survival of cells for spinal cord repair [17].
2.2 pH

Differences in the pH of different organs, tissues, organelles, or diseased tissues is a stimulus often used to trigger drug delivery from liposomes, micelles, and polymersomes [18]. As shown in Table 2, the pH of different tissues or cellular compartments varies greatly. These differences leave opportunities to design devices that change as they travel through or to locations with variable pHs, such as into the low pH environment of a tumor, or that change during infections (to low pH). Charged groups or acid-labile bonds in polymers enable them to change their properties in response to changes in pH.

### 2.2.1 Charged Polymers

Natural and synthetic polymers with charged groups (amine, carboxylic acid) undergo changes in protonation under different pHs. At physiological pH, acidic groups are deprotonated but then protonate at low pH which causes a change in charge of the groups, changing the overall charge of the polymer chains and also the swelling/collapse of crosslinked networks. The ionic to nonionic transitions are due to the gaining or loss of a proton indicative of the pKa of the respective polymers. Under higher pHs, anionic and cationic polymers will become hydrophobic, as a function of their pKa. Common groups on monomer chains that effect these changes are amino, carboxyl, sulfonates, or imidazolyl groups [18]. Commonly used charged polymers are shown in Fig. 3.

Anionic pH-sensitive polymers often have carboxylic groups that introduce a charge and include synthetic polymers, such as poly(acrylic acid), poly(glycolic acid), or poly (methacrylic acid), or natural polymers such as alginate. Carboxylic acid groups are deprotonated at physiological pH (7.4) allowing these polymers to dissolve in aqueous solutions but deprotonate at acidic pHs, which makes them insoluble which can allow for changes in polymer networks, including mesh size. The change in charge can also alter how local proteins or encapsulated drugs interact with the polymers themselves.

Methacrylic acid grafted with ethylene glycol (PMAAgEG) can deliver proteins specifically to the upper small intestine because it has a pH higher than the

<table>
<thead>
<tr>
<th>Location</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>7.34–7.45</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.0–3.0</td>
</tr>
<tr>
<td>Upper small intestine</td>
<td>4.8–8.2</td>
</tr>
<tr>
<td>Colon</td>
<td>7.0–7.5</td>
</tr>
<tr>
<td>Tumor, extracellular</td>
<td>7.2–6.5</td>
</tr>
<tr>
<td>Early endosome</td>
<td>6.0–6.5</td>
</tr>
<tr>
<td>Late endosome</td>
<td>4.5–5.0</td>
</tr>
<tr>
<td>Vagina</td>
<td>3.8–4.5</td>
</tr>
<tr>
<td>Inflamed tissue/wound</td>
<td>5.4–7.4</td>
</tr>
</tbody>
</table>

(Reprinted from [1, 12], with permission from Elsevier)
stomach. When crosslinked and at a pH below 4.8, PMAAgEG forms hydrogels that have a collapsed network with a small mesh size of 70 Å, and at pH above 4.8, they form a loose network with an increased mesh size of 210 Å [19]. Therefore, encapsulated proteins are entrapped and protected at low pHs of the stomach (pH = 1–3) and released at the increased pHs of the small intestine (pH >6). Furthermore, the upper small intestine has a high permeability that allows for the absorption of proteins. Insulin encapsulated into microparticles of PMAAgEG that are delivered into the gastrointestinal tract of Sprague-Dawley rats enabled the delivery of insulin into the upper intestine and its subsequent absorption into the bloodstream [20, 21].

Drug release may be controlled by the local charge of polymers. For example, nanoparticles made of terpolymers of poly(methacrylic acid), Polysorbate 80, and starch can encapsulate doxorubicin and release it into the acidic environment of tumors. Doxorubicin is positively charged and can associate with negatively charged (deprotonated) carboxyl groups on the methacrylic acid polymer at neutral pHs. If the local pH decreases below 6, then the carboxyl groups are protonated, losing their charge, and doxorubicin will be released [22].

Cationic pH-sensitive polymers often contain amine groups and include synthetic polymers such as poly($N,N'$-diethylaminoethyl methacrylate) (PDEAEM) or natural polymers such as chitosan. Amine groups are protonated at physiological pH (7.4) and donate protons below their pKa. The changes in solubility are exploited to enable the dissolution of polymers at low pHs, such as those found in the stomach, tumors, or the lysosomes (in cells). For example, chitosan is a commonly used cationic polymer because it is inexpensive and easy to process and has a pKa of 6.4. When used as a coating on tablets for oral delivery, chitosan protects the tablet in the neutral pH of the mouth (saliva) and then dissolves in the low pH of the stomach to enable the dissolution of the tablet in that targeted region.

Synthetic polymers composed of amine-containing monomers, such as PDEAEM or poly($N,N''$-dimethylaminoethyl methacrylate) (PDMAEM), can protect therapeutics until delivered to sites-of-action that have low pHs, such as tumors or intracellular compartments. For example, small interfering RNA are potential therapeutics to
prevent the transcription of key genes in the cytoplasm of tumor cells and other cells. For siRNA to properly function, it is crucial to protect siRNA until it is delivered to the cytoplasm of cells. PDEAEM used as coatings surrounding silica particles with siRNA successfully encapsulates and protects the siRNA until the nanoparticles enter the tumor cell. Once the particles enter the endosome (pH = 6–6.5), the PDEAEM becomes water soluble, releasing the siRNA into the cytoplasm. This method successfully decreased the expression of CXCR4 in a human breast cancer cell line [23].

### 2.2.2 Acid-Labile Polymers
Polymers that have acid-labile groups undergo hydrolysis at low pHs which can result in the degradation of gels or detachment of side-groups or grafts. For example, acid-labile groups have been used to detach PEG from polymers forming micelles to change their stealth, to detach other groups functionalized onto other chains to allow their release, or to degrade microspheres/gels through the cleavage of crosslinks. Fast acid-labile groups include hydrazones and slower groups include amide, ether, and ortho esters.

Hydrazone links are commonly used to introduce pH-labile bonds into polymers because they are very stable at physiological pH but rapidly cleave at acidic pHs [24]. Hydrazone is used to link drugs to polymer backbones, to conjugate polymers forming micelles, or to crosslink hydrogel networks, for example. There are numerous examples of hydrazones used to conjugate drugs to polymers to enable their release under acidic conditions, and these are well-summarized in various reviews [24]. For example, N-2-(hydroxypropyl)methacrylamide-drug conjugates allow for increased stability of drugs followed by their release in low pH environments such as intracellular compartments or tumors [25]. Similar strategies have been used to make PEG-conjugated proteins that release proteins at tumor sites [26].

Other links also have their own unique advantages. At low pH, polyacetals degrade into nontoxic alcohols and aldehydes which are considered nontoxic. Ortho esters (three alkoxy groups attached to one carbon) hydrolyze in mildly acidic conditions to produce esters, with further decreases in pH resulting in increases in the rates of hydrolysis [27, 28]. Ortho esters used to link block copolymers of hydrophilic PEG and hydrophobic polymethacrylates form micelles that undergo hydrolysis (resulting in solubilization of the micelles) in the low pH of tumor cells to release drugs into glioma cells [27].

### 2.3 Light
Light is a noninvasive stimulus for externally modifying the properties of polymer devices for applications in areas that are easily accessible to light, such as eyes and skin, or that may be exposed to light using a scope, such as the gastrointestinal tract. Light is easily introduced through lamps or lasers, and because molecules absorb very specific wavelengths of light, systems can be designed to respond to specific wavelengths (and to not respond to other wavelengths). The free radical polymerization of polymers is often initiated by light but is not the focus of this chapter, and
information is found in many reviews [29]. Photodegradable groups may be incorporated in polymer chains or crosslinks and used to enable the degradation of the polymer into smaller chains or the crosslinking/de-crosslinking of polymer networks; this is not reversible and is also not the focus of this chapter, and information is in reviews elsewhere [30, 31].

Reversible light-initiated changes in polymers are desirable to give external or internal control of device properties after implantation. When a molecule absorbs light, the absorbed energy is transformed by the molecule resulting in a myriad of possible outcomes such as fluorescence, luminescence, heat, degradation, intermolecular reactions, or intramolecular reactions (dependent on the molecule’s chemistry). Polymers modified with photochromic molecules that undergo inter- or intramolecular changes, such as photoisomerization or photodimerization, undergo property changes in response to different wavelengths of light. Molecules that dimerize and de-dimerize with specific wavelengths of light or that undergo isomerization with specific wavelengths of light are shown in Fig. 4.

2.3.1 Photodimerizing Systems

Polymers modified with molecules that reversibly dimerize can reversibly crosslink or reversibly bind polymer chains together. Photodimerizing molecules include anthracene that undergoes 4Pi + 4Pi cycloaddition with exposure to wavelengths above 300 nm and dissociates with wavelengths under 300 nm [32–35], as well as nitrocinnamate, coumarin, and cinnamylidene acetate which undergo reversible 2Pi + 2Pi cycloaddition under the same conditions.

The crosslinking of polymer networks with photodimerizing molecules can change the crosslinking density of hydrogels and the diffusion and release of macromolecules encapsulated within hydrogels. For example, alginate and hyaluronic acid functionalized with PEG-bound anthracene were shown to have increased crosslinking density with 365 nm light exposure, and the release rate of encapsulated protein (lysozyme and others) or small molecules (Coomassie Blue and others) into phosphate buffered saline (PBS) could be decreased with 365 nm light and with small increases shown with 254 nm laser exposures [36–38] (Fig. 5). Hydrogels made of anthracene-capped eight-armed PEG also show reversible swelling with 365 nm and 254 nm light exposures [39]. However, both of these systems showed minimal reversible swelling (increased swelling) with 254 nm of laser exposure. Hydrophobic systems made of polystyrene capped with anthracene have shown very reversible dimerization with 336 nm and 228 nm exposures. The ends resulted in chain extension or dissociation [40]. Elastomers of anthracene functionalized carboxylated nitrile butadiene rubber [41] as well as polynorbornene with pendant anthracene groups [42] produce films with reversible photo-induced crosslinking.

Cinnamylidene acetate-capped four-armed PEG forms membranes with reversible crosslinking properties and gels that also photoimmobilize other cinnamylidene acetate-modified molecules [43–46]. Nitrocinnamate-capped eight-armed PEG hydrogels also have photoreversible crosslinking which proved to be highly efficient and reversible when measured mechanically [47, 48]. When these gels encapsulated βFGF, they demonstrated slower release of βFGF from the gels when exposed to...
365 nm light [49]. Nitrocinnamate-modified gelatin has also been synthesized and then produced as microgels by photocrosslinking within reversible micelles [50] with reversible topographic changes with light exposures [51].

2.3.2 Photoisomerizing Systems

Azobenzene and spirobenzopyran both undergo trans-to-cis isomerization with UV light and cis-to-trans with visible light or heat. While both have been used to introduce photoreversible property changes in polymers, azobenzene has been the larger focus because its isomerization results in a change in its hydrophobicity, length, and ability to complex with β-cyclodextrin.

The hydrophobicity of the trans-form of azobenzene can be used to induce crosslinking of hydrophilic polymers. Copolymers of trans-4-methacryloyl
oxyazobenzene with $N,N$-dimethyl acrylamide form gels due to hydrophobic domains formed by the trans-azobenzene groups, which may be reversed with light [52]. Surface hydrophobicity may also be altered by UV-treating azobenzene that has been immobilized onto different inorganic materials, typically by self-assembly or adsorption with most applications in sensors or optical memory [53, 54].

The change in the length of azobenzene between 9.0 Å (trans) and 5.5 Å (cis) can also be used to induce stimuli-responsive changes in the stiffness of crosslinked hydrogels as a tool to study cell growth on substrates with different moduli. For example, 6-arm PEG crosslinked with azobenzene undergoes reversible changes in mechanical stiffness (modulus) with 365 nm and 254 nm light exposures [55]. This is presumed to be due to changes in the length of the azobenzene crosslinks, as shown in Fig. 6.

### 3 Biological Stimuli

Bioresponsive polymers are polymers that can change in response to biological stimuli in their local microenvironment (after implantation). Properties such as degradation, sol-to-gel transitions, or swelling of hydrogels can change in the presence of a variety of biological molecules such as proteins and large macromolecules or even whole cells [56]. Bioreponsive polymers have been used in applications such as drug delivery systems, tissue engineering, and bio-sensing. The three main classes of bioreponsive polymers that will be discussed are glucose-sensitive polymers, enzymatically degradable polymers, and aptamer-functionalized polymers.

#### 3.1 Glucose-Sensitive Polymers

Glucose-sensitive polymers are materials which change in response to free glucose in their environment. Glucose levels in a patient’s blood increase shortly after eating,
Azobenzene-based monomers (AMPB) incorporated into PEG hydrogels results in light-initiated changes in the distance between crosslinks and modulus. (Reprinted with permission from [55]. Copyright © 2015 American Chemical Society)
and insulin is secreted by the pancreas in a tightly regulated process to respond to this change in blood glucose levels. In diabetic patients, the pancreas doesn’t produce enough insulin, and therefore blood glucose levels need to be externally regulated by injected insulin [57]. Glucose-responsive medical devices (materials) that release insulin in response to higher glucose levels are under development to reduce monitoring and to ease the burden of patients.

Glucose-sensitive materials are commonly hydrogels and are used to form self-regulating, closed-loop feedback insulin release systems based on blood glucose levels for the treatment of insulin-dependent diabetes. Polymers that form these release devices have two main requirements: the ability to sense the concentration of glucose and to release encapsulated insulin in response to changes in blood glucose levels, as seen in Fig. 7. Glucose-sensitive polymers can recognize glucose by various mechanisms such as recognition by glucose oxidase, recognition by a glucose-binding protein, and recognition by phenylboronic acid [57].

3.1.1 Glucose Recognition by Glucose Oxidase

The enzyme glucose oxidase (GOx) is commonly used to introduce glucose sensing. It converts glucose to gluconic acid and hydrogen peroxide (Fig. 8) which causes a change in local pH that can drive changes in the properties of pH-sensitive polymers [58].

GOx, insulin, and catalase are typically incorporated into a pH-sensitive hydrogel matrix, such as copolymers of diethylenoaminoethyl methacrylate (DEAEM) and poly (ethylene glycol) monomethacrylate (PEGMA) and N,N-dimethyl acrylamide (DMAAm) and sucrose. Nearby glucose diffuses into the hydrogel, and the GOx catalyzes the reaction converting glucose into gluconic acid (Fig. 8). The newly formed acid decreases the pH within the hydrogel which then causes it to swell, resulting in the increase of the mesh size of the hydrogel and the release of insulin. Oxygen availability in this reaction is the limiting factor due to oxygen’s poor solubility in aqueous solutions, and therefore catalase is often incorporated to catalyze the decomposition of hydrogen peroxide to oxygen and water [57]. GOx was entrapped in copolymers of poly(2-hydroxyethyl methacrylate-co-\(N,N\)-dimethylaminoethyl methacrylate) (poly (HEMA-co-DMAEMA) to form glucose-sensitive hydrogels that had higher
swelling and faster insulin release rates in response to higher glucose concentrations (Fig. 9) [59, 60].

GOx has also been immobilized into copolymers of diethylaminoethyl methacrylate (DEAEM) and poly(ethylene glycol) methacrylate (PEGMA) crosslinked with tetra(ethylene glycol)dimethacrylate (TEGDMA). The resulting PDEAEM-g-EG gels showed strong pH-dependent swelling behavior in response to the increase in glucose concentration in their surroundings [61]. These gels also showed pulsatile, reversible swelling behavior when the pH in their microenvironment returned to the original value. This is important for glucose-responsive materials as the gels need to collapse to their original state once insulin release is no longer desired, i.e., when the levels of glucose decrease [61]. Other glucose-responsive polymers have been produced by copolymerizing \( N,N \)-dimethyl acrylamide (DMAAm) and sucrose with GOx and catalase, for example. Similar to the results obtained in the other studies, it was seen that the hydrogel underwent reversible swelling proportional to the concentration of glucose in its microenvironment [62].

3.1.2 Glucose Recognition by Glucose-Binding Proteins

Concanavalin A (Con A) is a protein in the saccharide-binding lectin family that has been commonly used in polymer glucose recognition systems because it is able to reversibly bind glucose [63]. Con A is a tetramer with four binding sites for glucose and can cause the crosslinking of polymers modified with glucose along their backbone. The Con A binds to the immobilized glucose (bound to the polymers) causing crosslinking of the polymers. When the resulting hydrogel is in a microenvironment containing free glucose molecules, these glucose molecules diffuse into the hydrogel and compete with the glucose molecules within the gel for binding to the Con A tetramer. Glucose binding to Con A causes de-crosslinking of the hydrogel. This is a reversible gel-to-sol phase transition. When glucose is no longer available and binding to Con A, the hydrogel re-crosslinks.

This glucose-dependent gel-to-sol transition has been used to encapsulate and release insulin. For example, glucosyloxyethyl acrylated chitosan (GEA-chitosan) microgels mixed with insulin and Con A form gels that entrap insulin and can release insulin in the presence of glucose with reversible release kinetics dependent on glucose concentrations [64]. Copolymers composed of allyl glucose and acrylamide have also shown similar transitions when mix with Con A and incubated with insulin [65].

![Fig. 8](image_url) The conversion of glucose to gluconic acid and hydrogen peroxide by glucose oxidase
Fig. 9 Glucose-dependent insulin delivery system using poly(HEMA-co-DMAEMA) hydrogels: (a) unswollen gel, (b) swollen matrix in the presence of glucose. (Reprinted from [59] with permission from Elsevier)
3.1.3 Glucose Recognition by Phenylboronic Acid
Phenylboronic acid (PBA) and its derivatives are grafted onto polymers to introduce glucose-responsive properties into materials. In water, PBA compounds exist in equilibrium between two forms – an uncharged trigonal form and an anionic tetrahedral form [66] – as seen in Fig. 10. The anionic form is able to covalently, reversibly bind glucose resulting in a shift in the equilibrium toward the charged form of PBA, resulting in swelling of pH-sensitive hydrogels.

For glucose-sensing materials, PBA is covalently grafted onto polymers, and in the presence of glucose, the anionic form of PBA compounds can form a stable complex with glucose [67] increasing the proportion of the charged form of PBA. With charge-sensitive polymers, this can induce reversible volume changes according to changes in the environmental glucose concentration [68]. Poly(N-isopropylacrylamide) (PNIPAM) has been used in the design of gels by the covalent attachment of PBA and its derivatives for the purpose of glucose sensing. Promising results from these systems have shown on-off insulin release in response to changing glucose concentrations in the gel’s microenvironment [69].

3.2 Enzymatic Degradation
Enzymes are present in different concentrations in different tissue states (tumor, healing, resting) and can be used as stimuli to induce changes in polymers at those sites. Many natural and synthetic polymers are degraded by enzymes, and polymers can also have enzyme-degradable peptides incorporated within their polymer chains or as crosslinks.

The rate of degradation of these polymers can be controlled through various factors. Firstly, the rate of enzymatic degradation in vivo will vary with its location (area of the body it is implanted in) due to the presence of specific enzymes and their concentration at this site [70] or its states (infection, healing). In addition to this, polymer characteristics such as their composition, molecular weight, surface properties, degree of crystallinity, and crosslinking can alter the rate of enzymatic degradation [71–73].

**Fig. 10** Equilibrium forms of phenylboronic acid. (Reprinted with permission from [66]. Copyright © 2003 American Chemical Society)
3.2.1 Enzyme-Degradable Polymers

Natural polymers have been exploited in applications such as drug delivery and tissue engineering due to their intrinsic characteristics, such as cytocompatibility or high water content, and because many will degrade due to enzymes present in vivo. Some examples of natural polymers that undergo enzymatic degradation are hyaluronic acid, chondroitin sulfate, chitosan, and alginate. Furthermore, some synthetic polymers can also undergo enzymatic degradation. Table 3 outlines some commonly used polymers and their respective key enzymes that degrade them.

The enzymatic degradation of polymers can be tailored by making copolymers or polymer blends that combine the characteristics of different polymers. For example, the enzymatic degradation in varying blend compositions of poly(lactic acid) (PLA) and poly(vinyl acetate) (PVA) was compared to the enzymatic degradation of PLA alone in solution containing proteinase K, a serine protease that has been known to degrade PLA. As the ratio of PVA in the blend increased, the degradation rate significantly decreased [74]. This decrease in degradation rate in PLA upon introduction of PVA could be exploited in biomedical applications where a slower or more tailored degradation rate is desirable.

3.2.2 Enzyme-Cleavable Peptides

The biodegradation of polymers can be altered by the integration of enzyme-cleavable peptides. These polymer-peptide hybrids are applied as drug delivery vehicles or tissue engineering scaffolds. They also present the opportunity for selective degradation by incorporating peptides that are cleavable by enzymes present in specific pathologies or microenvironments.

The incorporation of matrix metalloproteinases (MMP)-cleavable peptides with polymers is of particular interest because MMP-2 and MMP-9 are normally present in low levels in healthy tissues but have been shown to have increased expression in tumors [75]. Therefore, polymer-peptide conjugates can be produced for targeted treatment of tumors to deliver encapsulated drug in the presence of MMPs. For example, the peptide sequence PVGLIG, which is selectively cleavable by MMP-2 and MMP-9, was conjugated to poly(trimethylene carbonate) (PTMC) (by UV-initiated thiol-ene “click” chemistry) and then self-assembled into

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Key enzyme(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(ethylene glycol)</td>
<td>Dehydrogenase</td>
</tr>
<tr>
<td>Poly(propylene glycol)</td>
<td>Dehydrogenase</td>
</tr>
<tr>
<td>Poly(vinyl alcohol)</td>
<td>Oxygenase or dehydrogenase or hydrolase</td>
</tr>
<tr>
<td>Poly(caprolactone)</td>
<td>Lipase and cutinase</td>
</tr>
<tr>
<td>Poly(lactic acid)</td>
<td>Protease and lipase</td>
</tr>
</tbody>
</table>
nanoparticles. In the presence of MMP-2, there was selective degradation of the PTMC nanoparticles resulting in targeted drug release specific to tumor microenvironments [75]. Similarly, peptide-conjugated thermogelling polymers, such as Pluronic® copolymers and copolymers of poly(N-isopropyl acrylamide) and poly(acrylic acid), have the ability to gel at body temperature and also undergo selective degradation in the presence of MMPs [76–78].

The incorporation of cathepsin B cleavable peptides is another approach to introduce selective enzymatic degradation to polymers. Cathepsin B is an enzyme that is typically present in lysosomes although increased expression of cathepsin B has been shown to play a role in the malignancy of tumors as well as increasing the metastatic capability of tumors [79, 80]. Microparticles made of N-(2-hydroxypropyl) methacrylamide (HPMA) crosslinked with cathepsin B cleavable peptides are stable until exposed to cathepsin B. Once exposed to cathepsin B, for example, in tumor microenvironments, they undergo degradation and can be used as drug delivery vehicles [81].

### 3.3 Aptamer-Functionalized Polymers

Another method by which polymers can be designed to change in their local microenvironment is through the recognition of biologics through aptamers. Aptamers are single-stranded oligonucleotides (RNA or DNA) that are selected for in a lab to bind a target molecule with high specificity and affinity [82]. Aptamer target molecules can range from small organic molecules to large biomacromolecules, such as proteins, whole cells, and viruses. Aptamers are highly selective and bind with a high affinity because they are selected for in a lab through an in vitro selection process called SELEX (systematic evolution of ligands by exponential) amplification. There are four main steps involved in the SELEX process, including the incubation of a single-stranded DNA library with the target molecule, separation of the bound nucleic acids, followed by their elution, and then amplification [83].

In comparison to natural antibodies, there are many advantages of synthetic aptamers. Aptamers possess superior dissociation constant (k_d) values, in the nanomolar range when compared to antibody k_d values which are typically in the range of micromolar to millimolar [84]. Furthermore aptamers have high stability, are easy to synthesize and modify, and are of a relatively low cost [85]. The conjugation of polymer particles with aptamers leads to the development of materials that are responsive to the presence of certain biomolecular stimuli in their environment.

Due to their high selectivity toward specific targets, single-stranded aptamers have been used in biosensors and have shown potential as a powerful diagnostic tool [86, 87] or as indicators resulting in colorimetric change in the presence of a certain molecule of interest with high selectivity [88].

Because aptamers are made of oligonucleotides of RNA or DNA, complementary strands (of DNA/RNA) may reversibly bind to them. For example, an aptamer hybridized to its complementary strand can de-bind when incubated in the
presence of its target biomacromolecule (that it specifically binds to). Polymers modified with oligomers containing aptamers and their complementary sequences can be used to develop gels or strands that change in response to the aptamers target. For example, polyacrylamide modified with two different DNA oligomers can be crosslinked with an oligomer containing sequences complementary to each strand. When this crosslinker oligomer contains an aptamer sequence specific to gold nanoparticles, it dissociates with one of its complementary strands when incubated with gold nanoparticles, causing de-crosslinking of the polyacrylamide [89].

Furthermore, aptamer-modified microparticles may be bound to polymers modified with their complementary sequences. Polystyrene microparticles functionalized with aptamers specific to vascular endothelial growth factor (VEGF) and platelet-derived growth factor BB (PDGF-BB) have been immobilized to polymers with the complementary sequences [90]. The different microparticles could be specifically released in the presence of either VEGF or PDGF-BB.

Similar strategies have been used to immobilize and release gold nanoparticles (AuNP) from polyacrylamide hydrogels, as shown in Fig. 11 [91].

![Image](https://doi.org/10.1088/0957-4484/22/49/494011) (Republished with permission from [91]. © IOP Publishing. All rights reserved; permission conveyed through Copyright Clearance Center, Inc.)
4 Overview

Stimuli-responsive polymers are becoming more prevalent in biomedical engineering as they afford the ability for biomedical devices to be changed over time and to adapt to dynamic biological environments. Temperature, pH, light, glucose, enzymes, and other proteins are only six of many different stimuli that may induce changes in biomedical devices. The ability for physicians to use external stimuli to change devices after implantation or for the local microenvironment surrounding an implant to provide cues for devices to change are both useful mechanisms to tailor drug delivery, alter tissue engineering scaffold properties, and improve materials used in similar biomedical devices.

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Quince Seed Mucilage: A Stimuli-Responsive/Smart Biopolymer

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Biopolymers are functional materials that offer structural integrity to sense external stimuli such as temperature, pH, or ionic strength. Therefore, they are the ideal candidate for a variety of technological applications especially in biomedical sciences. Quince seed mucilage (QCM), composed of glucuronic acid and xylose (glucuronoxylan)-based biomaterial. Glucuronoxylan is an inexpensive and biocompatible polysaccharide hydrogel which is extruded from the seeds of different plants including quince (*Cydonia oblonga*). It is insoluble in water and swells significantly. QCM is also a smart stimuli-responsive biomaterial which can act as matrix for the sustained release of different drugs in response to environmental stimuli such as temperature, ionic strength, or pH. QCM normally swells in water or basic buffers and deswells under acidic conditions, organic solvents, or salt solutions. Therefore, QCM qualifies as potential smart biomaterial to be used in different biomedical as well as other industrial applications. This review will focus to summarize the current methods used for the isolation of QCM and various factors affecting the quality and yield of the product. It will further showcase their potential usage in advanced drug delivery systems (DDS), cosmetic industry, food industry (both as additives and packaging material), water purification, and in other biomedical applications. Moreover, the use of QCM as a smart/stimuli responsive biomaterial for food, medicinal and pharmaceutical applications is highlighted.

1 Introduction

The research to fabricate functional biomaterials either mimicked from natural systems or derived from biopolymers have emerged as one of the most promising methodology to develop green, biocompatible, environmentally friendly, and efficient advanced materials. These materials find many different potential applications especially in the field of biomedical sciences. This is mainly because biopolymers inherit the basic structural units which are compatible with physiological conditions. In this direction, quince seed mucilage (QCM) exemplifies the best biomaterial which is responsive to external stimuli such as pH, temperature, or ionic strength. QCM is mostly derived from seed coat of Quince (*Cydonia oblonga*), a small deciduous tree of family Rosaceae which is 4.5–6 m in height and distributed in all temperature ranges across the globe. The plant is cultivated for its yellow color fruit which are used to prepare its fruit preserves, marmalades, and confections. Each fruit consists of five carpels, and each ripened carpel contains 6–15 reddish brown slightly bitter seeds which are 6 mm in length and are arranged in two rows. Seeds are obtained by drying in air or allowing fruits to rot [1, 2].

Quince seeds extrude mucilage upon soaking in water called as quince seed mucilage (QCM). It swells in water; therefore, it is a naturally occurring hydrogel
material, mainly composed of glucuronoxylan (i.e., a polysaccharide) [3, 4]. Hydrogels are hydrophilic in nature that contain three-dimensionally (3-D) interconnected network of acidic, basic, or neutral components. Hydrogels, whether natural, semisynthetic, or synthetic, absorb and retain large quantities of water without being dissolved in water [5, 6]. Hydrogels swell in water due to the presence of certain important hydrophilic functional groups such as amino (–NH₂), carboxylic acid (–COOH), amide (–CONH₂), hydroxyl (–OH), and sulfonic acid (–SO₃H). Water retention capability of hydrogels also depends upon porosity and extent of cross-linking [7–9].

The insight to the structure-function relationship of QCM confirms that it fulfills all necessary criteria to qualify as an advance material, generally named “smart materials”. The smart materials have a wide range of biomedical applications, such as tissue engineering, biosensors, and cell culture supports. Additionally, biodegradability, availability, and stimuli responsiveness are the characteristics of these smart materials. The naturally occurring water-swellable QCM-based smart materials are attracting the attention of researchers across the globe especially for their use in advanced drug delivery systems (DDS) [3, 6, 10–12]. Hydrogels are normally characterized by various parameters such as swelling behavior, swelling indices, and pH-, solvent-, and salt-responsive swelling-deswelling exploiting their physical or chemical properties [6, 9, 13]. Such naturally occurring water-swellable polysaccharides are also known as smart biopolymers or smart biomaterials due to their external stimuli-responsive properties such as swelling-deswelling which make them potential candidates for designing advanced DDS [3, 7, 8].

Immense efforts have been made to develop the stimuli-responsive DDS [14, 15] based on synthetic polymers. Among them polyacrylamides, polyacrylic acids, poly (N-isopropylacrylamide), pluronic, and tetronics [9, 16–18] are leading from the front. However, QCM as naturally occurring hydrogels is current topic of interest in the development of advanced DDS because it is a better excipient for tablets than the synthetic polymers [11]. Non-biocompatibility, non-biodegradability, and cyst formation limit the use of synthetic polymers in pharmaceuticals especially DDS. Therefore, naturally occurring swellable polysaccharides like QCM have more potential than synthetic polymers because of their exceptional swelling indices such as pH- and saline-responsive swelling-deswelling (on-off switching) [3, 19–21]. There is plenty of room available in the area of using naturally occurring water-swellable and stimuli-responsive polysaccharides in advanced DDS-based applications.

Research of the past decades reveals the immense importance of QCM as naturally occurring water-swellable polysaccharides; our aim is to review the potentials of this biopolymeric material for the development of advanced DDS. Moreover, to the best of our knowledge, no comprehensive review is reported highlighting the broad-spectrum properties of QCM. This will add to the present literature to design and develop highly valuable stimuli-responsive, intelligent, and targeted DDS. We will summarize the current methods used for the isolation of QCM and various factors affecting the quality and yield of the product. In addition, their potential use
as food additives, in sustained release of drugs, in cosmetic industry, in food packaging, and in biomedical applications are showcased. Moreover, the use of QCM-based nanocomposites as biosorbent for water purification is highlighted. These properties will be discussed especially with reference to the biocompatibility, high swelling index, pH and salt responsiveness, and on-off switching properties of QCM.

2 Structure of QCM

The nuclear magnetic resonance (NMR) spectroscopy using $^1$H and $^{13}$C nuclei showed that QCM is a polysaccharide mainly consisting of glucuronoxylan (glucuronic acid and xylose). D-Xyl and 4-$O$-Me-D-GlcA are in the ratio of 2:1. These results supported the findings as reported by Prof. Gey and coworkers that 4-$O$-methyl-$\alpha$-D-glucopyranosyluronic and $\alpha$-D-glucopyranosyluronic residues are in the ratio of 9:1 [4].

Previous reports showed that upon hydrolysis, water-soluble portion of polysaccharide (QCM) yields L-arabinose, D-xylose, and aldobiouronic acid with $O$-methyl group. Arabinose, xylose, mannose, galactose, and glucose were also found in the hydrolysate of QCM in the ratio of 8:46:4:10:32, respectively. Amount of uronic acid in QCM was found to be 35% as determined by carbazole method. Methylation of QCM revealed the presence of 2,3,4-tri-$O$-methyl-D-xylose, 2,3-di-$O$-methyl-D-xylose, 3-$O$-methyl-D-xylose, and 2,3,4-tri-$O$-methyl-D-glucose [22].

Recently, monosaccharide analysis of QCM revealed the presence of xylose (40.43%) and mannose (31.11%) along with smaller amounts of glucose (5.75%), galactose (5.60%), and arabinose (6.39%). Higher amounts of xylose and mannose show that basic skeleton of QCM consist of xylan and mannan backbone with arabinose, galactose, and glucose as side chains. The QCM also consists of proteins (2.78%), uronic acid (13.16%), ash (5.64%), calcium (7331.1 ppm), and magnesium (2632.8 ppm). Average molecular weight of QCM was recorded as $9.61 \times 10^6$ g/mol, which is greater than commercially available gums such as xanthan gum ($4.05 \times 10^6$ Da), guar gum ($1.45 \times 10^6$ Da), gellan gum ($1.64 \times 10^6$ Da), and locust bean gum ($1.6 \times 10^6$ Da) [23]. Epidermis of quince seeds consists of greater proportion of mucilage [23]. During isolation of QCM, it was also noted that some water-soluble polysaccharides like partially $O$-acetylated (4-$O$-methyl-$S$-glucurono)-D-xylan with greater proportion of glucuronic acid residues also come in water.

Concluding, it can be stated that QCM is mainly a polysaccharide material called as glucuronoxylan which is responsible for its swelling-deswelling properties [11].

3 Isolation of QCM

Different strategies are used to isolate and to increase the yield of QCM. Generally, researchers soak the seeds in water and isolate QCM after swelling for certain time using muslin cloth or by centrifugation. Yield, viscosity, and purity of mucilage can
be improved by optimizing the extraction conditions. Extraction temperature, time, and isolation techniques also affect the yield of mucilage. Therefore, literature show highly variable conditions for the isolation of QCM which are being discussed herein with detail.

1. Fekri et al. mixed clean seeds of quince with water in glass erlans. The ratio between amount of water and seed was fixed to 20:1 (v/w) and stirred at 75 °C for 3 h. Seeds were separated from water by muslin cloth. The mucilage was precipitated using 96% ethanol thrice, and precipitates were separated by centrifugation at 6500 rpm for 15 min. The mucilage (10.9% yield) was then dried in an oven at 70 °C for 4 h [24].

2. In another attempt, mucilage was separated from seeds by initially washing the seeds with 96% ethanol for 5 min under continuous stirring using mechanical stirrer. After evaporating ethanol and drying seeds in an oven at 45 °C, the contents were shifted to distilled water using 25:1 (v/w) ratio between amount of water to seeds. Stirring of swollen seeds at 100 rpm at 45 °C for 15 min resulted in separation of mucilage from seeds [25].

3. Seeds of quince were soaked in distilled water. The ratio between amount of water and seeds used was kept 25:1 (v/w), and temperature was increased from 15 °C to 65 °C. Similarly, pH of the extraction medium was changed from 5.3 to 8.6 using HCl and NaOH solutions, respectively. Mucilage was extracted ultrasonically from all samples, and sonication time was kept in the range between 1 and 12 min. Mucilage was separated from seeds using centrifugation at 4000 rpm for 10 min before drying in an oven at 60 °C. Mucilage composed of 9.84% moisture, 90.16% dry weight with 12.59% ash, 71.6% carbohydrate, 3.16% fat, and 2.81% protein were obtained. The yield was greater than previous reports owing to change in extraction technique. Response surface methodology predicted the optimum conditions for the extraction of QCM which are as follows: Extraction temperature 38.03 °C, pH of 6.35, and sonication time of 7.68 min. The yield and viscosity of mucilage extracted under these optimized conditions were noted to be 14.09% [26].

4. In a recent report, seeds of quince were ground before soaking in water to increase surface area for rapid extraction of mucilage. Water and seeds mixture were used in the ratio of 50:1 (v/w) and agitated at 30 °C for 24 h. After centrifugation, mucilage was filtered using cheese cloth. Yield of dried mucilage was calculated to be 8% [27].

5. Turkoz et al. used hot distilled water to extract mucilage from seeds employing continuous stirring for 30 min. The mucilaginous extracts were squeezed through gauze and precipitated using 90% ethanol. The aqueous extracts were allowed to stand for 20 h before centrifugation and washing with ethanol, acetone, and diethyl ether, respectively. The mucilage was dried in vacuum desiccator over anhydrous calcium chloride. Final yield of extracted mucilage was found to be 11.2% [28].

6. Ashraf et al. [11] recently reported a more precise method. Clean seeds of quince (100 g) were soaked in 500 mL of water for 6 h and heated to 50 °C for 30 min. Seeds release mucilage which was separated using cotton cloth by gentle rubbing. The mucilage was defatted by frequent washing with n-hexane and then...
dried at 60 °C in an oven. Mucilage was stored in vacuum desiccator after grinding to desired particle size. Yield of mucilage was calculated to be 9.3% [11].

7. In another report, seeds were dried in sunlight followed by heating in an oven at 105 °C. The dried seeds were treated with petroleum ether to remove lipophilic substances. After defatting, seeds were again dried and soaked in water for 5 h. The soaked seeds were boiled for 30 min and allowed to stand for another 30 min. Mucilage extruded from these seeds was precipitated with acetone and separated using muslin cloth. The mucilage was dried below 50 °C and then ground to fine powder before passing through 20 mesh sieve [29].

8. Renfrew et al. introduced a novel protocol using first ethanol and ether separately or with ether-ethanol (50%), followed by extraction with water for 24 h. The mucilage from seeds was separated by filtering through cloth in the Buchner funnel. The mucilage was precipitated with alcohol and then dried with alcohol and acetone [30].

9. Abbastabar et al. used response surface methodology (RSM) to study the effect of temperature, pH, and water-seed ratio on the yield of quince seed mucilage. Maximum yield of mucilage was obtained at 60.77 °C and almost neutral pH (6.6) using water to seed ratio of 96.2 (w/w). The values of intrinsic viscosity (1530) and Huggins constant (0.32) proved that mucilage extruded under RSM conditions has strong gelling structure and hydrodynamic volume [31].

### 4 Physical Characteristics

The physical properties of excipient materials play a key role in the development of drug formulations. It is reported that QCM (dried and having 7.5% moisture) shows poor flowability owing to its strong cohesive forces among the particles. Poor flow properties are indebted to high values of angle of repose, Hauser ratio, and Carr’s index, which are 43°, 1.66, and 39%, respectively. Bulk density of QCM is 0.131 g/cm³ while tapped density is 0.217 g/cm³ [11]. Hence, the use of QCM as excipients requires some proportions of other excipients (lubricating and gliding agents) to increase the flowability of tablet grains containing QCM as excipient [6].

QCM is a good emulsifying and foaming agent. High molecular weight makes it a good thickening agent with greater viscosity. Intrinsic viscosity of QCM is 16.96 dL/g at 25 °C which decreases with increase in salt concentrations due to shielding effect of ions on polymer chain [23].

### 5 Biocompatibility of QCM

Biocompatibility of a material is one of the key factors to test the performance and suitability of excipients materials. Since these materials may get in contact with blood (intentionally or accidently), therefore, they normally require tests for both physiological and hemocompatibility [32]. Hemocompatibility of QCM is
generally studied using hemolysis and thrombogenicity. Directions of International Organization for Standardization (ISO) (ISO10993-4, 1999) are usually followed to evaluate abovementioned properties. Gravimetric method is applied to study thrombogenicity, and guidelines of American Society for Testing and Materials are followed to evaluate hemolysis. Sing et al. carried out the blood compatibility studies of a hydrogel for different biomedical applications in a new drug delivery system based on *Moringa oleifera* gum [33]. In a recent and the only study available on blood compatibility of QCM, it was noted that the weight of clot showed non-thrombogenic potential of mucilage. The value of hemolytic index noted was 3.91%. According to safety standards set by ISO document 10,993-3 2002, the value of hemolytic index less than 5% is considered safe. Therefore, we can assess that QCM could be safe for biomedical applications [34], but it still needs a lot more to test before declare it as completely biocompatible.

## 6 Applications

QCM being green, environmentally friendly, cost-effective, abundant amounts prepared from renewable sources, and with exceptional physical and chemical properties qualifies for many industrial applications. Some of its important applications are discussed in this section.

### 6.1 Pharmaceutical

QCM is a biocompatible, economical, and nontoxic biomaterial that can be used as excipient for targeted and sustained release of drugs for many different tablet formulations. Drug release of paracetamol from QCM-based tablet formulations has been evaluated using various concentrations of mucilage as an inactive pharmaceutical ingredient. As an example, Patel et al. studied the potential use of QCM as disintegrant for different pharmaceutical formulations and compared the results with cross-povidone, which is used as standard disintegrant in different pharmaceutical products [35]. Swelling behavior of hydrogel and its drug-polymer interactions are important parameters to evaluate it as a matrix in sustained DDS. Similarly, drug release from hydrogels depends upon pH of the medium, particle size, and swelling capability of the hydrogel. Keeping in view above properties of hydrogels, QCM has been successfully appraised for the targeted/sustained release of levosulpiride [11]. Being pH sensitive, QCM-based levosulpiride tablets showed sustained drug release in simulated gastric fluids (SGF) and simulated intestinal fluids (SIF). Due to low swelling and solubility in acidic media, release of levosulpiride was very small in acidic media of stomach. This property will help to protect the stomach from harmful effects of the acidic drugs, like NSAIDs. Moreover, tablet formulations showed enhanced and targeted release of drug in alkaline media of intestine due to high swelling index of QCM at neutral and near-neutral pH values. The drug release from the matrix followed super case II transport mechanism in which drug release
takes place by the erosion of tablets. On increasing the concentration of QCM in tablet formulations, the release of drug from tablets was delayed for almost 14 h. Similar results were also found for theophylline and diclofenac sodium [11, 34]. They also reported that QCM-based tablet surface appears smooth with microcracks on it and swells in water and maintains porous structures even after swelling (Fig. 1). Thus tablet formulations containing QCM as an inactive pharmaceutical ingredient will help to decrease the dose frequency and patient compliance.

### 6.2 Medicinal

The QCM decoction prepared from its seeds are used to treat pain and inflammation [36]. It is also being used to treat dysentery and diarrhea, skin burns, wounds, and sore throat perhaps due to its gelling and lubrication properties [11, 37]. Different industries such as food and medicine use QCM as an important ingredient in various preparations [38]. As an example and mentioned above that QCM containing compositions controls the slow
release of different bioactive molecules in the acidic pH regime, therefore, QCM containing formulations suppresses the stomach regurgitation. Treatment of gums and teeth can also be carried out using QCM. Food industries manufacturing chocolate milk, ice creams, and chocolate drinks also use QCM as product stabilizer. Siehers et al. has already patented a method to separate proteins from animals using quince, psyllium, or flax gums [39]. Recently, there are several reports on the utilization of QCM which proved its significance in wound healing. It healed the lesions induced by T-2 toxin on the skin of rabbits. Positive and negative control groups received T-2 toxin and eucerin, respectively. The study groups revealed that specimens which received 10 and 15% QCM showed complete healing of wounds after 9 days. It may be understood that QCM acts as a barrier between skin and T-2 toxin by decreasing water evaporation and increasing antioxidant potential. It was also noted in this study that QCM acts as growth promoter and prohibits synthesis of impaired proteins due to T-2 toxin. The QCM neutralizes the dermal toxicity by increasing granular tissue production [40] (Fig. 2) that is thought to be responsible for wound healing process [41]. Literature showed that QCM is a natural wound healing agent without any side effect. Actually, QCM acts as a barrier for oxygen and moisture and speeds up wound closure by collagen production [37].

In a recent and well-planned study, wounds and burns were treated with QCM in combination with Iranian folk medicines. The freshly extracted, dried, and ground QCM was dispersed in distilled water at concentrations of 50, 100, 200, and 400 μg/mL and after swelling applied on human skin fibroblast cell line (HNFF-P18) to explore its potential in wound healing. QCM stimulated the healing of wounds by increasing the proliferation of human skin fibroblast at all concentrations. Thus QCM can act as a natural wound healing agent [42].

In another recent study, synergistic effects of QCM with euracin cream were proved experimentally. Full thickness wounds were treated either with eucerin base or euracin base with different concentrations of QCM (5, 10 and 20%) twice a day. Rabbits treated with QCM in combination with euracin cream showed rapid healing of wounds than treated with only eucerin under similar set of conditions. Eucerin with 20% QCM healed the wounds completely in just 13 days and displayed higher values of growth factors such as hydroxyproline content, tissue resistance, and wound fluid levels (Fig. 3). The growth-promoting potential of QCM supports its use in Iranian traditional medicines [36].

Based on literature available, it can be concluded that some further efforts are required to prepare creams for wound healing and burn containing QCM. Moreover, researcher needs some clinical trials as well as physical pharmaceutics aspects like determination of shelf life and chemical stability before commercialization.

### 6.3 Cosmeceutical

QCM is separated from its seeds usually by gently heating using water as an extraction medium [43]. Dispersions prepared from QCM are thixotropic, and their viscosity decreases with increasing shear rate [2]. Viscosity of QCM is not
affected by changing temperature from 15 to 50 °C and pH from 4 to 10. It is also well established in the literature that viscosity of the QCM dispersions is not affected on adding the small concentrations of NaCl, i.e., up to 0.1 M [2]. Aforesaid properties make the QCM highly suitable material for cosmetics [44]. In fact, quince seed extract is already used as moisturizer in different cosmetic products. Quince mucilage (0.01–5%) is used in creams, lotions, and soaps to provide softness, cleaning, and comfort. Lotions used to set hair also contain QCM. The hairs can be combed into waves and curls by applying lotion containing QCM. It is due to the thickening properties of QCM. In cosmetics industry, a mascara cream is produced using QCM. The mucilage will prevent cream from drying in tubes and increase the

Fig. 2 (a) Photomicrograph of the section of rabbit skin 9 days after T-2 toxin administration without any treatment. Epidermal necrosis associated with inflammation in dermis layer is evident. (b) Photomicrograph of the section of rabbit skin 9 days after T-2 toxin administration and treatment with quince mucilage (5%). Incomplete epidermis granulation tissue forming can be seen. (c) Photomicrograph of the section of rabbit skin 9 days after T-2 toxin administration and treatment with quince mucilage (10%). Incomplete epidermis and inflammation is evident. (d) Photomicrograph of the section of rabbit skin 9 days after T-2 toxin administration and treatment with quince mucilage (15%). New epidermis layer with granulation tissue forming can be seen (H&E 200×). (Reprinted from Ref. [40] with permission from Elsevier)
stay of cream on eyelashes. QCM is also used in face cleansing and skin protection creams. QCM suspended in boric acid solution is used as a smoothening gel to get rid of cracks on breasts usually formed during breastfeeding [44, 45]. In conclusion, the QCM is a highly potential ingredient in different cosmetics; therefore, it has high potential to be commercialized. However, future research needs to explore the therapeutic effects of QCM on the keratinocyte associated with skin inflammation.

### 6.4 Food Packaging

Food products are usually packaged using biopolymer-based films because of their potential to act as barrier for oxygen and mechanical properties [46, 47]. Recently, it is reported that QCM-based biopolymer is highly suitable for blending with polyethylene glycol (PEG) and clays [25]. For the preparation of film emulsion, the QCM (10%) is mixed with polyethylene glycol (5% w/w) under constant stirring at 750 rpm and at 45 °C for 15 min. To increase thermal stability and mechanical properties, different amounts of nanoclay (0.5–2.0 g) were added to this emulsion. These films containing QCM were casted on Teflon plates and upon drying showed increased tensile strength and elongation properties up to 22 MPa and 6.5%, respectively. This study also showed the addition of nanoclay to the QCM films enhanced in gas

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**Fig. 3** Comparison of the wound healing in experimental groups. Data are expressed as mean ± SE. Values significantly different from eucerin-treated or no-treatment are indicated as *P < 0.05, **P < 0.01. (Reprinted from Ref. [36] with permission from Elsevier)
diffusion properties. Also these QCM films impregnated with nanoclay reduced the water vapor and oxygen permeability to $1.10 \times 10^{-7}$ g.m$^{-2}$.h$^{-1}$.Pa$^{-1}$ and 13.68 mL.day$^{-1}$.m$^{-2}$, respectively [25]. It is inferred from this work that QCM-based films containing nanoclay (2%) can be used as food preservatives.

Jouki et al. prepared QCM-based films by stirring it in glycerol and thyme or oregano essential oil and Tween 80 as a plasticizer, preservative, and surfactant, respectively. The films are prepared by continuous stirring at 45 °C for 15 min [48]. These films were used to wrap the rainbow fish fillets. The results indicated that the films prepared with this composition delayed more the lipid peroxidation of fillets than the film used as control after 6 days. It also significantly improved the quality of the fillets by reducing the color and texture changes. Additionally, the fillets wrapped with QCM-based films exhibited prominent reduction in pH after 12 days. The films inhibited the growth of S. aureus, E. coli, S. putrefaciens, and Y. enterocolitica and therefore act as a barrier for oxygen to improve antioxidant and antimicrobial potential. Scanning electron microscopy revealed that addition of glycerol resulted in smoothening the surface of the QCM-based films. However, surface morphology is not significantly affected by varying concentrations of glycerol. Thus QCM films can act as better packaging for the foods which are sensitive to oxidants [49].

Jouki et al. [43] also reported detailed antimicrobial investigations of QCM-based films containing different concentrations of thyme essential oil (1, 1.5, and 2.0%). The QCM films inhibited the growth of microorganisms such as Escherichia coli (ATCC 25922), Yersinia enterocolitica (ATCC 9610), Staphylococcus aureus (ATCC 25923), Bacillus cereus (PTCC 1154), Pseudomonas aeruginosa (ATCC 27853), Lactobacillus plantarum (ATCC 8014), Salmonella typhimurium (ATCC 14028), Escherichia coli O157:H7 (ATCC 25922), Listeria monocytoogenes (ATCC 1915), Vibrio cholera (ATCC 14033), and Shewanella putrefaciens (CECT 5346). Increasing the concentration of thyme essential oil resulted in the increase of free radical scavenging activity and percent elongation of films, whereas the glass transition temperature, tensile strength, and Young’s modulus of film were decreased. Thus, QCM film incorporated with thyme essential oil can save the food from microorganisms and oxidants and enhance the safety and shelf life of foods [43].

Recently, emulsions of whey protein isolate and sunflower oil were prepared using different concentrations of QCM and xanthan gum. The emulsions were homogenized at 7500 rpm for 3 min and further mixed with an antimicrobial agent, sodium azide (0.02%). QCM decreased the low-shear viscosities and shear thinning capabilities of emulsions, hence making the emulsions stable for up to 5 months [27]. Therefore, QCM proved a better emulsifying and stabilizing agent. The purified QCM can be used at industrial level.

Farahmandfar et al. coated the banana slices with low concentration of QCM (0.7%) before drying which stops their deterioration and speeds up drying process. Mucilage delayed shrinkage of banana slices with improved quality of tissues with low browning index [50]. Drying fruits in this way is an economical and time saving process.
Edible coatings are used nowadays to overcome the adverse effects of oil uptake during frying foods. Mixture of QCM and green tea extract were applied as coatings on shrimps before frying. This resulted in increased moisture content and decreased the oil uptake by shrimps. In addition, the antioxidant potential of green tea extract was increased [51].

### 6.5 Food Additive

Various mucilages such as xanthan, mustard, galactomannan, pectin, carrageenan, and guar are added to improve and control the quality of different characteristics of food products [52–54], e.g., yogurt is prepared from homogenized semi-fat milk (2.5% fat) with different amounts of QCM (0.003, 0.05, and 0.1%) as additives to evaluate its susceptibility [55]. Although the addition of QCM did not affect the color, acidity, stringiness, adhesiveness, and cohesiveness of the yogurt samples and also decreased the hardness, chewiness, gumminess, and amount of syneresis. The amount of syneresis was decreased due to interaction of the negative charge on mucilage surface with casein micelles.

Farahmandfar et al. in a recent study studied the impact of different concentrations of QCM (0.1 and 0.3% w/w) addition to whipped cream with low fat content (30%) which affected its texture, rheology, and physical parameters. Addition of QCM improves quality of ice creams by increasing viscosity, hardness, and overrun. Mixture of ice cream with mucilage exhibits thixotropic and pseudoplastic behavior; therefore, QCM can act as fat replacer in food products [50].

Low-fat hamburgers were prepared by adding different concentrations of QCM (2, 4, 6, and 4.8%) to evaluate loss during cooking, antioxidant activity, water holding tendency, and hardness during 9 days’ study [56, 57] (Fig. 4). By increasing the QCM concentration and decreasing oil content, cooking loss and lipid oxidation were decreased by capturing free radicals. It also helped to improve texture, flavor, appearance, and acceptability of the hamburgers owing to greater water retention capacity. The storage time of hamburgers without compromising the quality and taste can also be improved by adding QCM.

### 6.6 Water Purification

Water pollution is an alarming issue, the world population is facing nowadays. The hydrogels containing polysaccharides and polysaccharide-based composites are widely used for the uptake of heavy metal ions or organic contaminants such as dyes from water [58, 59]. In this direction, QCM-based magnetic nanocomposites containing iron oxide nanoparticles (Fig. 5) removed the methylene blue from aqueous solution more efficiently than only nanoparticles [60]. QCM makes the magnetic nanoparticles better adsorbents for methylene blue. Adsorption of dye from aqueous solution increases with increasing the concentration of dye, magnetic
Fig. 4. (a) Effects of different quince seed gum (QSG) amounts on the cooking loss (CL %), (b) water holding capacity (WHC), (c) oxidative stability (mg of Malondialdehyde), (d) oxidative capacity (DPPH assay), (e) firmness in raw, and (f) fried hamburgers during the storage time (A Oil, B QSM, and C Time (day)). (Reprinted by permission from Springer Ref. [57]).
nanoparticles, and pH. However, increase in temperature and ionic concentration resulted in the reduction of dye uptake.

This study also reported on the thermodynamic parameters which confirm spontaneous and exothermic nature of biosorption process. The removal of methylene blue follows Langmuir isotherm and pseudo second-order kinetics. Adsorbent once used can be regenerated and utilized again and again with almost same efficiency; hence, QCM is an efficient and economical adsorbent for water purification.

7 QCM-Based Smart Materials

Smart materials or intelligent materials have the ability to perform a material property change in response to an external stimuli in their environment. The fact that QCM-based hydrogels are responsive to different external stimuli such as pH, ionic strength, and solvent makes it an ideal smart biomaterial for number of applications. The responsiveness of QCM-based hydrogels is mainly related to its unique chemical structure and inherits physical properties. Literature showed that some other hydrogels like tragacanth, arabinoxylans, xanthan gum, and guar gum are state-of-the-art water-swellable materials used for sustained release of the drugs at the target site [21, 61–63], however, most of them fail to meet the criteria to be used as excipients in more specialized areas, e.g., in gastric system-related DDS. Enormous efforts have been made by the researchers to develop gastro-retentive systems with increased bioavailability of drugs based on naturally occurring water-swellable biopolymers [64–66]. In this direction, only arabinoxylans, due to high swelling index, have attracted the vigil eye of researchers working in targeted drug delivery systems, and several formulations of different drugs have been successfully designed for targeted and sustained delivery [65–68].

QCM a water-swellable biomaterial, (Fig. 6) with manyfold high swelling index than arabinoxylans and other gums, offers stimuli-responsive swelling-deswelling behavior, especially pH-sensitive on-off switching which leads to the developments of pH-responsive DDS. Ashraf et al. [11] have recently reported an elaborative and leading study on QCM swelling-deswelling, stimuli-responsive on-off switching behavior. The authors explored its potential use in the development of stimuli-responsive and sustained release-based advanced DDS. Following subsections will
detail about different parameters which influence the stimuli-responsive swelling-deswelling behavior, their benefits in DDS, and also explaining the mechanisms of on-off switching of QCM.

### 7.1 pH Response

Hydrogels are generally sensitive to external stimuli such as pH and are considered important for the controlled release of drugs [18, 69]. QCM contains greater portion of glucuronic acid residues (carboxylic groups) which makes glucuronoxylan-based QCM a slightly acidic hydrogel. In acidic buffer of pH 1.2, QCM showed lesser swelling. It is because at acidic pH, carboxylic acid groups are protonated and strength of hydrogen bonding is increased. Hydrogen bonding brings polymer chains close to each other and hydrogel shrinks. In buffers of alkaline pH (6.8 and 7.4), QCM swells considerably by absorbing a lot of water [11]. Actually, at alkaline pH, carboxylic acidic groups ionize to carboxylate anions. These negatively charged groups repel each other, and polymer chains relax to allow water penetration [6, 7]. Hydrogel follows second-order swelling kinetics which can be determined from normalized degree of swelling and normalized equilibrium degree of swelling values [70].

### 7.2 Ionic Response

Polysaccharide-based hydrogels are responsive to different concentrations of salt solutions [71, 72]. With increase in salt concentrations, swelling of hydrogels decreases. Similar behavior is observed for glucuronoxylan hydrogel (i.e., QCM) as reported in a recent study by Ashraf et al. [11]. Low swelling in salt solutions is due to enhanced charge screening effect. Another reason for less swelling in salt solutions is the decrease in osmotic pressure between salt solution and QCM.
Swelling of hydrogel is relatively higher in NaCl than KCl. This is due to smaller size, greater charge density, and hydration energy of sodium ions which helps the sodium ions to develop greater forces of attraction with anions of hydrogel [7].

### 7.3 Effect of Solvents

Solvents play key role to impact the physical properties of hydrogels. QCM-based hydrogels possess greater swelling in distilled water. This is due to polar nature and greater dielectric constant of water [7]. When swollen polysaccharide hydrogel is placed in less polar solvent such as ethanol, it will deswell by releasing some amount of water. On shifting the hydrogel again in water, ethanol will be washed, and hydrogel will gain water and swells again [6]. It is obvious from a recent work by Ashraf et al. that QCM-based hydrogel shows swelling and deswelling (on-off switching) behavior in water and ethanol, respectively [11].

### 7.4 Stimuli Responsiveness of Tablet

Similar to powdered hydrogel (QCM), theophylline and diclofenac sodium tablets employing the formulations based on QCM are also found highly responsive to various stimuli such as water, pH, and salt solutions. Tablet formulations show greater swelling in deionized water due to interactions of hydrophilic functional groups on polymer chain with water. However, swelling of tablets is less than powdered QCM. It is because tablets are in compressed state and have lesser surface area to interact with water. Likewise, QCM-based aforesaid tablet formulations of theophylline and diclofenac sodium were found salt responsive as well as they showed on-off switching behavior in the pH range from 7.4 to 1.2 media, respectively [34]. Due to this innate property of QCM in compressed tablets, release of drugs was also found negligible in SGF and sustained in SIF. This pH-responsive nature of tablet formulations based on naturally occurring hydrogels supports its application in intelligent drug delivery systems, which provide targeted and sustained release of drugs in intestinal tract [6]. It will help to decrease drug dose and increase the therapeutic potential.

### 8 Conclusions

In conclusion, quince seed mucilage (QCM) has attracted the attention of researchers in the last two decades not only due to its nutritive value but also because of its inherit physical and chemical properties which makes it a potential candidate for a number of applications in pharmaceutical, in food sector, and healthcare industry. Chemically QCM is glucuronoxylan, a biopolymer or polysaccharide containing glucuronic acid and xylose. It has many fold high swelling index than other pharmaceutical admired polysaccharides like guar gum, arabinoxylan,
rhamnogalacturonan, and galactomannans. QCM also showed remarked stimuli-responsive on-off switching (swelling-deswelling) which make it a material of high commercial value. Keeping in view the immense importance of this water-swellable polysaccharide, i.e., glucuronoxylan (of natural origin), we foresee its bright future in a wide range of industrial applications including pharma, healthcare, and food sectors. QCM could be highly valuable for the development of stimuli-responsive intelligent/smart formulations for the targeted and sustained release of drug delivery systems on commercial scale because of its biocompatible nature, high swelling index, pH, salt responsiveness, and on-off switching properties of QCM.

The reported literature reveals that QCM in combination with wound healing creams helps in promoting the cell growth and hence improves the wound healing applications and burn treatments. QCM is highly useful ingredient (as it improves different physical aspects) in different cosmetics. However, future research need to explore the therapeutic effects of QCM on the keratinocyte associated with skin inflammation to explore its potential use at commercial scale. QCM proved to be a good film forming, emulsifying, and stabilizing agent, and purified QCM can be used at industrial level especially in food packaging and as food additive.

To further explore the prospective applications of QCM, researcher needs to concentrate to develop methods for its isolation and purification without compromising the structural integrity. Since the QCM is considered as a green and renewable biomaterial, effort should be put forward for its cultivation to increase the yield to meet the commercial demands of market.

References


Blood Compatible Polymers

Sara Alibeik and Kyla N. Sask

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Abstract

Medical devices made from polymeric materials come in contact with blood in a wide range of applications, including stents, artificial vascular grafts, hemodialysis membranes, catheters, and sutures, among others. In this chapter,

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an overview of the ongoing investigations with blood compatible polymers is provided. A summary of polymers used in blood contacting devices will be given, followed by details focusing on each of the types of polymers that are most commonly used. Furthermore, a description of the efforts made in improving the blood compatibility of these polymers will be provided, as most synthetic polymers are required to go through some level of modification in order to be used in blood contacting devices. Most modification strategies address the changes in surface properties of these polymers with the aim of controlling the interactions between blood components and the polymeric surface. Among these modification techniques, use of bioinert molecules, bioactive molecules, and a combination of the two molecules are the subject of most studies.

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<th>List of Abbreviations</th>
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1 Overview of Blood Compatible Polymers

Polymeric biomaterials have widely been used in blood contacting devices, with the primary use in cardiovascular applications. These include both devices in contact with blood for a short time such as catheters and hemodialysis membranes, as well as long-term blood contacting devices such as stents, vascular grafts, left ventricular assist devices, and heart valves.

A list of synthetic polymers extensively tested and used in blood contacting devices is provided in Table 1. Various modification strategies have been carried out on these polymers and a summary of some of the main references is given. These studies will be discussed throughout each polymer section of this chapter.

It should be noted that despite using the term blood compatible polymers for this chapter, in general, synthetic polymers and other biomaterials are not considered completely blood compatible. However, significant research has been carried out to develop polymeric materials that have improved blood compatibility and create less

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Applications</th>
<th>Modification strategies [Reference numbers]</th>
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<tbody>
<tr>
<td>Polyurethane (PU)</td>
<td>Catheters, left ventricular assist devices (LVAD), pacemaker lead insulation, artificial vascular grafts</td>
<td>[2–49, 154]</td>
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<tr>
<td>Silicones</td>
<td>Catheters, blood pumps, heart valve structures, pacemaker leads, blood oxygenators, tubing, microfluidic devices</td>
<td>[51–62, 155]</td>
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<td>Polytetrafluoroethylene (PTFE) (Teflon)</td>
<td>Artificial vascular grafts, catheters</td>
<td>[64–86, 158]</td>
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<td>Polyethylene terephthalate (PET) (Dacron)</td>
<td>Artificial vascular grafts, sewing rings on artificial heart valves, fabrics in implants, surgical mesh, catheter cuffs</td>
<td>[88–104, 159]</td>
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<tr>
<td>Polyvinylchloride (PVC)</td>
<td>Catheters, blood bags, tubing</td>
<td>[106–112, 114, 161, 162]</td>
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<td>Polysulfone (PSF), polyethersulfone (PES)</td>
<td>Hemodialysis membranes</td>
<td>[116–128], [129–133, 168]</td>
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<tr>
<td>Polyacrylonitrile (PAN)</td>
<td>Hemodialysis membranes</td>
<td>[135–143, 169]</td>
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<td>Polyamide (nylon)</td>
<td>Sutures, balloons for catheters</td>
<td>[145–147, 177]</td>
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<td>Polypropylene (PP)</td>
<td>Membranes in blood oxygenators, disposable syringes</td>
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<tr>
<td>Polyglycolide (PGA)</td>
<td>Sutures</td>
<td>N/A</td>
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<tr>
<td>Polylactide (PLA), poly (L-lactide) (PLLA)</td>
<td>Sutures, stents</td>
<td>N/A</td>
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of a reaction in blood. An understanding of polymer-blood interactions is important to achieve this goal and minimize adverse responses.

1.1 Polymer-Blood Interactions and Testing

Similar to other types of biomaterials, polymeric biomaterials induce numerous adverse responses in contact with blood such as thrombosis, infection, as well as immune and inflammatory responses. Such responses can ultimately lead to the failure of the device. Thrombosis and the complications associated with it remain the main challenge in the context of blood contacting polymers. Figure 1 shows the sequence of events during blood-material interactions [148]. As shown, protein adsorption happens as the first event upon polymer-blood contact. This results in activation of the coagulation cascade, platelets, and the complement system. Blood coagulation involves various proteins, cells, and other components in a series of complex reactions. Thrombin is a central enzyme in coagulation and thrombosis and is responsible for converting fibrinogen to fibrin. Fibrinogen also plays an important role in platelet adhesion and activation. Thrombus formation can cause issues at the site of the device or can embolize and cause issues elsewhere in the body.

Testing of polymers in contact with blood should thus investigate reactions not only at the polymer surface but also in circulating blood. Protein adsorption and platelet adhesion are often studied as initial indicators of how well a material may interact with blood. However, in vitro measurements don’t always correlate with the in vivo performance of a material and rigorous characterization and analysis is needed. ISO 10993-4 provides guidance on the selection of tests for blood interactions including thrombosis, coagulation, platelets, leukocytes, and the complement system. It is important for studies to examine multiple factors, use appropriate controls, and adhere to standardized methods. The future of this area relies on continual development to better understand the mechanisms involved in blood-material interactions along with improvement towards testing standardization.

1.2 Polymer Modification for Blood Contacting Devices

For most of the currently used blood contacting devices, thrombosis is prevented therapeutically through anticoagulation and antiplatelet therapies [149]. Bleeding associated with the use of such therapies, however, necessitates the use of other approaches such as materials modification. Hence, there has been ongoing research to modify materials to reduce thrombotic complications. The general approaches used in the modification of polymers to reach this goal include: bulk and surface modifications. Bulk modifications of copolymers used in blood contacting devices such as polyurethanes have been addressed in several studies as described in Sect. 2.1. Surface modification, however, has been of greater interest due to the fact that polymer interactions with blood mainly happen on the surface. In the context of surface modification, the following strategies have generally been
Fig. 1 The sequence of events in blood-material interactions. (Taken from Ref. [148] with permission)
employed: passivation of the polymer surfaces that would prevent protein adsorption (bioinert surfaces), incorporation of bioactive molecules that would inhibit coagulation (bioactive surfaces), or modification with bioactive molecules in combination with the protein-resistant molecule.

### 1.3 Bioinert Surfaces

The goal of creating bioinert (also known as nonfouling, antifouling, or protein-resistant) surfaces is to minimize blood protein adsorption and passivate the surface by using “protein resistant” molecules. The most common molecule studied to create such surfaces is polyethylene oxide (PEO), also referred to as polyethylene glycol (PEG). PEO is a linear polymer with repeating units of $-\text{CH}_2\text{-CH}_2\text{-O-}$. The protein-resistant properties of PEO are associated with hydration and steric effects (Fig. 2). Numerous research studies have looked into improving blood compatibility of polymers by incorporating PEO. Typically, the surface of the polymer is modified to introduce functional groups. These functional groups can then react with PEO. Other protein-resistant molecules studied for polymer modification include PEO-containing copolymers, albumin, poly(2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)), poly(2-hydroxyethyl methacrylate) (PHEMA), poly (sulfobetaine), poly(carboxybetaine), poly(2-methoxyethylacrylate) (poly(MEA)), polyacrylic acid (PAA), poly(methacrylates), hydroxyethylmethacrylate phosphatidylcholine, poly(vinyl alcohol) (PVA), polyvinylpyrrolidone (PVP), and glycidyl methacrylate (GMA). Almost all studies in this area have indicated that despite the success of such surfaces in reducing protein adsorption, they still need further modification to inhibit thrombosis.

### 1.4 Bioactive (Biofunctional) Surfaces

The aim of creating bioactive surfaces is to support specific interactions between the polymer surface and blood, mainly to inhibit coagulation. The most commonly
used approach for inhibition of coagulation is heparinization. Heparin is a widely used anticoagulant that binds to the protein antithrombin in blood and inhibits thrombin, along with other coagulation factors, including factor Xa. It does this through its active pentasaccharide sequence. Many commercially available blood contacting devices are surface modified with heparin. Examples of such surfaces are the Carmeda® Bioactive surface (CBAS® Heparin) by W.L. Gore & Associates, Inc., and the Trillium® Biosurface by Medtronic (Fig. 3) [150]. Hirudin is another anticoagulant that also inhibits thrombin; however, unlike heparin that acts indirectly through antithrombin, hirudin directly binds to thrombin’s active site. Other molecules that have been used to create bioactive surfaces include antithrombin, an antithrombin-heparin covalent complex, a combination of heparin with other molecules such as chitosan, and small peptides. Certain bioactive molecules such as lysine have also been incorporated to take advantage of the fibrinolytic system and to lyse the clot that is formed on the polymer surfaces. Another approach in developing bioactive surfaces has focused on mimicking the nitric oxide releasing properties of endothelial cells to prevent platelet adhesion and activation. Endothelialization of polymeric surfaces has also been explored as a way of preventing thrombosis and in order to mimic the lining of blood vessels. Finally, in the past decade, researchers have focused on modification of polymers to contain both protein resistant and bioactive molecules (Fig. 4). More details on these strategies are presented throughout this chapter.

In the next sections, the major polymer types that are used in blood contacting devices are described along with the efforts that have been carried out to improve each of these polymers for such applications.

![Fig. 3 Examples of commercially available heparinized surfaces: Schematic of (a) Trillium® Biosurface and (b) CBAS® Heparin [150]](image-url)
2 Polymers Used in Current Blood Contacting Devices

2.1 Polyurethane (PU)

Polyurethanes (PUs) are block copolymers made of alternating blocks of soft elastomeric and hard crystalline segments. Figure 5 shows the typical polyurethane synthesis procedure. The hard segment is usually made of a diisocyanate and contains a diol or diamine as the chain extender (Table 2). The soft segment is typically a high-molecular-weight long chain macrodiol such as a polyester, a polyether, a polycarbonate, or a polybutadiene (Table 2). The phase segregation between the hard and soft segment provides PUs with their exclusive physical and mechanical properties. Furthermore, the wide range of choices available for soft and hard segment make PUs ideal polymers to be used in biomedical devices [151]. Medical grade PU is available from many different manufacturers. Table 3 shows a list of these medical grade PUs under various trade names. Pellathane® and Biomer® are among the very first commercial biomedical grade PUs synthesized. These PUs are made of polytetramethylene oxide (PTMO), methylene-bis-phenyldiisocyanate (MDI), and butanediol (BD) or ethylenediamine (ED) as the chain extender, respectively. Despite their current successful performance as catheters, these PUs lack properties required for long-term blood contacting devices. To improve blood compatibility, new polydimethylsiloxane (PDMS)-based PUs, polycarbonate-based PUs, and PDMS-polycarbonate-based PUs were commercially synthesized and are currently available commercially (Table 3).

Some of the most common applications of PU in blood contacting devices are in the development of catheters, left ventricular assist devices, and pacemaker lead insulation [152]. They have become one of the preferred polymer types as a catheter.
for long-term use in contact with blood. This is mainly due to lower instances of infection, relatively better hemocompatibility and tunable mechanical properties when compared with other polymers. Furthermore, compared with many other polymers, application of PU in pacemaker lead insulation has been promising [153]. A number of PUs have also been used to make left ventricular assist devices (LVADs). When used as the chamber wall material in LVADs, PU showed desired mechanical properties compared with several other polymers [1]. Without any modification, however, PU would fail as a long-term LVAD due to associated thrombosis and infection.
There is ongoing research on further improving the blood compatibility of PUs for long-term blood contacting devices. In this section, major efforts in bulk and surface modification of PU for improved blood compatibility are described.

Many initial studies on segmented polyurethanes (SPUs) were focused on using a selection of different hard and soft segments to improve blood compatibility. In one study, PEO-containing PUs showed more thrombogenicity than the pure PTMO-based PUs. It was also found that there was a threshold concentration of PEO in the PUs (when PEO/PTMO mixture was used as the soft segment) affecting the blood contacting properties of this polymer [2]. The polydimethylsiloxane (PDMS)-based polyurethanes showed the lowest platelet adhesion when compared with PUs synthesized with either of the PEO, PTMO, hydroxyl-terminated polybutadiene or polyisobutylene as the soft segments [3]. Another study showed no significant improvement in blood compatibility when PUs were synthesized by substituting the PTMO soft segment with 5 and 15 wt% of PDMS [4]. Increasing molecular

### Table 2

<table>
<thead>
<tr>
<th>Common macrodiols used as soft segment</th>
<th>Common diisocyanates</th>
<th>Common chain extenders</th>
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</thead>
<tbody>
<tr>
<td>Polytetramethylene oxide (PTMO)</td>
<td>Methylene bis(p-phenyl isocyanate) (MDI)</td>
<td>Ethylene glycol</td>
</tr>
<tr>
<td>Polyethylene oxide (PEO)</td>
<td>Methylene-bis(p-cyclohexyl isocyanate) (H13MDI)</td>
<td>1,4-Butane diol</td>
</tr>
<tr>
<td>Polypropylene oxide (PPO)</td>
<td>Toluene diisocyanate (TDI)</td>
<td>Ethylene diamine</td>
</tr>
<tr>
<td>Hydroxyl-terminated polybutadiene (HTPB)</td>
<td>1,5-Naphthalene diisocyanate (NDI)</td>
<td>Hexane diol</td>
</tr>
<tr>
<td>Polyhexamethylene carbonate glycol</td>
<td></td>
<td>1,4-Butane diamine</td>
</tr>
<tr>
<td>Polycaprolactone</td>
<td></td>
<td></td>
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### Table 3

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Biomer™ (Ethicon)</td>
<td>Polyether urethane urea</td>
</tr>
<tr>
<td>Pellethane® TPU (Lubrizol) (Dow)</td>
<td>Aromatic polyether urethane</td>
</tr>
<tr>
<td>Bionate® PCU (DSM)</td>
<td>Polycarbonate polyurethane</td>
</tr>
<tr>
<td>BioSpan® SPU (DSM)</td>
<td>Segmented polyurethane</td>
</tr>
<tr>
<td>CarboSil® TSPCU (DSM)</td>
<td>Silicone-poly carbonate-urethane</td>
</tr>
<tr>
<td>ChronoSil®</td>
<td>Silicione-poly carbonate-urethane</td>
</tr>
<tr>
<td>Elastane™ TPU (DSM)</td>
<td>Thermoplastic polyether-urethane</td>
</tr>
<tr>
<td>PurSil® TSPU (DSM)</td>
<td>Thermoplastic silicone-poly ether-urethane</td>
</tr>
<tr>
<td>Tecoflex HR™ (Thermedics)</td>
<td>Aliphatic polyether urethane</td>
</tr>
<tr>
<td>Cardiothane-51™ (Kontron)</td>
<td>Polyether urethane modified with PDMS</td>
</tr>
<tr>
<td>Corathane® (Corvita)</td>
<td>Polycarbonate polyurethane</td>
</tr>
<tr>
<td>Rimplast™ (Petrarch Systems)</td>
<td>Silicone-polyurethane mix</td>
</tr>
</tbody>
</table>
weight of various soft segments in polyurethaneureas (PUUs) impacted the blood compatibility of these polymers due to creating a more microphase-separated structure. This indicated that the hydrophilic and hydrophobic balance of PUU film surfaces could affect their blood compatibility [5].

Incorporating ionic groups into segmented PUs to mimic the structure of heparin, a known anticoagulant, has widely been investigated. Substituting urethane hydrogen with sulfonate groups in PU resulted in lower platelet adhesion and activation but higher fibrinogen adsorption [6]. Use of sulfonated chain extenders in PU synthesis has also been investigated. These studies, however, have not been conclusive in evaluating blood compatibility. In PTMO-based PU, sulfonation resulted in decreased platelet activation but higher fibrinogen adsorption whereas in PEO-based PU, sulfonation caused both enhanced fibrinogen adsorption and platelet activation [7, 8]. Another study showed increased fibrinogen adsorption, platelet adhesion, and albumin adsorption but prolonged thrombin times for PUs synthesized with sulfonated chain extenders [9, 10]. Reduced platelet adhesion was observed on PUs with carboxylated chain extenders compared with PUs with sulfonated chain extenders [11]. Protein adsorption and platelet adhesion was studied on PUs with carboxylate groups grafted into their soft segment [12]. This work showed that the protein adsorption and platelet adhesion were dependent on the graft chain length with the thiopropionic graft being the most hemocompatible followed by thiosuccinic and thioglycolic grafts. Polybutadiene-based PUs with carboxylate groups incorporated into their soft or hard segment were synthesized and were investigated for protein adsorption and platelet adhesion [13, 14]. This study showed that for these types of PUs, and in the absence of known protein-resistant chains such as PEO, ion-containing PUs did not show low protein adsorption and platelet adhesion compared with the non-ion-containing PU.

Blood-polymer interactions mainly take place on the surface. Hence, many studies have been focused on improving the interactions on surfaces while preserving bulk properties of the PU biomaterials. As mentioned previously (in Sect. 1), one of the main strategies in this regard, is to create “bioinert” or “nonfouling” surfaces by the attachment of hydrophilic or other protein-resistant molecules to the surface of PUs.

In one study, PU surfaces modified with PEG and PEG-SO$_3$ were compared with unmodified PU in terms of fibrinogen and albumin adsorption from plasma [15, 16]. PU grafted with PEG only (no sulfonate groups) was found to have the least fibrinogen and albumin adsorption compared to the other surfaces. In another study, PUU was modified with hydroxyl- and amino-terminated PEGs of different chain lengths [17, 18]. A decrease in protein adsorption was observed with an increase in PEG chain length. Amino-terminated PEG surfaces showed lower protein adsorption compared with hydroxyl-terminated PEG. Although PEG modification reduced protein adsorption, the reduction was independent of protein size or charge. When PU substrate containing monobenzyloxy PEG was modified with PEG and monomethoxy PEG as fillers, higher protein adsorption was observed [19]. This study concluded that backfilling with PEG caused the benzyloxy-containing PEG chains to stretch, which resulted in higher protein adsorption.
Use of other protein-resistant molecules such as poly(oligo(ethylene glycol) methacrylate) (poly(OEGMA)) has also been explored. Attachment of poly (OEGMA) on PU through atom transfer radical polymerization (ATRP) significantly reduced fibrinogen adsorption [20]. Similarly, zwitterionic compounds such as sulfobetaine, carboxybetaine, and phosphorylcholine have been shown to reduce protein adsorption and to improve antithrombogenic properties of PU [21]. PU surfaces modified with poly(2-methacryloyloxyethyl phosphorylcholine) (poly (MPC)) using ATRP showed significantly lower fibrinogen and lysozyme adsorption compared with nonmodified surfaces [22]. Another study showed reduced protein adsorption and platelet adhesion on poly(carbonate urethane)s containing fluorinated alkyl phosphatidylcholine side groups [23]. Yuan et al. reported decreased platelet adhesion on PU modified with sulfobetaine [24–26]. In an additional study, they used a three-step functionalization reaction to introduce carboxybetaine to PU surfaces and showed low platelet adhesion on the modified surfaces [27].

Approaches to modify the surface of PUs by attaching functional or “bioactive” molecules have also been frequently studied as a means to improve blood compatibility. This strategy is often combined with the approach to provide a bioinert layer, for example, with PEO grafting on the PU surface. These bioinert molecules can then provide functional groups to attach bioactive molecules of interest, as will be described in the studies detailed below.

Immobilization of anticoagulants on the surface of PU is frequently used to prevent thrombus formation. As discussed previously, the anticoagulant heparin has been used as a surface modifier on various polymeric materials due to its ability to catalyze thrombin inhibition. Heparinization of PU has been extensively studied and there have been various different approaches taken to create antithrombogenic heparin materials on a range of PUs [28, 29]. Immobilization of different types of heparin on PU has also been explored. Low-molecular-weight- (LMWH) and unfractionated heparin (UFH) were compared for their thrombogenicity in terms of protein and platelet interactions on PU [30]. Both LMWH and UFH modified PU surfaces demonstrated less protein adsorption and platelet adhesion than the PU controls, but variations were observed with molecular weight, particularly at shorter incubation times. An important factor in attaching bioactive molecules to polymer materials is maintaining functionality of the molecule of interest, and this can be especially important for heparin due to its need to bind antithrombin through its active pentasaccharide sequence. Liu et al. used spacer chains of different lengths and terminal functional groups to immobilize heparin to a PUU [31]. Thrombus formation on the heparinized PU was less than on controls and the adhesion and activation of platelets was suppressed.

Molecules such as PEO can also be used as spacer molecules for attachment of bioactive moieties in addition to providing a passivating layer. Various studies have investigated the combination of both PEO and heparin modification on PU [32–34]. Park et al. used PEO of different chain lengths to attach heparin onto a segmented PUU (Biomer) [35]. Protein adsorption and platelet adhesion were reduced and heparin bioactivity was enhanced with the spacers. Furthermore, a prolongation of occlusion times with immobilized heparin demonstrated the ability to create a thrombo-resistant material.
As an alternative to heparin, a covalent antithrombin-heparin (ATH) complex was developed and various studies have immobilized it on PU substrates to provide active anticoagulant function. Attachment has been through direct covalent grafting [36] and also through a base coat applied to PU catheters and grafts with PEO [37–39]. Additional studies have investigated the chemical and physical properties of ATH-modified PU [40] and have also explored the grafting of PEO with a range of MWs to elucidate the mechanisms of protein and platelet interactions [41, 42]. Overall, ATH-modified PU has demonstrated the ability to provide both noncatalytic and catalytic thrombin inhibition and shows potential as an anticoagulant surface in a range of blood contacting applications.

Surface modification of polyurethane with the direct thrombin inhibitor hirudin has also been explored. Recombinant hirudin was covalently immobilized on a poly(carbonate) urethane surface and a vascular graft using an albumin basecoat [43, 44]. In both studies, the PU modified with hirudin showed greater thrombin inhibition and binding than controls and was able to maintain potent antithrombin activity.

Promoting fibrinolysis on PU surfaces has been investigated as a method to lyse clots and decrease thrombosis [45–47]. Chen et al. created dual functioning PU surfaces by combining PEG grafting, to provide resistance to nonspecific protein adsorption, and immobilizing lysine, to promote plasminogen binding. Fibrinogen adsorption was significantly reduced on the modified PU and plasminogen bound to the surfaces with high selectivity [48]. Although the surfaces also bound tissue plasminogen activator (t-PA) from plasma and were able to lyse clots, a greater concentration of t-PA may be required. Wu et al. continued this work and loaded PU with t-PA as an exogenous plasminogen activator source. The rapid release of t-PA into plasma was confirmed and formed blood clots were dissolved [49]. These methods demonstrate significant potential for reducing thrombus formation by clot lysis on PU materials in contact with blood.

Another strategy that involves modification of PU to provide an active function is nitric oxide release and generation for inhibiting platelet adhesion and aggregation. There have been many different chemistries studied to incorporate these materials in PUs for a wide range of applications, and they have particular advantages in contact with blood for catheters and extracorporeal circuits. This area has been discussed in detail in a recent book chapter [154].

### 2.2 Silicones

Silicones consist of a chemical backbone with alternating silicon-oxygen bonds and organic groups attached to each silicon. The most common silicone elastomer is polydimethylsiloxane (PDMS), and it has significant use in many areas including the biomedical field (Fig. 6). Common applications where silicones are used in contact with blood include catheters, blood pumps, heart valve structures, pacemaker leads, devices for extracorporeal circulation (ECC), including blood oxygenators and tubing, as well as microfluidic devices. PDMS has also been used as a primary
reference material in hemocompatibility, biocompatibility, inflammatory response, and in vivo studies [50].

Some of the main advantages of silicones are their thermal and chemical stability, low toxicity, oxygen permeability, ease of processing, and low cost. However, as biomaterials, in particular for blood contacting applications, their high degree of hydrophobicity is a disadvantage, since it promotes interactions at the blood-material interface including protein adsorption, platelet adhesion, red blood cell lysis, and other potentially adverse responses. To reduce the risk of thrombosis, systemic anticoagulation is typically needed with the use of silicone devices.

In order to improve their compatibility with blood, silicones can be modified using various strategies to alter their surface properties. These modification methods can be classified as surface (chemical and physical), bulk (blending, copolymerization, IPNs, functionalization), and other methods as reviewed by Abbasi et al. [51]. Since silicones do not naturally possess suitable functional groups for subsequent attachment of molecules of interest, in many cases they must first be activated. Some techniques that have been used to functionalize and modify silicone surfaces will be described in further detail below.

PDMS rubber has been modified by an irradiation and grafting method to create materials with a range of wettabilities for comparing relative blood compatibilities [52]. PDMS surfaces were treated with a CO$_2$-pulsed laser, creating superhydrophobic surfaces, and also grafted with hydroxyethylmethacrylate phosphatidylcholine, resulting in superhydrophilic surfaces. The two extreme hydrophobic and hydrophilic surfaces demonstrated a reduction in platelet adhesion and activation compared to the control PDMS.

Zwitterionic polymers, including sulfobetaine and carboxybetaine, can provide nonfouling characteristics to PDMS and have been investigated for their ability to reduce nonspecific protein adsorption. PDMS was functionalized with carboxybetaine and blended with PDMS elastomer to create films. These materials demonstrated reduced protein adsorption (bovine serum albumin), bacterial adhesion and show promise as antifouling surfaces in various applications, including blood contacting devices [53]. Carboxybetaine groups were also covalently grafted on the surface of Si-H functionalized PDMS through a hydrosilylation reaction. These modified materials displayed a reduction in bovine serum albumin adsorption, a prolonged dynamic clotting time, and were able to decrease bacterial adhesion, demonstrating their antifouling properties [54].
As with many other polymers, modification with polyethylene oxide (PEO) has been studied extensively to provide greater hydrophilicity on PDMS. Chen et al. have completed numerous studies where silicones have been modified with different PEO polymers. PDMS-based elastomers were prepared by incorporating monofunctional PEO during curing with classic room temperature vulcanization chemistry \[55\]. PDMS with PEO was able to decrease fibrinogen adsorption from both buffer and plasma. Further protein adsorption studies were completed on PDMS incorporated with both mono- and bifunctional PEO using a similar rubber formation process \[56\]. Again, reductions in protein adsorption (fibrinogen, albumin, and lysozyme) were observed with the addition of PEO to PDMS. Compared to the controls and the bifunctional PEO surfaces, the monofunctional modification showed greater protein selectivity.

PEO of various molecular weights and functionality have also been grafted to PDMS after introducing a high density of Si-H groups on the surface by acid-catalyzed equilibration in the presence of methylhydrosiloxane (MeHSiO)\(_n\) and linking PEO by a platinum-catalyzed hydrosilization reaction \[57\]. These surfaces demonstrated protein-resistant properties due to their ability to reduce fibrinogen adsorption from buffer and plasma, and albumin from buffer. Reductions of greater than 90% fibrinogen adsorption were achieved on the modified PDMS versus the controls. This modification method was extended to a generic process where after functionalization of PDMS with Si-H groups, hydrosilylation with PEO-NSC then provides activated esters that various biomolecules containing amines can readily react, including proteins, peptides, and glycosaminoglycans such as heparin \[58\]. For blood contacting applications, heparin was successively grafted to the PEO functionalized PDMS and covalently bound antithrombin providing effective thromboresistant properties.

A unique antithrombin-heparin (ATH) covalent complex was immobilized on PDMS using PEO as a linker, providing an anticoagulant surface in contact with blood \[59\]. Using thromboelastography ATH-modified PDMS was shown to delay the initiation of coagulation and clotting time. Polydopamine was also used as a bonding agent in additional studies investigating ATH-modified PDMS in the design of microfluidic blood oxygenators \[60\].

Surfaces with both resistance to nonspecific protein adsorption and fibrinolytic properties have been obtained with modification of PDMS. A PEG spacer was used to attach ε-lysine for selective adsorption of plasminogen from plasma. The modified surfaces significantly reduced the adsorption of fibrinogen, were able to bind plasminogen, and through conversion to plasmin effectively dissolved fibrin clots \[61\].

Platelet adhesion and activation can be naturally inhibited by nitric oxide released from endothelial cells. As with other polymers, this idea has been applied to silicones to create nitric oxide releasing surfaces. Zhang et al. used a three-step process to synthesize nitric oxide releasing silicone rubbers with covalently linked diazeniumdiolate groups. The materials were coated on the inner walls of silicone rubber tubing for extracorporeal circulation (ECC) in a rabbit model. The nitric oxide releasing materials showed a decrease in platelet consumption and platelet
activation and overall had improved thromboresistance [62]. In a similar rabbit model, a solvent swelling method based on S-nitroso-N-acetylpenicillamine (SNAP) was used to load silicone rubber tubing with nitric oxide [155]. The SNAP-loaded silicone rubber ECC circuits achieved efficient release of nitric oxide, preserving the blood platelet count at 64% of baseline and providing a 67% reduction in thrombus formation compared to controls. The results suggest that these modifications have the potential to improve the hemocompatibility of silicones used in blood contacting devices.

2.3 Polytetrafluoroethylene (PTFE; ePTFE)/Teflon

Polytetrafluoroethylene (PTFE), also known as Teflon, is a thermally and chemically stable, hydrophobic polymer (Fig. 7). The main application of this polymer in blood contacting devices has been for catheters and vascular grafts. PTFE was developed by DuPont in 1938 and was marketed under the trade name Teflon. Later on, W.L. Gore & Associates Inc. introduced expanded PTFE (ePTFE). ePTFE is more porous than regular PTFE and is used as prosthetic vascular conduits.

In vascular surgery, use of autologous saphenous vein as the graft is desirable. When this choice is not available for reasons such as insufficient size or length, prosthetic grafts are used. Along with polyethylene terephthalate (PET)/Dacron, ePTFE is the most common polymer used in this application. ePTFE has shown better performance compared with Dacron when used in certain locations in the body [156, 157]. Examples of commercially available ePTFE-based grafts include the following: Gore-Tex (W.L. Gore & Associates, Inc.); VascuGraft (B. Braun Medical Inc.); Exxcel Soft Vascular Graft (Boston Scientific); Impra CenterFlex (Bard Peripheral Vascular); Exxcel Soft (Atrium Medical Corporation), which are all unmodified; Gore Propaten (W.L. Gore & Associates, Inc.), which is heparin modified with Carmeda Bioactive Surface (CBAS); Impra Carboflo, Venaflo II, Distaflo, Dynaflo (Bard Peripheral Vascular), which are all carbon coated, Advanta VXT, Advanta SST, Advanta Supersoft (Atrium Medical Corporation), which are all reinforced; Lifespan (Angiotech/Edwards Life Sciences), Rapidax (Vascutek Ltd.), Advanta SST (Atrium Medical Corporation), which are all trilaminar; and Maxifo, SealPTFE, Taperflo, CannulaGraft (Vascutek Ltd.), which are all unsealed and gelatin sealed [63]. Most of these are either approved or in clinical trials for peripheral bypass and hemodialysis applications.

**Fig. 7** PTFE structure
Despite the successful performance of ePTFE when used as a large diameter vascular graft, it is prone to failure as a small diameter graft (DI < 6 mm). This failure happens due to thrombus formation as well as mismatch between the graft and the native vessel. The mismatch causes the development of neointimal hyperplasia. Efforts to improve the performance of ePTFE grafts range from antithrombotic surface modifications and coatings to endothelial cell seeding and nitric oxide modification.

Surface coating of ePTFE with polymers, proteins, and other molecules known to improve blood compatibility has been studied by a number of researchers. Yang et al. modified ePTFE grafts with poly(1,8-octanediol citrate), a biodegradable polymer, and studied the biocompatibility of these grafts in vitro [64]. They observed delayed plasma clotting as well as enhanced attachment of porcine endothelium-like cells on modified grafts compared with nonmodified ones. In a study by Sato et al. [65], ePTFE films were coated with poly(2-methoxyethyl acrylate) (PMEA) and seeded with human umbilical vein endothelial cells or aorta smooth muscle cells. Both of these cells adhered and proliferated well on these films while platelets did not adhere, showing promise for applications involving endothelialization and the development of blood vessels. In another study, Karrer et al. modified ePTFE with polypropylene sulfide-PEG and showed decreased thrombogenicity on modified grafts in a porcine shunt model [66]. Photopolymerization of an acrylate phospholipid on ePTFE grafts showed low platelet adhesion and fibrinogen adsorption in a baboon shunt model [67]. Jin et al. functionalized ePTFE capillaries with poly(vinyl alcohol) (PVA)—glycidyl methacrylate (GMA) copolymers. This was followed by immobilization of human serum albumin (HSA) onto the functionalized surfaces. Their data showed a sharp suppression of platelet adhesion on PVA coated and PVA–GMA–HSA coated PTFE capillaries [68]. The protein, P15, a cell-binding peptide, has also been attached to ePTFE grafts. These grafts exhibited a higher amount of endothelialization in a sheep model [69]. In another study, Rotman et al. coated ePTFE grafts with anti-CD34 antibodies, a protein that captures circulating endothelial progenitor cells and showed improved endothelialization on these grafts after 72 h in vivo [70]. Among other molecules used for ePTFE graft modification is carbon. Kapfer et al. compared carbon impregnated ePTFE vascular grafts with standard unmodified grafts in patients but observed no statistical difference in patency [71].

Several studies have used bioactive reagents with antithrombotic properties to modify ePTFE grafts. The most widely studied and commercially available reagent in this regard is heparin. One of the most known commercially available ePTFE grafts modified with heparin is the Gore-Tex Propaten graft by W.L. Gore. The modification of this graft is done by the covalent end-point attachment of heparin (Carmeda BioActive Surface, CBAS). A few studies have looked at these heparin-modified ePTFE grafts in a canine model. Significant reductions in platelet deposition and thrombus formation were observed on heparin-coated grafts [72, 73]. In a clinical study carried out on 86 patients, Carmeda Bioactive modified ePTFE grafts showed an improved outcome compared with unmodified ePTFE grafts [74]. A more recent clinical study with 569 patients concluded that the overall risk
of primary graft failure was significantly reduced (by 37%) on these heparin-modified ePTFE grafts [75].

Modifying ePTFE with heparin through other methods has also been studied. ePTFE tubes were modified with aminated poly(1,8-octanediol-co-citrate) (POC) via POC carboxyl functional groups [76]. Heparin was then attached to the amino terminal of the POC. POC-heparin-coated ePTFE grafts showed significant reduction in platelet adhesion and performed better in whole blood clotting kinetic studies. Lu et al. coated ePTFE grafts with an anti-CD133 antibody-functionalized heparin/collagen multilayer [77]. Prolonged coagulation time and less platelet activation and aggregation was observed on modified ePTFE grafts compared with the bare ePTFE grafts. Furthermore, these modified surfaces showed a higher rate of endothelialization in a porcine carotid artery transplantation model. Zhu et al. studied ePTFE grafts modified with a chitosan/heparin complex [78]. These grafts exhibited reduced platelet adhesion in vitro and no clot formation 2 weeks post-implantation in a canine vein model. Greisler et al. used a combination of fibroblast growth factor-1/fibrin glue with heparin to improve ePTFE graft performance [79]. They observed greater endothelialization on these grafts compared with control grafts in a canine model.

Other antithrombotic molecules explored for ePTFE modification are hirudin and tissue plasminogen activator (t-PA). Heise et al. used hirudin in combination with iloprost (a vasodilator and an inhibitor of platelet aggregation) along with PEG to modify ePTFE [80]. They demonstrated improved blood flow in modified grafts and less restenosis compared with unmodified ePTFE. Greco et al. modified ePTFE grafts with a tissue plasminogen activator-iloprost combination and observed improved patency in a rat model [81].

Another approach to improve the blood compatibility of ePTFE grafts has been surface modification by seeding endothelial cells. Endothelial cells (ECs) are a natural lining of the blood vessels and secrete substances that prevent thrombosis and neointimal hyperplasia. In most studies utilizing this approach, ECs were isolated from the artery or vein of a patient, expanded in cell culture, and seeded onto the graft. In a clinical study by Deutsch et al., the EC-seeded ePTFE grafts showed significantly better patency than a control group after 9 years [82]. Magometschnigg et al. investigated the EC-seeded grafts for crural reconstruction. These grafts exhibited 39% improvement in patency after 30 days compared with the control nonmodified ePTFE grafts [83]. Laube et al. used a similar technique by seeding venous endothelial cells onto ePTFE grafts for coronary arteries. Their results demonstrated that 91% of the grafts used (out of 21 grafts) were patent after 28 months [84]. Griese et al. coated ePTFE with fibronectin and modified it further with endothelial progenitor cells (EPCs). Rapid endothelialization and reduced neointima deposition on these grafts were observed in a rabbit model [85].

Endothelial cells in the body are constantly producing nitric oxide (NO), which is essential for vascular regulation. Furthermore, NO prevents platelet aggregation, a necessary requirement for blood compatibility. Thus, modification of vascular grafts with NO to improve graft patency has been investigated [86, 158]. In a study by Pulfer et al., pores of ePTFE grafts were modified with polymeric diazeniumdiolate.
polyethyleneamine/NO microspheres. These grafts exhibited NO release in vitro for more than 150 h [158]. There is potential for this technique to produce grafts with improved resistance to thrombosis.

2.4 Polyethylene Terephthalate (PET)/Dacron

Polyethylene terephthalate (PET) is one of the most common polymers of the polyester family (Fig. 8). It is frequently referred to by its trade name Dacron, which is PET manufactured into fibers that can be in knitted or woven form. When PET is in film form, it is often called Mylar, also a DuPont trade name. The aromatic rings in the backbone of PET and its stiff polymer chains provide it with numerous advantages including a high melting point, excellent tensile strength, toughness, fatigue resistance, and chemical stability. When in fiber form, PET also has good crease and abrasion resistance [87].

PET has been used for many years in blood contacting medical device applications, most commonly for vascular graft prostheses. It has also been used for sewing rings on artificial heart valves, fabrics for fixation of implants, surgical mesh, and as a catheter cuff material. As an artificial blood vessel, PET has been successful for large and medium diameter sites with high flow, however, similar to PTFE, in small diameter graft applications, such as coronary artery bypass, there are major issues. Acute graft failure can occur from surface-induced thrombogenic events and delayed graft failure results from the development of intimal hyperplasia along with thrombosis.

In order to overcome these problems, a range of strategies to modify PET have been explored including physical adsorption through coatings and chemical coupling to the surface. Since PET does not possess any chemically active functionality, appropriate functional groups must first be introduced on the surface to achieve covalent grafting. Molecules that have been immobilized on PET include those that provide a passivating effect, such as PEO, and those with a particular active function, such as anticoagulants.

Studies have investigated a range of methods of immobilizing PEO on PET. Desai and Hubbell used a solution technique to incorporate PEO molecules of various molecular weights (MWs) into several biomedical polymers including PET. PEO-modified PET of MW 18,500 showed a substantial decrease in albumin adsorption compared to the PET control. Platelet adhesion was reduced to a large extent with this MW PEO and to a less extent with PEO of lower and higher MWs [88]. These authors also covalently grafted PEO to the surface of PET and investigated

![PET structure](image)
biological responses [89]. High MWs of PEO, specifically 18,500, again demonstrated decreases in albumin adsorption, along with fibrinogen adsorption, and platelet adhesion compared to the control. Gombotz et al. also covalently grafted various MW PEO molecules to better understand the mechanisms of low protein adsorption. In this study, it was found that a lower surface density of high MW PEO was more effective in providing decreases in protein adsorption than a higher density of low MW PEO [90].

Other methods of immobilizing PEO containing molecules on the surface of PET have been explored including modification with block copolymers and polymer brushes. A two-step procedure was used to graft two types of PEO triblock copolymers to PET, first PEO-polybutadiene-PEO (PEO-PB-PEO), and then the commercially available Pluronic PEO-poly(propylene oxide)-PEO (PEO-PPO-PEO) [91]. The surface was primed with PEO-PB-PEO to create a double bond rich layer and PEO-PPO-PEO was then coated on the surface followed by γ-irradiation. PEO-grafted surfaces showed a decrease in fibrinogen of more than 90% compared to control PET along with a reduction in platelet adhesion. PEO-containing polymer brushes have also been grafted onto PET. Li et al. utilized surface initiated atom transfer radical polymerization (SI-ATRP) to tether various chain lengths of poly(ethylene glycol) methacrylate (PEGMA) to the surface of PET. The relationship between polymer chain length and resistance to protein adsorption was assessed and a reduction in fibrinogen adsorption was achieved on PET surfaces grafted with PEGMA [92].

In early years, collagen and gelatin were used to coat PET grafts. However, these grafts have continued to cause issues due to their high thrombogenicity. Albumin coatings on PET grafts have also been explored for many years. The use of albumin as a surface modifying agent has been investigated due to its ability to provide a relatively inert passivating layer. An in vitro study of an albumin coating on Dacron arterial prostheses was carried out to assess thrombogenicity in terms of platelet activation, fibrin formation, and leucocyte interactions [93]. The albumin-coated surfaces prevented platelet accumulation on the surface, diminished coagulation activation and fibrinopeptide A formation, and had minimal leucocyte deposition on the surface. There have been several albumin-coated PET grafts used clinically including the Bard albumin-coated DeBakey Vascular® II and the albumin saturated, autoclaved DeBakey Soft Woven graft [94]. Marois et al. performed in vivo studies to compare the Bard albumin-coated vascular graft (ACG) to the uncoated Vascular® II. With the albumin-coated graft, there was no increased presence of an immune reaction and the healing response was equivalent to the uncoated prosthesis after albumin was resorbed. There was a decrease in platelet and fibrin uptake on the luminal surface of albumin-coated grafts; however, the differences were small [95].

The widely used anticoagulant, heparin has been attached to PET to provide it with bioactive functionality. To immobilize heparin, Kim et al. exposed PET to oxygen plasma glow discharge and the resulting peroxides on the surface were used to graft acrylic acid. PEO was then coupled to the surface to act as a spacer and for subsequent attachment of heparin and insulin [96]. On the surfaces that contained heparin, the plasma recalcification time and activated partial thromboplastin time
were prolonged and a decrease in platelet adhesion was observed. Various heparin-modified grafts have been commercially available, including the InterVascular Hemaguard grafts and InterGard Knitted Heparin-Bonded Vascular Prosthesis by InterVascular [63]. Clinical studies with the Intervascular Hemaguard heparin-bonded collagen-coated grafts have been reported [97]. For infrainguinal bypass, these grafts produced comparable primary patency rates to other synthetic grafts. When used for femoropoliteal bypass grafts, collagen-coated heparin-bonded Dacron from InterVascular were found to have significantly better patency rates than PTFE at 3 years, but no difference at 5 years [98].

Another anticoagulant that has been immobilized on PET is the highly specific direct thrombin inhibitor, hirudin. Phaneuf et al. modified the surface of PET fibers using alkaline hydrolysis to create carboxyl groups and attach a bovine serum albumin (BSA) base coat. Recombinant hirudin (rHir) was then covalently linked to the modified surface and thrombin inhibition was assessed [43]. The PET with immobilized rHir had significantly greater thrombin adhesion and high thrombin inhibition compared to controls. Studies were continued and these r-Hir-BSA-PET vascular grafts were evaluated to determine the stability of surface bound rHir and the interaction of thrombin-rHir in an in vitro flow model [99]. The grafts coated with rHir demonstrated their stability and reduced local thrombin concentration under physiological flow. A further in vivo assessment of PET with covalently linked rHir was carried out with patches implanted in a canine thoracic aorta under high flow and shear rates [100]. After exposure to nonheparinized arterial blood flow for 2 h, the Dacron-BSA-r-Hir explanted patches demonstrated retained antithrombin activity, and compared to controls, had a much thinner pseudoinima of proteins and platelets along with significantly less thrombus formation on the graft surface. These studies have shown potential for covalent linkage of biologically active proteins like hirudin to the surface of PET vascular grafts.

Another study coated a polydopamine mussel adhesive inspired layer on PET films to then covalently graft zwitterionic cysteine [101]. Following surface characterization of the materials, protein adsorption, platelet adhesion, and hemolytic testing were carried out. The cysteine-modified PET demonstrated a significant decrease in BSA adsorption, reduced platelet adhesion and aggregation, along with a low hemolysis rate.

Various studies have utilized nitric oxide’s ability to inhibit platelet adhesion through particular surface modifications on PET. Duan and Lewis covalently grafted L-cysteine to PET to promote NO transfer to the polymer and release from it. In this study, platelet adhesion on the cysteine-modified PET was reduced by more than 50% compared to the control [102]. In a subsequent study, various thiol-containing groups, including those with L-cysteine moieties and more L-cysteine sites, were attached to PET and compared for their ability to promote NO transfer and release [103]. All polymers demonstrated a significant reduction in platelet adhesion with L-cysteine having the greatest decrease.

L-arginine immobilization has also been investigated for its potential ability to release NO when in contact with blood leading to a reduction in thrombus formation. Studies have covalently attached L-arginine to the surface of PET using
glutaraldehyde as a crosslinker [104, 159]. In initial work, successful surface modification of PET with L-arginine was confirmed and the modified films demonstrated a slight reduction in protein adsorption and significant decreases in platelet adhesion and thrombus formation [159]. Further investigations with L-arginine immobilized on PET looked at various properties of anticoagulant activity and hemolysis [104]. A blood clotting test showed good antithrombogenic activity for modified PET and in clotting time assays the plasma recalcification time (PRT) and activated partial thromboplastin time (APTT) were both prolonged compared to unmodified PET. The hemolysis ratio for L-arginine modified PET was found to be low indicating that when the surfaces contact blood, red blood cells remain intact. Overall, these modifications on PET show promise for blood contacting medical device applications.

2.5 Polyvinylchloride (PVC)

Polyvinyl chloride (PVC), also known as polychlorehene, is a thermoplastic polymer made by addition polymerization of vinyl chloride monomer (Fig. 9). Additives, known as plasticizers, are typically added in biomedical applications to make this hard polymer soft and flexible. The ease of sterilization as well as the flexibility of the plasticized PVC has made this polymer a good candidate for blood contacting devices, in applications such as catheters and blood bags. The plasticizers, however, can leach out of the polymer in vivo, which leads to adverse effects such as inflammation.

Examples of commercially available PVC blood bags include the following: Transfufol3226 (Cerus Corporation); PL146 and PL 1813 (Fenwal Inc.); Compoflex® (Fresenius Kabi); CPD bag (Grifols); A (MacoPharma); Standard PVC (Pall Corporation); XT-150 and bags with different volumes (TerumoBCT) which are all made of PVC with di(2-ethylhexyl)phthalate (DEHP) as the plasticizer; PL2209 (Fenwal Inc.); Compoflex® (Fresenius Kabi); CPP (Haemonetics); B (MacoPharma), which is made of PVC with butyryl-tri-n-hexyl-citrate (BTHC) as the plasticizer; B (MacoPharma), which is made of cyclohexane 1,2-dicarboxylate (DINCH); PL1240 (Fenwell Inc.); and Compoflex® (Fresenius Kabi), Transfer bag (Grifols), A (MacoPharma), ELX (Pall Corporation), which are all made of PVC with tris-octyl tri-mellitate (TOTM) as the plasticizer [160]. PVC bags can be used to store blood components such as red blood cells, platelets, and plasma. The plasticizer used in such applications has typically been di(2-ethylhexyl)phthalate (DEHP). Reduced red cell haemolysis has been observed when blood bags were made of.

![PVC structure](Fig. 9 PVC structure)
DEHP-plasticized PVC compared to storage in glass. Exposure to DEHP has also shown to cause a range of adverse effects such as toxicity in animal models. In 2002, the FDA issued a notification regarding the exposure to DEHP-plasticized PVC. In recent studies, DEHP is labeled as a category 2 toxic compound, with recommendations on finding an alternative additive to replace this plasticizer in certain applications [105].

In order to minimize or eliminate the problems associated with DEHP-plasticized PVC, two main approaches have been explored. The first approach focuses on modification of DEHP-plasticized PVC to improve blood compatibility of this polymer and to reduce DEHP migration and release. Surface modification of DEHP-plasticized PVC is one of the strategies employed by researchers to reduce DEHP migration. One study showed that sulfonated DEHP-plasticized PVC has reduced DEHP migration compared to a nonsulfonated version of this polymer [106]. In contact with blood, the sulfonated polymer also exhibited decreased inflammatory response in vitro and in rodent models. Another study looked into improving blood compatibility of DEHP-plasticized PVC via surface heparinization [107]. This modification technique caused decreases in fibrinogen and Factor XII adsorption, and improvements in thrombin-antithrombin (TAT) complex and complement component C3a generation. Furthermore, lower content of DEHP at the surface of the modified polymer was reported. Gamma radiation was explored as another technique to modify DEHP-plasticized PVC surfaces [108]. Higher doses of gamma radiation were shown to minimize the amounts of leached DEHP. Bulk modification of DEHP-plasticized PVC has also been studied. Yu et al. attempted to stabilize DEHP by incorporating 2,3,6-per-O-benzoyl-β-cyclodextrin (Bz-β-CD) [109]. This modification technique had no negative impact on physical properties of DEHP-plasticized PVC but decreased the leaching of DEHP.

The second approach to eliminate problems associated with leaching of DEHP suggests the use of alternative plasticizers that can be safe in blood contacting applications. Di-(2-ethylhexyl)-adipate (DEHA), acetyl-tri-n-butyryl-citrate (ATBC), di-iso-nonyl phthalate (DINP), glycerides, castor-oil-mono-hydrogenated acetates (COMGHA), cyclohexane 1,2-dicarboxylate (DINCH), di(2-ethylhexyl) terephthalate (DEHT), tri-2-ethylhexyl trimellitate (TETM), tris-octyl tri-mellitate (TOTM), and butyryl-tri-n-hexyl-citrate (BTHC) are some of the main alternative plasticizers under investigation for this purpose. Information related to these alternative plasticizers is available in several review papers [110–112, 161]. Based on these reviews, studies on alternative plasticizers have tried to evaluate the alternatives based on the following criteria: how the plasticizer affects the stored blood component (most of the plasticizers have been shown to impact the storage of red blood cells); how toxic the plasticizer is; and how much of it is going to leach over time. There are, however, limited data on toxicity of DEHP and the alternative plasticizers. These reviews suggest that there is still a need for systematic toxicological studies of the alternative plasticizers in PVC. They recommend continued investigation on the development of DEHP-free PVC as well as alternative polymers that can be used in lieu of PVC in blood bag applications, especially red blood cell containers.
Another application of PVC in medical devices is in catheters and tubing, mostly for short-term purposes [113, 114, 162]. Studies have shown that in contact with blood, similar to other polymers, complications such as thrombosis on the surface of noncoated PVC can occur. Incorporation of heparin or hydrophilic polymers have been tested to improve blood compatibility and have proven to be effective in reducing thrombus formation [114, 162].

2.6 Membrane Materials: Polysulfone (PSF), Polyethersulfone (PES), Polyacrylonitrile (PAN), and Polymethylmethacrylate (PMMA)

In this section, polymers that are used as membrane materials in blood contacting applications will be discussed. The focus will be on polysulfone (PSF), polyethersulfone (PES), polymethylmethacrylate (PMMA), polyacrylonitrile (PAN), and ethylene vinyl alcohol copolymer (EVA), all materials that are widely used for hemodialysis membranes.

Hemodialysis involves the use of semipermeable membranes to filter waste products from the blood due to loss of kidney function. The membranes are usually configured as hollow fibers, which have a high surface area that contacts and interacts with blood and its components. The blood compatibility of polymers used as hemodialysis membranes is therefore extremely important.

One of the initial materials used as a membrane in hemodialysis was cellulose. Cellulose membranes, however, showed complications in contact with blood, mainly by activating the complement system as well as reducing patients’ immune function leading to infection [163–166]. Due to these complications, use of synthetic polymers such as PSF, PES, PAN, PMMA and EVA as the membrane in blood contacting applications were tested and provided evidence of minimizing or eliminating such complications [167].

Polysulfones (PSFs) are thermoplastics that contain aromatic groups joined by an SO2 group (Fig. 10). The multiple aromatic rings can be connected directly, as in the case of polysulfone (PSF), or can be joined by an oxygen, resulting in polyethersulfone (PES) (Fig. 10). Due to this structure, PSFs possess high mechanical strength, thermal stability, good chemical resistance and are able to be easily processed into flat sheets and hollow fibers. They have an asymmetric microstructure and are inherently hydrophobic. For medical applications, their ability to withstand a wide range of sterilization techniques including steam, ethylene oxide, and gamma radiation is a great advantage. However, their hydrophobicity makes them especially prone to inducing adverse reactions such as complement activation, blood coagulation, platelet adhesion and activation, and other cellular responses when in contact with blood. Since the blood compatibility of both PSF and PES membranes is insufficient, systemic administration of anticoagulants is required during their use in hemodialysis.

Polysulfone was introduced as a commercial hemodialysis membrane by Fresenius [115]. Fresenius continues to manufacture various lines of hollow
fiber membranes for hemodialysis including high performance membranes, along with both Toray and Asahi. Numerous strategies to modify PSF membranes have been investigated in order to improve their blood compatibility. Polyvinylpyrrolidone (PVP) is added during the manufacturing of PSF in order to decrease the hydrophobicity and to change pore size distribution. PSF membranes have been modified with PVP by various methods including physical blending, chemical grafting, and other surface modifications [116, 117]. PSF membranes blended with PVP were also evaluated on the nanoscale using atomic force microscopy (AFM) [118]. The study determined that the PSF/PVP surface was covered with PVP and protein adsorption greatly decreased with increasing content of PVP.

Many other methods have also focused on decreasing the hydrophobicity of PSF using hydrophilic molecules such as PEO, phophorylcholine, and other bioinert molecules. PEG was incorporated into PSF membranes through the synthesis of amphiphilic graft copolymers with PSF backbones and PEG side chains (PSF-g-PEG) [119]. These membranes were hydrophilic and exhibited high resistance to protein adsorption and cell attachment, showing potential for use in hemodialysis. In another study, PSF was coated with Pluronic™, triblock copolymers of polyethylene oxide and polypropylene oxide (PEO-PPO-PEO), with varying PEO block length [120]. Pluronic™-coated PSF membranes showed a decrease in the adsorption of plasma proteins (albumin, globulin, and fibrinogen) along with a reduction in platelet adhesion. PSF has also been blended with both PVP and PEG and these membranes were compared to a surface treatment method using trimesoyl chloride and m-phenylene diamine, and a polyacrylonitrile (PAN) unmodified membrane [121]. Although all membranes displayed similar protein adsorption, the blend and surface-modified membranes exhibited less platelet adhesion and thrombus formation indicating improved blood compatibility.

Fig. 10  PSF and PES structures
Ishihara et al. have modified PSF membranes with the phospholipid polymer 2-methacryloyloxyethyl phosphorylcholine (MPC) using a blending method. Surface characterization showed that the MPC additive was concentrated at the surface and provided a reduction in fibrinogen adsorption [122]. Further studies found that with an increase in MPC composition, the density of the adsorbed total protein from plasma decreased. The MPC modified membranes were able to reduce platelet adhesion and aggregation, demonstrating their potential for improving blood compatibility [123]. Asymmetric porous membranes were also prepared with the MPC modifications and the decreases in protein adsorption and platelet adhesion were still evident [124].

Strategies to attach functional molecules to PSF membranes have also been investigated. PSF membranes have been grafted with a layer of poly(acrylic acid) to covalently bind conjugated linoleic acid (CLA), a naturally occurring substance with antioxidant properties [125]. These CLA-immobilized membranes reduced protein adsorption and hemolysis, prolonged coagulation time, and effectively improved blood compatibility of PSF. Heparin, the well-known anticoagulant, was covalently immobilized on PSF after activation of the surface with amino groups and EDC/NHS chemistry to bind heparin [126]. The modification demonstrated increased hydrophilicity of the membranes along with improved blood compatibility, in terms of longer coagulation times and reduced platelet adhesion [126]. PSF has also been modified with both chitosan and heparin. In a study by Yang and Lin, membrane surfaces were first treated with ozone to introduce peroxides and then grafted with either acrylic acid or chitosan, followed by the covalent immobilization of heparin with glutaraldehyde [127]. The hydrophilicity of PSF membranes increased with the addition of chitosan and heparin and both protein adsorption and platelet adhesion decreased, along with a prolongation of coagulation time. A recent study utilized the popular mussel-inspired method of polydopamine coatings to graft heparin and bovine serum albumin (BSA) covalently on PSF membranes through the reactive polydopamine layer [128]. After modification, the membranes showed improved hydrophilicity, reduced protein adsorption and platelet adhesion, and extension of blood coagulation times. Overall, these methods show promise for improving the blood compatibility of PSF membranes used in hemodialysis applications.

More recently, polyethersulfone has been investigated and used as a membrane material in hemodialysis due to its advantageous properties including its strength and stability. Although surface modification can be difficult due to its stable chemical structure, a range of methods have been utilized to provide better blood compatibility including bulk modification, blending, coating, and grafting methods. Like PSF and other polymers, PVP and PEG have been added to PES to increase its hydrophilicity [129, 130]. Surface modifying macromolecules (SMMs) have also been blended with PES to create surfaces with fluorinated functional groups [130]. These materials show great potential for improving the blood compatibility of PES as well as other polymers in contact with blood [132]. Many other modification strategies for PES have been studied and several review papers discuss these in further detail [133, 168].
Polyacrylonitrile (PAN) (Fig. 11) is a semicrystalline polymer with the general formula \((C_3H_3N)_n\). It offers some advantages when used as a membrane material in hemodialysis systems, including good mechanical and thermal stability. Furthermore, its nitrile groups (−CN) can be easily modified to offer functional groups for surface modification. In hemodialysis, the PAN hollow fiber membrane allows small- to middle-size proteins to be removed from blood. Use of these membranes in blood-related systems is usually accompanied by the injection of anticoagulants. This is due to the hydrophobic properties of PAN membranes, which can cause biofouling, thrombosis, and immunoreactions in blood contacting applications. One of the early PAN membranes made was AN-69, a copolymer of acrylonitrile and sodium methallylsulfonate (made by Rhone-Poulenc, France). These membranes demonstrated improved outcomes in terms of their immune response when compared with cellulose membranes [134]. Despite wider use of PSF membranes, PAN membranes still demonstrate some advantages over PSF membranes. Easier surface modification can be achieved on PAN membranes to improve blood compatibility. Numerous research studies have been carried out in this area, mainly to increase hydrophilicity of the PAN membrane. The modifications range from incorporating hydrophilic polymers to immobilizing biomolecules.

Blending PAN with 20 wt% polyvinylidene fluoride (PVDF) increased the hydrophilicity of the membrane and was shown to reduce protein adsorption, platelet adhesion, and thrombus formation [135]. In another study, membranes made of a copolymer of acrylonitrile with N-vinyl-pyrrolidone showed protein-resistant properties compared with the blended PAN with poly (N-vinyl-2-pyrrolidone) alone [136]. Both platelet adhesion and albumin adsorption decreased with an increase in N-vinyl-pyrrolidone content in the copolymer [137]. Copolymerization with maleic anhydride or 2-hydroxyethyl methacrylate to obtain poly(acrylonitrile-co-maleic anhydride) (PANCMA) or poly(acrylonitrile-co-HEMA) (PANCHEMA), respectively has also been studied. These copolymers were specifically useful in grafting further hydrophilic polymers or biomolecules onto the surface of the membrane. Attaching polyethylene glycol (PEG) on this copolymer improved protein-resistant properties and reduced platelet adhesion and macrophage attachment [138]. Immobilization of heparin, insulin, or chitosan onto the PANCMA membrane decreased platelet and macrophage adhesion [139, 140].

Ubricht et al. modified the surface of PAN membranes using plasma treatment. They further grafted poly hydroxyl-ethylmethacrylate (PHEMA) on the plasma-treated surfaces. Both plasma-treated and poly(HEMA)-grafted surfaces showed
reduced protein adsorption [141]. Ulbricht et al. also grafted polymerized hydrophilic monomers such as acrylic acid, 2-hydroxymethyl methacrylate and poly (ethylene glycol) methacrylate on PAN membranes using UV radiation graft polymerization. They observed very little bovine serum albumin adsorption on these surfaces. Another approach to modify the PAN membranes took advantage of hydrolyzing nitrile groups of PAN into carboxyl groups. These carboxyl groups can then be further used to attach biomolecules. Yang and coworkers used this strategy to attach a chitosan/heparin complex onto PAN membranes. This modification provided a reduction in protein adsorption, platelet adhesion, and thrombus [142]. The same technique was also used to graft conjugated linoleic acid onto the PAN surface. These modified membranes showed increased coagulation time in vitro compared with the unmodified PAN membrane [143]. In another study, Yang and coworkers immobilized platelet-adhesion promoting collagen (COL) and platelet-inhibiting human serum albumin (HSA) onto PAN membranes [169]. HSA-modified membranes demonstrated lower platelet adhesion, longer blood coagulation time, and higher thrombin inactivity levels, as well as lower complement activation compared with the unmodified and COL-modified PAN.

Polymethylmethacrylate (PMMA) is a synthetic polymer with the structure shown in Fig. 12. Its use as a membrane in hemodialysis has been of interest due to its good solute permeability and relatively good biocompatibility [170, 171]. Several studies have shown the advantages of using PMMA over other synthetic membranes in reducing a complication known as uremic pruritus. This complication happens when there is excessive urea in the blood due to renal failure. Some studies have shown that PMMA membranes could remove some of the high-molecular-weight uremic toxic solutes, which cannot be typically removed by membranes made of other synthetic polymers [172, 173]. This property was associated with the ability of the PMMA to adsorb such solutes and its improved permeability [174]. The main manufacturer of commercial PMMA membranes is Toray Medical Co. Ltd. This membrane is made by mixing isotactic (iso) and syndiotactic (syn) PMMAs. Their newly developed PMMA membrane called the BG-U series has an increased homogenized number of pores and has shown to be very effective in removing a range of solutes with low platelet adhesion, low complement activation, and high degree of cytokine removal.

**Fig. 12** PMMA structure
There are other less studied synthetic polymers used as hemodialysis membranes. An example of one is ethylene vinyl alcohol copolymer (EVA). EVA is a hydrophilic polymer with protein-resistant properties. Two commercial versions of this polymer, EVAL and EVOH (by Kawasumi Laboratories, Japan) have been produced. In several research studies, EVA membranes have exhibited reduced activation of platelets and decreased formation of platelet-neutrophil aggregates compared with cellulose diacetate, PSF, and PMMA membranes in in vivo and in vitro models [175, 176].

2.7 Other Polymers

In the previous sections, polymers that have been most extensively studied for blood contacting applications were discussed. In this section, a brief overview is given of a few additional polymers that have been used in specific blood contacting applications. For these polymers there have been less research studies completed addressing the enhancement of their blood compatibility, but they still warrant discussion.

2.7.1 Polyamide

Polyamide is the general term used for any polymer containing an amide linkage. In this section, we use this term for synthetic polyamides, mainly nyons, used in blood contacting applications such as sutures. The high tensile strength of nylon makes it a suitable candidate to be used in sutures (specifically as nonresorbable sutures), and in tendon and ligament repair [144]. Another application of synthetic polyamides is in balloons for catheters, where they are typically used as a copolymer such as a polyamide-polyurethane copolymer. Research studies on the biocompatibility of polyamide sutures are generally focused on complications associated with infection. Modification strategies to render antimicrobial properties have, hence, been studied by several researchers. These modification techniques have been addressed in a review paper by Shmack et al. [145]. Several studies have also been carried out on the modification of polyamide for blood compatibility. For example, a copolymer of amidoamine and methylmethacrylate with heparin binding capacity was studied for blood compatibility [146, 147]. The heparin-bound copolymer showed low thrombus formation (<0.22 mg in 60 min) and a low percentage of hemolysis (<5%). In another study, Nagase et al. synthesized polyamides containing phospholipid (PC) moieties. After incubation with platelet rich plasma for 1 h, the modified polyamides showed reduced platelet adhesion compared with nonmodified polyamides (ten times reduction) [177].

2.7.2 Polypropylene

Polypropylene (PP) is widely used in syringes as well as in membranes for blood oxygenators. Most disposable syringes today are made of polypropylene. The first microporous polypropylene membrane oxygenators were developed in the 1970s. These membranes can act as an artificial lung in clinical extracorporeal circuits. An example of a commercially available oxygenator with a polypropylene membrane is
the Affinity NT Oxygenator by Medtronic. In most applications, these membranes are coated with anticoagulants such as heparin for improved blood compatibility. Medtronic, for example, offers two heparin coated Affinity NT membranes: the Carmeda® BioActive Surface (CBAS) and Trillium® Biosurface.

2.7.3 Polyglycolide (PGA), Polylactide (PLA), and Poly(lactide-co-glycolide) (PLG)

PGA, PLA, and PLG are all biodegradable polymers used in applications such as tissue engineering scaffolds, drug delivery systems, and biodegradable sutures. Two examples of biodegradable sutures currently used in clinical applications are Vicryl® (Ethicon Inc.) (composed of a copolymer made from 90% glycolide and 10% L-lactide) and Dexon (composed of PGA). The degradation of these sutures happens by hydrolysis and they are typically used in ophthalmic and orthopedic surgeries.

PLA and PLG have also been used for the development of bioresorbable stents and for coating drug eluting stents. These types of stents were made in response to the limitations associated with metallic stents (restenosis, thrombosis, and impairment of vessel geometry) [178]. Lincoff et al. showed that stents coated with low-molecular-weight PLA (80 kD) elicit an intense inflammatory response as opposed to stents coated with high-molecular-weight PLA (321 KD) in a porcine coronary artery model [179]. Using a porcine animal model and high-molecular-weight PLA stents, Tamai et al. observed no thrombosis (6 months postimplantation) and no inflammation (16 weeks postimplantation) [180]. Similar promising results were observed for stents made of PLG [178].

3 Conclusions

Polymers are frequently used in devices that come in contact with blood in both short- and long-term applications. Their advantageous mechanical properties have provided improved function in many cases, but due to their foreign nature in the body, these synthetic materials continually illicit adverse responses. As a result, thrombosis remains a major issue and a limiting factor for the extended use of polymeric blood contacting devices. Some devices function sufficiently, particularly in short-term applications, for example, polyurethane and silicone peripheral catheters. However, in other applications, such as small diameter vascular grafts, current PTFE and PET materials have been unsuccessful and thrombus formation is detrimental to their use. In hemodialysis, membrane materials include PSF, PMMA, and PAN, but due to the large membrane surface area in contact with blood, adverse interfacial reactions create limitations. As discussed in this chapter, there are numerous other polymers used in blood contacting applications, but improvements to these are also still needed.

To overcome thrombosis and attempt to improve the blood compatibility of polymers, both bulk and surface modification methods have been researched. The common surface modification strategies that have been explored among polymers include the creation of bioinert surfaces, bioactive surfaces, and a combination of the
two. Although bioinert modification with PEO and other molecules has provided improved results, these approaches have been inadequate when used in devices clinically. Functionalizing surfaces with bioactive molecules has shown potential but further work is required. It is expected that a combination of these strategies to best mimic the multifunctionality of native endothelium may lead to improved blood compatibility. Furthermore, continued investigations are needed to fully understand the complicated processes involved in blood-material interactions along with more standardized approaches to testing polymers for their level of blood compatibility.

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Abstract

The utilization of polymers for biomedical applications ("biomedical polymers") has led to significant advancements in medicine. Biomedical polymers have made a profound impact on human health and improved the quality of life for

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many patients. Current and evolving biomedical challenges posed by disease, environmental triggers, and physiological processes demand the development of biomedical polymers with specific properties and function. To address these challenges, the design of biomedical polymers has become of paramount importance. Designing polymers with specific structures opens the door to tailored properties and function. In this chapter, we cover the design of biomedical polymers for a variety of applications. We show that key polymer structures and properties are crucial to desired functionality for a given application. The biomedical applications we cover include (1) drug delivery, (2) imaging and tracking biomedical polymers in vivo, (3) scaffolds for tissue engineering, (4) medical devices, (5) surgery and wound repair, and (6) biosensors. By looking at the polymer structure-property-function relationships provided herein, we hope that this will enable improved designs of biomedical polymers to realize enhanced performance and efficacy in transforming human health.

**Abbreviations**

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CD</td>
<td>Cyclodextrin</td>
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<tr>
<td>DETOSU-HD</td>
<td>3,9-bis (ethylidene 2,4,8,10-tetraoxaspiro [5,5] undecane) and 1,6-hexanediol</td>
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<tr>
<td>DPCs</td>
<td>Dynamic polyconjugates</td>
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<td>EPR</td>
<td>Enhanced permeability and retention</td>
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<tr>
<td>GPa</td>
<td>Gigapascal</td>
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<tr>
<td>HA</td>
<td>Hyaluronic acid</td>
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<tr>
<td>hMSCs</td>
<td>Human mesenchymal stem cells</td>
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<tr>
<td>HPMA</td>
<td>N-(2-hydroxypropyl)methacrylamide</td>
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<tr>
<td>IC_{50}</td>
<td>Half maximal inhibitory concentration</td>
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<tr>
<td>MDa</td>
<td>Megadaltons</td>
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<td>MMP2</td>
<td>Matrix metalloproteinase 2</td>
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<td>MPa</td>
<td>Megapascals</td>
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<td>PAMAM</td>
<td>Poly(amidoamine)</td>
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<td>PANI</td>
<td>Poly(aniline)</td>
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<tr>
<td>PAsp</td>
<td>Poly(aspartic acid)</td>
</tr>
<tr>
<td>PBH</td>
<td>Poly(butyl and amino vinyl ether)s</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCL</td>
<td>Poly(caprolactone)</td>
</tr>
<tr>
<td>PCPH</td>
<td>Poly[1,6-bis(p-carboxyphenoxy)hexane]</td>
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<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
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<td>PEI</td>
<td>Poly(ethyleneimine)</td>
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<td>PEO</td>
<td>Poly(ethylene oxide)</td>
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<td>PGA</td>
<td>Poly(glycolic acid)</td>
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<td>PGlu</td>
<td>Poly(glutamic acid)</td>
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<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
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<tr>
<td>PLCL</td>
<td>Poly(lactide-co-caprolactone)</td>
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Biomedical polymers embrace a wide range of different classes with unique structures and characteristics. Most notably, there are natural and synthetic biomedical polymers that may be utilized directly, modified, or synthesized from starting monomers. The molecular weight of polymers greatly impacts their physical properties. In a typical polymerization, as the polymer chains grow, the critical molecular weight for chain entanglements is reached. At this point, many of the physical properties become notable such as the glass transition temperature ($T_g$) and melting temperature ($T_m$). $T_g$ refers to long-range segmental motion of the polymer chains, which are in a vitrified state above the $T_g$ with limited mobility. Other polymers are able to pack into domains that afford a semicrystalline morphology with a melting temperature ($T_m$). The morphology of the polymer greatly affects the physical properties. For a given polymer, there may be dispersity in the distribution in the molecular weight of the chains, which is measured as the polydispersity index (PDI). A completely uniform molecular weight of a polymer has monodispersity (PDI = 1); however, the majority of polymers are polydisperse (PDI > 1). The PDI can influence polymer processing and the uniformity of properties. In this chapter, we highlight the physical properties of polymers and how these properties can be utilized for biomedical applications.

To be classified as a biomedical polymer, biocompatibility must be satisfied. That is, the biomedical polymer must be tolerated by the body and not elicit side effects, allergies, or toxicity. Biodegradable polymers break down into monomers and constituents that may be absorbed in the body. These breakdown products must be safe and biocompatible as well. Polymers and their components clear from the body via different means depending on size and molecular weight. For example, polymers smaller than 5 nm are rapidly excreted in the kidney, while 10–100 nm polymers can circulate systemically for hours [1]. The mechanism of absorption or clearance of the polymer may depend on the biomedical application. The target application must be matched to the function of the polymer. For instance, in gene therapy, polymers must be able to effectively deliver nucleic acids without cytotoxicity. In this book chapter, the goal is to provide a foundation of structure-property-function relationships within different classes of biomedical polymers and applications. As a result, we want this information to elucidate the design of specific polymers and provide a platform to improve contemporary biomedical polymers.
2 Biodegradable Polymers

The biologically induced breakdown of polymers into their monomers and constituent parts provides a means for absorption and clearance from the body in biomedical applications. In many applications, it is crucial that polymers break down into their monomers. For example, in drug delivery, the degradation of polymers may facilitate the release of drug. For implants, the degradation of biomedical polymers evades the necessity for extra surgery to remove implants. In tissue engineering, the breakdown products of polymers can facilitate natural tissue growth.

Polymers may be biologically degraded through hydrolytic, enzymatic, acidic, and reducing mechanisms. Here, we cover the different classes of biodegradable polymers followed by their physical properties. Important physical properties relevant to synthetic biodegradable polymers include their morphology, thermal transition temperatures, and modulus. Next, we discuss the polymer’s mechanism of degradation and rate of degradation. This information should serve as a toolbox to select a biodegradable polymer with specific physical and degradation properties for a particular biomedical application. The chemical structures of biodegradable polymers can be found in Fig. 1 for synthetic biodegradable polymers and Fig. 2 for natural biodegradable polymers.

2.1 Synthetic Biodegradable Polymers

(a) Poly(ester)s

Within the polyester family, notable materials include aliphatic poly(ester)s, poly(ortho ester)s, and poly(phosphoester)s. These polymers can embrace a wide range of morphologies from completely amorphous rubbery or glassy materials to semicrystalline rigid polymers. The main mechanisms of degradation are by hydrolysis and enzymatic cleavage. Enzymes involved in the hydrolysis of esters include esterases and lipases. The rate of degradation can vary on time scales from hours to years based on the chemical structure and composition. Furthermore, acidic environments increase the rate of hydrolysis.

(i) Poly(lactic acid) (PLA), Poly(glycolic acid) (PGA), and Poly(lactic-co-glycolic acid) (PLGA)

PLA is a glassy semicrystalline polymer with a $T_g$ of 60–65 °C and $T_m$ of 150–160 °C. The degradation times of PLA can last for months [2, 3].

PGA is a glassy semicrystalline polymer with a $T_g$ of 35–40 °C and $T_m$ of 225–230 °C with degradation times on the order of months. By creating of copolymers of PLA and PGA, namely, poly(lactic-co-glycolic acid) (PLGA), an amorphous material is created with a $T_g$ ranging from 40 to 60 °C. Depending on the molecular weight and ratio of glycolic acid to lactic acid units, this copolymer can provide degradation times on a much shorter time scale from hours to days [4, 5]. PLA, PGA, and PLGA are FDA-approved and offer high mechanical strength with moduli between
1 and 10 gigapascals (GPa). These polymers can be degraded hydrolytically and through enzymatic cleavage by esterases and lipases.

(ii) Poly(caprolactone) (PCL)

PCL has a long aliphatic unit that provides flexibility and semicrystallinity, resulting in a $T_g$ of $-60^\circ$C and a $T_m$ of 60 $^\circ$C. PCL has a modulus between 0.1 and 1 GPa. Furthermore, the increased chain length of methylene groups provides a high degree of hydrophobicity [6]. Consequently, the
hydrophobicity minimizes the ability of water to hydrolyze the ester bonds. PCL has notably slow degradation that is influenced by crystalline microstructure [7]. The degradation of PCL can last for months to years, which occurs through hydrolysis and hydrolase-type enzymes, i.e., lipases, esterases, and proteases.

(iii) Poly(ortho ester)s

Polymer surface erosion is another mechanism by which degradation occurs and can be noted for poly(ortho ester)s. In surface erosion, the exterior of the polymer surface degrades first before the inside bulk of the material. This may be exemplified most notably for poly(ortho ester)s, such as those based on 3,9-bis (ethyldiene 2,4,8,10-tetraoxaspiro [5,5] undecane) and 1,6-hexanediol (DETOSU-HD), which has a Tg of 22 °C. The degradation of DETOSU-HD proceeds over years [8]. Tunable degradation kinetics from days to months can be realized based on the amount of comonomer incorporated. DETOSU-based copolymers can have a modulus between 0.5 and 1 GPa. With hydrolysis being the primary route of degradation in surface erosion, the backbone of poly(ortho ester)s may further be designed to degrade rapidly under acidic conditions.

(iv) Poly(phosphoester)s

With a backbone similar to nucleic acids, poly(phosphoester)s are a versatile class of poly(ester)s that are amenable to designed functionality. Examples of poly(phosphoester)s include poly(phosphate)s, poly(phosphite)s, poly(phosphonate)s, and poly(phosphoramidate)s. These structures allow for chemical modification in the backbone or side chain emanating from the phosphorous group. Moreover, these groups attached to the phosphorous heavily influence the physical properties and rate of degradation. For example, poly(bisphenol A-ethyl phosphate) (BPA-EOP) has a Tg of 103 °C, breakdown temperature of 310 °C, elastic modulus of 508 MPa, and shear modulus of 183 MPa [9]. By incorporating a phenyl group through a phosphonate linkage, poly(bisphenol A-phenylphosphonate) (BPA-PP) has Tg of 115 °C, breakdown temperature of 539 °C, elastic modulus of 627 MPa, and shear modulus of 230 MPa. The in vivo degradation of these materials showed that greater than 80% weight loss of BPA-EOP was encountered after 70 weeks, while less than 20% weight loss was found for BPA-PP. Therefore, by controlling the side chain for poly(phosphoester)s, the rate of degradation could be tuned dramatically. Poly(phosphoester)s can be degraded under physiological conditions by hydrolysis as well as by enzymes such as phosphatases and phosphodiesterases.

(b) Poly(anhydride)s

Similar to poly(ester)s, the degradation rate and drug release properties of poly(anhydride)s can be controlled by chemical structure and composition. Poly(anhydride)s provide a surface erosion degradation mechanism through hydrolysis of the anhydride bond. Two common poly(anhydride)s are poly(sebacic anhydride) (PSA) and poly[bis(p-carboxyphenoxy)propane] (PCPP). PSA has a
T_g and T_m of 62 °C and 79 °C, while PCPP has a T_g and T_m of 92 °C and 230 °C. PCPP has a flexural strength of 2.76 MPa and Young’s modulus of 8.84 MPa [10]. PCPP alone showed constant erosion degradation kinetics over several months. By creating copolymers of PCPP and PSA, the rate of degradation has been increased by increasing the amount of PSA. Furthermore, a simultaneous decrease in T_g and T_m is observed when increasing the amount of PSA in the copolymer. With PCPP-SA (21:79) copolymer, complete degradation was reached in less than 2 weeks.

2.2 Natural Biodegradable Polymers

(a) Polysaccharides

Composed of sugar monomers, polysaccharides are mostly water-soluble polymers that are inherent in nature, biocompatible, and biodegradable. Each polysaccharide has a unique structure and particular functional groups. They are mainly degraded by enzymes specific to the sugar and polymer. For example, dextran may be degraded by dextranases such as dextranhidrolase, glucodextranase, and dextran glucosidase, in addition to others. Their natural origin and biodegradability make polysaccharides appealing for biomedical applications. Here, we list common polysaccharides found in biomedical applications.

(i) Dextran

Dextran consists of alpha-1,6 glycosidic linkages between glucose units. Branches are often found in dextran and occur via alpha-1,3 linkages. Dextran is a water-soluble, neutral polysaccharide. Dextran can easily be modified and rendered insoluble for therapeutic applications. For example, dextran has been derivatized with acetal groups by modification of the hydroxyl groups with 2-methoxypropene [11]. This derivatization produces a pH-sensitive acetalated dextran wherein acetal groups hydrolyze under mild acid conditions. For vaccine application, the protein ovalbumin was encapsulated in nanoparticles formed by a double emulsion. Ovalbumin particles increased major histocompatibility complex class I presentation when compared to free protein. Given the promise of these particles for vaccines against tumors and viruses, the acetalated dextran may be useful in sutures and scaffolds.

(ii) Alginate

Alginate is a carboxylic acid-containing, negatively charged polysaccharide composed of mannuronate and guluronate units linked by glycosidic bonds. The carboxylic acid moiety allows for facile and specific chemical modification. Furthermore, alginate may be chemically crosslinked by calcium to form gels, which find great biomedical use. For example, alginate has been crosslinked ionically and photochemically to form macroscopic gels incorporating small interfering RNA and cells for gene knockdown [12]. Alginate was ionically crosslinked using calcium and
photochemically crosslinked using an initiator combined with UV irradiation. The photocrosslinked alginate provided controlled degradation through hydrolysis of ester linkages, sustained release of small interfering RNA, and gene knockdown.

(iii) **Hyaluronan**

Hyaluronan is a negatively charged polysaccharide containing carboxylic acid and amide groups. Specifically, hyaluronan is composed of $N$-acetylglucosamine and glucuronic acid units. It is crucial in tumor development and can be harnessed by nanomaterials as a targeting ligand [13]. For example, lipid nanoparticles have been functionalized with hyaluronan to target epithelial cancer cells that overexpress hyaluronan receptors (CD44 and CD168). The effect of hyaluronan molecular weight on biological responses has been evaluated using nanoparticles [14]. Low molecular weight hyaluronan-decorated nanoparticles have low binding to CD44 receptor, while high molecular weight shows high binding and affinity for CD44 receptor. Furthermore, low molecular weight hyaluronan may serve as a substitute for poly (ethylene glycol) in stealth nanoparticles for passive delivery.

(iv) **Chitin and Chitosan**

Chitin is mostly composed of $N$-acetylglucosamine units connected by beta-1,4 linkages. Deacetylated glucosamine units are found, and their percentage depends on the source for production. Chitin is insoluble in water due to extensive hydrogen bonding. Chitosan exists largely as the deacetylated derivative of chitin with some acetylated glucosamine units present. Chitosan can be made soluble under acidic conditions and has many advantageous properties for biomedical applications [15]. Specifically, it is renewable, nontoxic, and hydrolyzable by lysozyme. In addition, chitosan has film-forming, hydrating, and wound healing properties wherein it can be used for surgical sutures, dental implants, artificial skin, and rebuilding of the bone. The primary amine on chitosan allows for facile functionalization with chemical moieties that enhance interaction with cells or delivery of hydrophobic drugs. For instance, $N$-trimethyl chitosan can provide interaction with negatively charged cell membranes, and $n$-lauryl-carboxymethylchitosan can form micelles that encapsulate paclitaxel [15].

(v) **Chondroitin Sulfate**

Chondroitin sulfate is a negatively charged polysaccharide containing sulfate, carboxylic acid, and amide functional groups. It is composed of glucuronic acid and $N$-acyethylgalactosamine units and can form proteoglycan aggregates through binding to protein. The aggregate can interact with tissue via electrostatics. Chondroitin sulfate has anti-inflammatory properties, prevents production of cartilage cytokines, and elicits apoptosis of articular chondrocytes [16]. The mineralization process and repair in the bone has been accelerated by using chondroitin sulfate. This anionic polysaccharide has been used to treat osteoarthritis [17]. Chondroitin sulfate was found to be more effective than glucosamine in reducing knee pain.
(vi) **Fucoidan**

Fucoidan is an anionic polysaccharide containing sulfate groups and fucose units. Fucoidan encompasses a family of fucoids that can differ in chemical composition. For example, some fucoids are composed of monosaccharides like glucose or galactose, acetyl groups, or proteins. Fucoidan has a number of desirable pharmacological characteristics such as anti-inflammatory, anticoagulant, antithrombotic, and anti-oxidative properties [18]. Fucoidan has been used for drug delivery by crosslinking with chitosan to form a “fucosphere” [19]. Bovine serum albumin was encapsulated in fucospheres, and its release was controlled by varying concentration of polymers, albumin, and preparation method. In addition the cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), has been encapsulated in fucospheres [20]. Fucospheres demonstrated slow and sustained release from 90 to 140 days with retention of released cargo. Neutropenia and aplastic anemia can be treated with delivery of GM-CSF.

(b) **Polypeptides**

Polypeptides are polymers composed of amino acid monomer units connected via amide bonds in the backbone. They are natural, water-soluble polymers with a variety of functional groups and properties. Notable polypeptides in biomedical applications contain functional groups such as carboxylic acids and amines. Polypeptides are degraded by proteases that cleave the amide backbone of the polymer.

(i) **Poly(\text{\text{l}-lysine}) (PLL)**

PLL is a cationic polypeptide composed of lysine units, which bear a free primary amine. This primary amine allows for facile functionalization chemistry. PLL exists in two primary forms, most commonly as α-PLL where the long aliphatic amine group exists as the side chain. In ε-PLL, the long aliphatic amine resides in the backbone of the polymer. The inherent cationic nature of PLL enables strong complexation with polyanions. This property can find great use in complexing with nucleic acids for gene delivery or silencing. For example, PLL has been complexed with DNA and exhibited effective transfection [21].

(ii) **Poly(aspartic acid) (PAsp)**

PAsp is an anionic polypeptide composed of aspartic acid residues. It exists in two main polymeric forms: alpha and beta. In α-PAsp, there is a methylene group between the carboxylic acid and the backbone, while for β-PAsp, this methylene group is found in the backbone. The degradability, biocompatibility, low cytotoxicity, and functional carboxylic acid group of PAsp make it a good candidate for biomedical applications. For instance, PAsp-based supramolecular assemblies have been prepared for gene delivery [22]. Benzene-functionalized PAsp was combined with cyclodextrin-core PAsp polycations through host-guest interactions to form assemblies that were then mixed with DNA to form complexes that could effectively transfect cells.
(iii) Poly(glutamic acid) (PGlu)

PGlu is another anionic polypeptide that is composed of glutamic acid units. It mainly exists in two forms: alpha and gamma. In the alpha form, PGlu has an ethylene group between the carboxylic acid and the backbone, while in the gamma form, this ethylene group is in the backbone; therefore, there is no spacer between the backbone and carboxylic acid group. The anionic polyglutamic acid can be tethered to a functional group and can bind electrostatically to cationic nanoparticles. For example, PGlu-based ligands have been used as nanoparticle coatings to enable ligand-specific gene delivery in vitro [23] and alter biodistribution and gene delivery efficacy in vivo [24].

(c) Proteins

Proteins are large assemblies of polypeptides. They may consist of a variety of polypeptides connected by linkages such as disulfide bonds. Proteins can adopt unique tertiary structures such as beta sheets and alpha helices. Proteins serve many important roles biochemically that facilitate physiological processes. Similar to polypeptides, proteins are degraded enzymatically by proteases.

(i) Albumin

Albumin is a water-soluble protein that represents a group of proteins that make up the largest fraction of proteins in the blood. Human and bovine serum albumin are the most common albumin proteins. Human serum albumin is a non-glycosylated, anionic protein with enzymatic and antioxidant properties. It contains numerous disulfide bridges and at least one free thiol. Human serum albumin adopts an ellipsoidal structure and is largely composed of alpha helices. Bovine serum albumin (BSA) PRINT® particles encapsulating self-replicating RNA have been prepared toward vaccine use. To avoid dissolution, BSA particles were rendered transiently insoluble by using a reducible disulfide crosslinker [25]. These particles were complexed with cationic and transfecting lipid and effectively delivered RNA replication to Vero cells [25].

(ii) Collagen/Gelatin

Gelatin is a derivative of collagen that has been partially and irreversibly hydrolyzed. Collagen serves as the major protein in connective tissue while being the most abundant protein in mammals. Collagen contains a large number of glycine residues, followed by proline. The linkages between collagen strands are broken in gelatin, which can assume a variety of supramolecular structures ranging from globular to fibrillar with triple-stranded helices. Acid-solubilized collagen is a liquid at 4 °C and a hydrogel at 37 °C and neutral pH. This thermogelling property enables the encapsulation of cargos for biomedical applications. For example, small interfering RNA and cells have been encapsulated in macroscopic collagen hydrogels for gene silencing [12]. This may serve as an injectable biodegradable polymer for sustained inhibition of gene expression.
3 Nondegradable Biocompatible Polymers

A polymer is considered biocompatible when it does not pose side effects, allergic reactions, and toxicity while allowing the body to function normally. This opens the area of broad classes of polymers to biomedical applications. Here we will cover different nondegradable, biocompatible polymers that have found use in biomedical applications. Then, their physicochemical, thermal, and mechanical properties may be covered. The intent in this section is to provide a repertoire of biocompatible polymers that may be used in biomedical applications. The chemical structures of these biocompatible polymers are illustrated in Fig. 3.

(a) Poly(ethylene glycol) (PEG)

PEG is a water-soluble, neutral polyether composed of ethylene oxide repeat units. PEG also has broad solubility in organic solvents. PEG can be identified as polyoxyethylene or poly(ethylene oxide) (PEO). PEG is generally used for polymers with a molecular weight less than 20 kDa, PEO for polymers greater than 20 kDa, and polyoxyethylene is used for any molecular weight. It has a T\textsubscript{g} of \(-66\,^\circ\text{C}\) and T\textsubscript{m} of \(66\,^\circ\text{C}\) \cite{26}. PEG is utilized in a wide range of molecular weights down to oligomers. For general biomedical applications, the molecular weight of PEG is commonly between 2 and 10 kDa. PEG has notably been used on the surfaces of particles to provide a hydrophilic stealth layer that minimizes interactions with serum proteins and the mononuclear phagocytic system \cite{27}. For instance, it was found that high density of PEG in brush conformation

![Chemical structure of nondegradable, biocompatible polymers](Fig. 3)
provided the longest circulation half-life, lowest protein binding, and lowest macrophage association when compared to low-density PEG and unPEGylated nanoparticles [28].

(b) Poloxamers
Poloxamers (or commercially “Pluronics®”) are ABA triblock copolymers of PEO-poly(propylene oxide)-PEO. A wide range of Pluronics are commercial with different molecular weights and percentage of PEO [29]. These copolymers have a distinct critical micelle concentration and hydrophilic-lipophilic balance. Examples of two Pluronics are F127 and L61. Pluronic F127 has 200 ethylene oxide units, 65 propylene oxide units, and a molecular weight of 12,600 Da. Pluronic L61 has 4.6 ethylene oxide units, 31 propylene oxide units, and a molecular weight of 2 kDa. The water solubility of Pluronics decreases as the relative fraction of propylene oxide units increases. Given the large amount and percentage of ethylene oxide units in Pluronic F127, it has water solubility greater than 10%. Conversely, the greater relative number of propylene oxide units in Pluronic L61 renders it insoluble water. Poloxamers exhibit a reversible solgel transition when going from low temperature to high temperature. To improve stability of the hydrogel, poloxamer 407 was derivatized with thiol and acrylate groups [30]. The solgel transition was achieved at body temperature, and the crosslinking was realized under physiological-mimicking conditions. The resulting stability was enhanced, and release of drug was prolonged four times relative to the normal hydrogel. This study demonstrated the potential of poloxamer hydrogels for controlled drug release, cell encapsulation, and tissue engineering.

c) Poly(2-oxazoline)s (POx)
As PEG is utilized in several biomedical formulations, a functional analogue class of polymers to poly(ethylene glycol) has been pursued: poly(2-oxazoline)s (POx). POx are a class of polymers that have a polypeptide-like structure and tunable water solubility. POx consists of an ethyleneimine backbone where the nitrogen atom is substituted as an amide with a variety of groups and solubilities. For example, poly(2-ethyl-2-oxazoline) is water soluble, while poly (2-nonyl-2-oxazoline) is water insoluble. POx fit into a variety of biomedical applications ranging from drug and gene delivery to membranes and stimuli-responsive materials [30, 31]. POx have been conjugated to biomolecules such as bovine serum albumin and insulin to tailor properties [32]. For insulin POx conjugates, blood glucose levels were lowered for four times longer than insulin alone. Similar to PEG, albumin POx conjugates mitigated immunogenic properties.

d) Poly(N-(2-hydroxypropyl)methacrylamide) (PHPMA)
PHPMA is a water-soluble, neutral polymer that is nontoxic and non-immunogenic. PHPMA has an alcohol-containing side chain that allows for functionalization in biomedical applications. PHPMA is commonly used as a drug carrier by conjugating drugs through peptide linkers that can be cleaved by intracellular enzymes. For instance, the protein synthesis inhibitor puromycin and the antibiotic daunomycin have been bound to PHPMA copolymer via
When incubated with lysosomal cathepsin proteases, the drugs were released over 20 h. The conjugates exhibited differential toxicity in a mouse leukemia model depending on the identity of the peptide linker.

e) Poly(ethyleneimine) (PEI)

PEI is a water-soluble cationic polymer with an ethyleneimine backbone, which provides a secondary amine. PEI’s $T_g$ is $-28 \, ^\circ C$ and $T_m$ is between 40 and 47 $^\circ C$ [34]. One of the most prominent uses of PEI in biomedical applications involves non-viral gene delivery. The amino groups and cationic nature of PEI facilitate polyplex formation with DNA through electrostatics. The molecular weight and structure of PEI have a notable effect on the physicochemical properties and activity of the polyplex, specifically, condensation, size, charge, biodistribution, and transfection efficiency. High molecular weight PEI has shown greater DNA condensation and cytotoxicity, while low molecular weight has exhibited higher transfection efficiency [35].

f) Poly(ethylene) (PE)

PE is a simple poly(olefin) that is composed of two methylene groups in the backbone per repeat unit. PE molecular weights typically range from 1.4 kDa to 3.5 MDa for solid material. Waxy solids are found for lower molecular weights with degree of polymerization between 8 and 100. At degrees of polymerization less than 8, the alkanes exist as liquids or gases. Ultrahigh molecular weight (UHMW) PE is most commonly used in biomedical applications such as a bearing surface in joint replacements. UHMW PE has a molecular weight range from 2 to 6 Mg/mol, a melting point of 125–135 $^\circ C$, and a tensile modulus of 0.8–1.5 GPa [36]. UHMW PE joint replacement devices have lifetimes greater than 15 years if implanted correctly, and current research into the design and properties of the material may further improve performance.

g) Poly(styrene) (PS)

PS is a glassy, hydrophobic thermoplastic polymer with a $T_g$ of 100 $^\circ C$ [26]. It contains an aromatic phenyl group side chain and may be produced in several forms. Polystyrene has an ultimate tensile strength of 40 MPa, elongation of 7%, and tensile modulus of 3 GPa. PS has found use in biomedical applications for the fabrication of shape- and size-specific particles for biological studies. Specifically, the phagocytosis of PS particles by alveolar macrophages has been investigated [37, 38]. The shape of PS particles was found to play a dominant role in phagocytosis initiation, while size was found to dictate completion of phagocytosis depending on volume compared to the size of the cell. Wormlike PS particles, with aspect ratio greater than 20, showed minimal phagocytosis compared to equal volume spherical particles.

h) Poly(methyl methacrylate) (PMMA)

PMMA is glassy, hydrophobic thermoplastic polymer with a $T_g$ of 115 $^\circ C$ for its syndiotactic form [26]. PMMA has a Young’s modulus ranging from 1.8 to 3.1 GPa. The tensile strength of PMMA ranges from 48 to 76 MPa. PMMA may be used in amphiphilic block copolymers to form nanoparticles for biomedical applications. For example, PMMA-core/PEG-shell block
copolymers have been prepared and functionalized with a ligand that can chelate radioactive copper-64 to study structural effects on biodistribution [39]. In another study, the effect of plasma protein nanoparticle coating on biodistribution and phagocytosis by the reticuloendothelial system was studied for radiolabeled PMMA nanoparticles [40].

(i) Poly(acrylic acid) (PAA)

PAA is a glassy, water-soluble polymer with a $T_g$ of 105 °C [26]. PAA is an anionic polymer bearing a carboxylic acid residue directly off the vinyl backbone. This acid group allows for facile functionalization and also provides additional properties. For example, the acid group allows PAA to swell several times its volume and finds use as a super-absorber. For functionalization of PAA, the carboxylic acid groups have been modified with PEG to serve as stealth coating. Specifically, cationic hydrogel nanoparticles were complexed with PAA-PEG through electrostatics to neutralize surface charge and alter biodistribution in vivo, facilitating accumulation in hepatocytes [41]. Furthermore, PAA-coated nanoparticles encapsulating small interfering RNA enabled gene knockdown in vivo.

(j) Poly(vinyl ether)s

Poly(vinyl ether)s consist of a vinyl backbone with an ether side chain. A variety of chemical groups may be selected as the side chain, which influences properties. For example, poly(methyl vinyl ether) has a $T_g$ of −31 °C and $T_m$ of 144 °C, while poly(butyl vinyl ether) has a $T_g$ of −55 °C and $T_m$ of 64 °C [26]. For biomedical applications, additional groups such as primary amines may be chosen as the side chain functionality. One particularly interesting example is that of an amphipathic poly(vinyl ether) containing butyl and amino side chains, which is termed PBAVE. Due to its amphiphatic nature, PBAVE has membrane lytic activity and was able to transfect DNA efficiently in vitro [42]. When PBAVE was conjugated with small interfering RNA and targeting ligands, in vivo gene knockdown was achieved [43].

4 Polymer Architectures and Structures

Polymers may adopt a wide variety of architectures and structures. Here we will cover the different polymer architectures and structures along with some of their properties. The architecture of a polymer chain refers to the branching from a linear chain, hence the term branched polymer. Dendrimers represent a particular treelike architecture. Crosslinked polymeric networks are chemically, physically, or ionically connected completely to other polymer chains. Polymer complexes are formed from complementary polyelectrolytes that self-assemble into unique structures. Micelles are self-assembled particles from amphiphilic block copolymers. Nanoparticles are a broad class of self-assembled or top-down fabricated polymeric particles with sizes between 1 and 100 nm. Covering a few of the different polymer architectures and structures will supply a foundation for understanding the properties of biomedical
polymers. Figure 4 shows representative cartoons of linear polymers, block copolymers, polymeric networks, and branched polymers.

(a) Linear

Linear polymers consist of linear chains of monomeric units. They may adopt conformations ranging from random coils to globules. Linear polymers can have different tacticities, that is, the order of adjacent chiral center’s stereochemistry. These include isotactic (all substituent on the same side), syndiotactic (substituent alternate on the chain), and atactic (substituents are random along the chain). These conformations can influence the packing of chains and corresponding physical properties. Nonetheless, the properties of linear polymers depend largely on their molecular weight, which influences the chain entanglement and packing.

(b) Branched

Branched polymers contain side chains emanating from the backbone based on the original monomer. An example of branched PEI shows the architecture in Fig. 5. The degree and type of branching influence polymer properties such as rheology, melting, and degradation in addition to application efficacy [44]. For example, the intrinsic viscosity of a branched polymer increases with molecular weight less than that for a linear polymer. Furthermore, the thermal transition temperatures ($T_g$, $T_m$) of poly(ethylene) are influenced by branching. Linear high-density poly(ethylene) has solid crystallization with a $T_m$ of 135 °C [44]. The addition of a few branched units (known as low-density poly(ethylene)) depresses the melting temperature to 115 °C [44]. Introducing many branches in poly(ethylene) renders the polymer amorphous without a $T_m$. Additionally, for poly(ester)s, the rate hydrolysis is slower for branched polymers versus linear polymers.

(c) Polymer Networks

Polymeric networks are a special type of polymer structure in which all chains are connected via crosslinks. The crosslinks may take form through chemical linkages, physical domains (such as glassy nodules in rubbery matrices), or ionic bonds (using complementary ions to connect polyelectrolytes). For chemical
means, this may be accomplished by using monomers and crosslinkers with appropriate functionality. For example, PEG diacrylate can be polymerized to yield a crosslinked network (Fig. 6). Similarly, gelatin can be functionalized with acrylamide groups that can be crosslinked through free radical polymerization. For ionic crosslinking, the carboxylic acid groups of alginate may be crosslinked using calcium(2+).

(d) Dendrimer

Dendrimers constitute a unique synthetic three-dimensional class of polymers with monodispersity, nanometer sizes, and treelike structures. The structures are composed of a core from which branched units emanate. Each subsequent branching point is referred to as generation X (number). The degree of branching, or number of generations, contributes to the size, molecular weight, and number of surface groups. Dendrimers terminated with reactive end groups allow for further functionalization with different moieties such as other polymers (e.g., PEG). There are two main synthetic approaches for dendrimers: (1) divergent growth, development of a dendrimer originates from a core, and (2) convergent growth – the dendrimer surface is reacted together inward to a focal core. The flexible synthetic approaches to dendrimers allow access to tunable cores, branches, and surface groups with defined physical properties.

One of the most well-known dendrimers is poly(amidoamine) (PAMAM), which is illustrated in Fig. 7. PAMAM dendrimers are synthesized largely from ethylenediamine and methyl acrylate where the surface end groups can be controlled based on the final reaction.

(e) Micelle

Polymeric micelles are generally composed of amphiphilic block copolymers that self-assemble into core-shell structures where the core is hydrophobic and the shell is hydrophilic. The size and physical properties of the polymeric...
micelles can be tuned by the identity and molecular weight of the blocks of the amphiphilic copolymer. One polymer class that has already been covered, Pluronics, readily forms micelle architectures. In aqueous solution, the hydrophobic segments of the block copolymer self-assemble into a core with the hydrophilic blocks on the exterior. These micelles can find use as surfactants in a variety of technologies such as drug delivery, lubricants, detergents, and soaps.

(f) Nanoparticles
Polymeric nanoparticles can be formed through a variety of processes such as nanoemulsification, complexation, nanoprecipitation, stretching, and Particle Replication In Non-wetting Templates (PRINT®). Through these processes, nanoparticles are obtained with sizes based on the material and methodology employed.

(i) Nanoemulsification
Nanoemulsions consist of a lipid oil core stabilized by surfactants to yield stable dispersions. To prepare nanoemulsions, conventional approaches utilize high-shear or high-pressure homogenization and ultrasonication. Exemplar surfactants include poly(ethylene glycol) with aliphatic tails and triglycerides. For instance, a formulation was developed from D-alpha-tocopheryl polyethylene glycol 1000 succinate (TPGS), polyoxyethylene 20-stearyl ether (Brij 78), and miglyol 812 to yield 200 nm “BTM”

Fig. 7  Chemical structure of PAMAM generation 1
nanoparticles [45, 46]. A microwave synthesis approach was employed for the BTM composition to obtain sub-30 nm nanoparticle sizes by accessing the phase inversion temperature [47]. Similar to nanoemulsions, solid lipid nanoparticles have been extensively studied [48] and consist of a solid lipid core instead of an oil core. These nanoparticles provide physical and chemical stability of the dispersion.

(ii) Complexation
When mixing oppositely charged polyelectrolytes together such as a polycation with a polyanion, nanoparticle formation may take place. An example of this type of nanoparticle would be observed from the combination of a negatively charged nucleic acid like DNA with a positively charged amine-containing polymer such as PEI [49].

(iii) Nanoprecipitation
Hydrophobic polymeric nanoparticles can be synthesized using nanoprecipitation. In this approach, the polymer is dissolved in a water-miscible organic solvent, which is dispensed generally dropwise into an aqueous solution containing surfactant. By rapid solvent diffusion, nanoparticles are formed and can reach sub-100 nm sizes. Notable examples involve the synthesis of PLGA nanoparticles stabilized by poly(vinyl alcohol) or Pluronics [50, 51].

(iv) Stretching
A unique approach was taken to fabricate elliptical disk PLGA nanoparticles starting from spherical particles [52]. Initial spherical particles were embedded into a polymer film that was stretched to yield non-spherical particles. These particles were capable of shape-switching modulated by temperature, pH, or chemical means from minutes to days.

(v) PRINT®
Particle Replication In Non-wetting Templates (PRINT®) technology is a specialized particle molding process spun-off from soft lithography to fabricate nanoparticles with control over size, shape, composition, and surface properties [53, 54]. Perfluorinated elastomeric molds with shape- and size-specific cavities are filled with liquid monomer precursors, polymers, or a variety of materials through capillary or melt-fill processes. After curing or solidification, nanoparticles are extracted from the cavities generally using an adhesive polymer, which then may be dissolved to harvest the particles. PRINT technology has been utilized to synthesize biocompatible hydrogel PEG-based particles to biodegradable PLGA particles with a variety of shapes and sizes such as cylinders, cubes, and hex nuts [55, 56].

5 Biomedical Applications

After covering the different types and identities of polymers as well as their corresponding physicochemical properties and structures, it is now possible to delve into their applications. We will present the use of biomedical polymers in
the following applications: (1) drug delivery, (2) imaging and tracking biomedical polymers in vivo, (3) scaffolds for tissue engineering, (4) medical devices, (5) surgery and wound repair, and (6) biosensors. Each application and sub-focus therein embraces key design criteria of biomedical polymers to realize desirable performance. Here, we review the literature on efficacious approaches and polymer systems that provide target behavior for given applications. Further, we highlight the crucial features of polymers that achieved success in their respective application. This may serve as a reference to understand the design of polymers for a variety of biomedical applications. In turn, we hope that this will lead to further iterations, designs, and development of polymeric systems for a range of applications with improved performance.

5.1 Drug Delivery

In the battle against human disease, drug delivery has come to the forefront of intensive research. Treatment of diseases using therapeutic interventions can be accomplished by delivering drugs that can remedy malfunctioning and restore normal physiology. Delivering treatments can be enhanced with the use of polymeric materials. Herein, we will cover the literature of biomedical polymers used for drug delivery, which will include different polymer architectures and cargos such as chemotherapeutics and nucleic acids. This literature may serve as a repertoire of biomedical polymers for researchers to select or adopt for their particular disease treatment goal.

5.1.1 Mechanism of Drug Release

The therapeutic drug may be released from the biomedical polymer through chemical and/or diffusive processes. In chemical processes, the drug may be bound to the polymer through a covalent linkage as a conjugate. There are a variety of linkers used for polymer-drug conjugates with specific degradation mechanisms. In diffusive processes, the drug may be encapsulated within a nanoparticle or polymer matrix and diffuse out. The diffusion of drug from the polymer can depend on the pore size, degree of complexation, crystallinity, or hydrophobicity.

Conjugation of therapeutic drugs to polymers can enhance their efficacy in vivo. For example, polymer conjugates can provide prolonged circulation in the blood, increased tissue accumulation and cell uptake, improved stability, and triggered release in specific biological locations. Polymer-drug conjugation is notably different than other self-assembled and top-down systems in that polymer-drug conjugates use a covalent linkage for drug delivery. Hydrophilic polymers, such as PEG, are often conjugated to drugs to enhance their solubility and provide in vivo stealth-like behavior.

Between the polymer and drug, the linker structure is of crucial importance as this determines how and when the drug is released. Extensive efforts into designing linkers for polymer-drug conjugates have enabled triggered release in specific tissues and cellular compartments for effective drug delivery. Hydrolyzable and acid-sensitive linkers include esters, hydrazones, beta-thiopropionate, and maleamate.
bonds. Reductively cleavable linkers include disulfides, while enzymatically cleavable linkers involve the use of peptide sequences. Examples of these linkers are provided with literature references under the different architectures that will be covered.

For diffusion of the drug from a polymeric material, there are several factors that govern the rate of release. For example, with a crosslinked nanoparticle, the pore size and crosslink density influence the rate of release: higher crosslink density corresponds with a slower rate of drug release. For micelles, the degree of hydrophobicity will dictate the noncovalent interaction strength and tendency of the drug to stay in the core of the micelle. In polyelectrolyte complexes, the binding between polyanion and polycation will influence the rate of release based on the ionic strength required to break the ionic interactions. For solid polymers, the rate of dissolution may be influenced by the crystallinity of the material and may determine the diffusion of drug from the polymer matrix. Examples of these drug diffusion systems will be covered for the different polymeric architectures.

5.1.2 Therapeutic Cargos

Chemotherapeutics
Cancer is a devastating health problem that is expected to be the leading cause of death over the next few years. In 2015, the United States is expected to have 549,430 cancer deaths, which accounts for nearly 1 of every 4 deaths [57]. Direct medical costs for cancer in the United States were estimated at $88.7 billion. Given the huge economic and healthcare burden created by cancer, there is a critical need for effective cancer treatments. Chemotherapeutic drugs are effective in eradicating cancer cells; however, they inflict damage to healthy cells as well [58]. Biomedical polymers have enabled improved drug delivery to tumor tissues [54, 59–61].

DNA
DNA used for gene therapy can adopt multiple conformations and has a size ranging from 1 to 200 kb pairs. After entry into the cytoplasm, DNA must cross the nuclear pore membrane to reach the nucleus for its expression. For gene therapy, DNA must place the important genes at particular locations in the chromosome for the production of target proteins. For efficient delivery to target cells and the nucleus, the identity and structure of biomedical polymer surrogates make a tremendous impact on translation for expression of desired proteins. For example, amine-containing cationic polymers are often utilized to provide electrostatic complexation with nucleic acids, cellular internalization, and endosomal escape into the cytoplasm.

Small Interfering RNA (siRNA)
siRNAs are short (20–25 nucleotides), double-stranded RNA that serve an important role in RNA interference. The antisense strand of siRNA targets the complementary strand of a certain gene. After entry into the cytoplasm, siRNA may be recognized and incorporated into the RNA-induced silencing complex where siRNA is unwound and binds the complementary messenger RNA sequence. This binding
results in the cleavage of the duplex, which prevents the expression of the target protein. To efficiently deliver siRNA into the cell and cytoplasm using biomedical polymers, the structure and physicochemical properties play a huge role in achieving effective gene silencing. Similar to the delivery of DNA, amine-containing cationic polymers are often utilized in transfection with siRNA.

5.1.3 Polymer Architectures for Drug Delivery

To deliver a drug to a particular tissue and cell, certain polymer architectures may provide distinct advantages. A variety of polymer architectures have been developed with specific physicochemical properties that enable delivery of select drugs. The sizes observed with the different architectures range from sub-5 nm to greater than 100 nm. Chemical compositions of the biomedical polymers vary from homopolymers to block copolymers and may include hydrophobic, hydrophilic, ionic, and targeting moieties. Here we cover the different architectures found for biomedical polymers used in drug delivery. These architectures include polymer conjugates, dendrimers, micelles, and nanoparticles.

Polymeric Conjugates

Delivery of chemotherapeutic drugs has been realized using β-cyclodextrin (CD)-based polymers. β-CD linear polymers were synthesized and conjugated with camptothecin. When incorporated into the polymer, camptothecin had more than three orders of magnitude greater aqueous solubility [62]. Camptothecin conjugates showed cell-line- and structure-dependent half maximal inhibitory concentrations (IC50s) in vitro where the parent polymer had low cytotoxicity. The different structures investigated included different peptide linkers (Fig. 8). The rate of drug release from the conjugates was significantly faster in plasma compared to phosphate buffered saline (PBS), which may be attributed to cleavage by enzymes found in the bloodstream. The peptide linker and molecular weight of the CD conjugates were then evaluated in vivo [63]. High molar mass (97 kDa) polymer conjugates showed greater antitumor efficacy than the native drug. Conjugates with the shorter peptide linker (glycine) were found to be less toxic than the longer peptide linker (triglycine). The CD conjugates afforded long-term control over tumor growth, which may be attributed to sustained release of the drug in acidic, intracellular compartments.

A hydrophilic polymer that has been used in polymeric drug conjugates derives from N-(2-hydroxypropyl)methacrylamide (HPMA). In one study, a conjugate between poly(HPMA) and an angiogenesis inhibitor (TNP-470) was established using a peptide linker [64]. This peptide linker (Gly-Phe-Leu-Gly) was found to be enzymatically degradable and demonstrated selective release from the polymer by the lysosomal enzyme cathepsin B. The conjugate accumulated selectively in tumor tissue, minimized accumulation in normal organs, and enhanced the activity of the drug in vivo.

siRNA polymer-drug conjugates have been pursued by many groups. siRNA is a prime candidate for conjugation due to its moderate size, negative charge, and high susceptibility to degradation by RNAses. siRNA conjugates have been synthesized with acid and reductively labile linkers. For example, siRNA was conjugated to PEG
via acid-labile beta-thiopropionate bond [65] (Fig. 9). The PEG was end-functionalized with lactose to target hepatoma cells. When this conjugate was combined with PLL, targeted polyion complexes were formed, which were capable of significant gene silencing in hepatocarcinoma cells. Another example of an siRNA conjugate with a reductively labile linker involves siRNA conjugated to PEG via a disulfide bond [66]. This siRNA conjugate was mixed with poly(ethyleneimine) (PEI) to form polyelectrolyte complex, which effectively silenced target gene expression in prostate cancer cells.

Amphipathic cationic polyvinyl ethers were synthesized with different alkyl group sizes [42] to evaluate potential for transfection. Transfection of DNA increased with alkyl group size where the butyl group showed the greatest transfection. The butyl group may provide the best transfection due to its hydrophobicity and ability to lyse membranes. This polymer, termed PBAVE, which is composed of poly(Butyl and amino vinyl ether)s, was translated into dynamic polyconjugates (DPCs) for the delivery of siRNA [43]. This was accomplished by reversibly masking the amines with acid-labile PEGs and targeting ligands via maleamate bonds (Fig. 10). Furthermore, siRNA was conjugated to the polymer through a reductively labile disulfide bond. After endocytosis of the polyconjugate by the target cell, the endosomal vesicle gradually becomes more acidic. At this point, the acid-labile groups hydrolyze from the conjugate and reveal the PBAVE, which is able to lyse the endosome for entry into the cytoplasm. Herein, the intracellular environment is reducing and can cleave the disulfide bond between siRNA and the polymer to facilitate delivery of the cargo to the RNA-induced silencing complex. This dynamic polyconjugate system was able to target and effectively deliver siRNA to liver cells in vivo for gene knockdown.

**Polymeric Micelles**

For chemotherapeutic drug delivery, biocompatible polymeric micelles have shown high drug loading, encapsulation of poorly water-soluble drugs, prolonged
For example, filamentous micelles (filomicelles) with high physical stability were prepared from block copolymers of poly(ethylene oxide) (PEO) and PCL or poly(ethylethylene) as degradable or inert materials, respectively [67]. In one particular study, the hydrodynamic diameter of filomicelles ranged from 25 to 60 nm, and the persistence length ranged from 0.5 to 5 μm. The size of the filomicelles was tuned by varying the block length of the copolymer with higher molecular weight yielding longer filomicelles. Compared to their spherical counterparts, the filomicelles persisted in circulation ten times longer, which was about 1 week after intravenous injection. The non-spherical shape allows for evasion of macrophage phagocytosis, and circulation time increased with persistence length of the filomicelle. Paclitaxel-loaded filomicelles showed efficacious drug delivery and improved therapeutic effect compared to spherical counterparts. Further investigation of paclitaxel-loaded micelles showed that filamentous micelles nearly double the maximum tolerated dose compared to spherical micelles [68]. Incorporation of fluorescent dye into the filomicelles showed that they migrate into a tumor and the micellar fragments penetrate into the tumor stroma.

Another micellar example with block copolymers involves poly(glutamic acid) (PGlu) and PEG in the delivery of cisplatin [69]. The formed micelles adopted a diameter of 28 nm, and the incorporation of cisplatin occurred via ligand exchange of Pt(II) from the chloride to the carboxylate of glutamic acid residues. Steady release of cisplatin occurred under physiological conditions. Drug-loaded micelles showed prolonged circulation and high tumor accumulation with complete tumor regression without toxicity. Combining PGlu-PEG block copolymer with a platinum anticancer drug and PGlu homopolymer at different concentrations allowed access to different sizes of micelles below 100 nm for evaluation in tumor models [70]. In highly permeable colon tumors, all particles accumulated and showed similar anticancer efficacy. However, in poorly permeable pancreatic tumors, the accumulation of drug increased with decreasing micelle size, and anticancer efficacy was greatest for the smallest, 30 nm micelles. Further derivatization of the PEG-PGlu polymeric micelle system with cyclic RGD integrin-targeting peptide and a platinum anticancer drug showed rapid tumor accumulation and penetration into tumor parenchyma [71]. Targeted micelles showed enhanced tumor delivery and anticancer efficacy in vivo, which may be attributed to a proposed selective active transcytosis internalization pathway via integrin receptors.
Given the anticancer efficacy observed with polymeric micelles, they have been utilized in clinical trials mainly due to their size, improved pharmacokinetics, and enhanced tumor accumulation of drug. A block copolymer composed of PEG and poly(D,L-lactide) was combined with paclitaxel to form 20 to 50 nm polymeric micelles, referred to as Genexol-PM® [72]. This formulation has been studied in clinical trials and is approved in South Korea for the treatment of breast cancer and non-small cell lung cancer. This formulation exhibited a higher maximum tolerated dose, better tumor accumulation, and improved efficacy compared to free drug and Taxol®, a commercially developed form of paclitaxel formulated with Cremophor EL®.

For the clinical trials using Pluronics, the formulation consisted of doxorubicin and Pluronics F127 and L61 (named SP1049C), which yielded ~25 nm micelles. Phase II clinical trials have been carried out using SP1049 C for advanced adenocarcinoma of the esophagus and gastroesophageal junction [73]. This study showed SP1049C provided substantial antitumor activity and a positive safety profile. In a murine leukemia model, SP1049C was found to decrease tumorigenicity and aggressiveness of cancer cells in vivo with enhanced activity against cancer stem cells [74].

Anticancer activity has also been seen in vivo for a polymeric micelle NK911, which has gone into clinical trials. NK911 is a block copolymer of PEG and poly(aspartic acid) (PAsp) and is partially conjugated with doxorubicin [75]. This copolymer is formulated with free doxorubicin to produce ~40 nm micelles that gradually releases doxorubicin over 8–24 h. The micelle showed prolonged blood circulation and high tumor accumulation. Furthermore, NK911 exhibited higher anticancer activity than free doxorubicin in multiple tumor models. In clinical trials, NK911 was found to be well tolerated [76].

A variety of poly(2-oxazoline)s (POx) block copolymers have been synthesized and studied for drug delivery [77]. One interesting example involves the first demonstration of formulating third-generation taxoids with POx micelles [78]. These POx were composed of methyl and butyl POx with a molecular weight of 10 kg/mol and PDI of 1.14. All taxoids could be loaded at nearly 1:1 ratio (50% drug) with resulting sizes around or below 100 nm. One of the taxoids showed enhanced efficacy in a multidrug resistant cancer cell line compared to paclitaxel in vitro. Furthermore, this taxoid demonstrated enhanced tumor inhibition in two orthotopic multidrug resistant tumor models in vivo.

The stability of polymeric micelles can be improved through crosslinking approaches. One approach harnesses block copolymers with ionic and nonionic hydrophilic segments. These block ionomers were combined with oppositely charged polymers to form block ionomer complexes. This approach has been utilized to deliver small molecule drugs, proteins, and nucleic acids [79]. In addition to electrostatically crosslinked micelles, covalently crosslinked micelles have been explored. For example, a biodegradable PAsp-based, PEG-terminated triblock copolymer was synthesized with crosslinkable thiol and pH-responsive tertiary amino groups [80] (Fig. 11). This system was termed interlayer-crosslinked micelle, which was capable of loading doxorubicin in the partially hydrated core and had a disulfide-crosslinked interlayer to prevent core expansion at neutral pH.
After internalization by cells, triggered release of drug occurred in the acidic, reducing environment of the lysosome. In vitro and in vivo anticancer efficacy was observed for these micelles with improved performance compared to free drug and PEG-PCL micelles.

Polymeric micelles have been utilized for the delivery of nucleic acids. For example, siRNA was linked to phosphothiol ethanol through a disulfide bond, and this conjugate was mixed with phosphothiolethanol-PEG to form reducible micelles [81]. This system showed protection of siRNA from nuclease degradation, cytocompatibility, and gene knockdown 50-fold more effective than free siRNA. Another example of micellar delivery of siRNA in combination with paclitaxel used matrix metalloproteinase 2 (MMP2)-sensitive copolymers [82]. Specifically, PEG was linked through a cleavable peptide linkage to 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine. This system provided high stability, siRNA condensation, drug encapsulation, MMP2-triggered tumor targeting, and increased cell uptake after MMP2 exposure.

**Dendrimers**

Efficacious chemotherapeutic delivery via dendrimer conjugates has been demonstrated. For example, camptothecin was conjugated to PEGylated PLL dendrimer through aspartic acid [83]. The dendrimer conjugate had a long blood circulation time and ca. 14 times tumor uptake of the drug relative to free drug. With a single injection of the dendrimer conjugate in tumor-bearing mice, there was prolonged survival, while in another model all mice survived to the end of the study when injected with multiple doses. Another example involved the derivatization of a bow-tie dendrimer (Fig. 12) with doxorubicin through an acid-labile hydrazone bond, which provided prolonged blood circulation of the drug and nine times higher tumor accumulation relative to free drug [84]. A single initial intravenous injection resulted in complete tumor regression and survival of 60 days with efficacy similar to that of commercially available Doxil (liposomal formulation of doxorubicin).

The incorporation of drugs with dendrimers through noncovalent approaches has been pursued, harnessing a variety of interactions. For example, high-generation poly(propylene imine) dendrimers were utilized for the encapsulation of Rose Bengal within their cavity via host-guest-like interactions [85]. However, for drug
release, specific hydrolysis conditions were required to remove the dendritic shell. Additional noncovalent approaches include multiple hydrogen bonding and hydrophobic interactions. Poly(aryl ether)s have been utilized to enhance the water solubility of hydrophobic compounds [86]. The hydrophobic nature of certain dendrimers such as poly(arylene ether)-based polymers can be exploited to form micelles by functionalizing the outer surface with hydrophilic groups such as PEG. Micellar dendrimers have an intriguing advantage over conventional polymer micelles composed of amphiphilic block copolymers. Specifically, the micellar dendrimers remain stable under dilute conditions while the stability of block copolymers depends on the critical micelle concentration.

PLL has been used for dendrimer synthesis and DNA transfection. Dendritic PLL was synthesized with different generations [21], and the fifth- and sixth-generation PLL dendrimers were able to effectively transfec cells in vitro. These fifth- and sixth-generation dendrimers have 64 and 128 surface amine groups and were subsequently functionalized with arginine and histidine to increase the positive charge for complexation [87]. Arginine-modified dendritic PLL enhanced DNA transfection 3- to 12-fold in multiple cell lines, while the histidine derivative did not. For the histidine derivative, acidic conditions were required to effectively complex DNA. Using this effective complexation, subsequent transfection showed enhanced DNA delivery compared to the native polymer.

Fig. 12 Chemical structure of poly(ester) dendrimer-PEG bow-tie hybrid
Nanoparticles
Prodrugs have been designed for controlled release of chemotherapeutics conjugated to polymeric nanoparticles [88]. These prodrugs were a follow-up iteration from the development of acid-sensitive silyl ether crosslinkers with tunable degradation rates and cargo release kinetics [89]. For the prodrugs, asymmetric bifunctional silyl ether conjugates of camptothecin, dasatinib, and gemcitabine were synthesized. The silyl ether bond between the drug and polymerizable unit allowed for release of the native, active therapeutic after hydrolysis (Fig. 13). These prodrugs were synthesized in one step and were polymerized into PEG-based nanoparticles. By controlling the steric bulk of the substituent on the silicon atom, the release of drug could be tuned and was accelerated under acidic conditions. Corresponding in vitro studies showed that drug release could be tuned to elicit cytotoxicity similar to free drug in addition to eliciting minimal toxicity without regard to drug loading.

Docetaxel was loaded into 200\,\mu{}m cylindrical PLGA nanoparticles with encapsulation efficiencies greater than 90%. PRINT allows for high drug encapsulation regardless of loading while maintaining the physical properties of nanoparticles. By controlling the loading of docetaxel, the IC\textsubscript{50} was tuned and reached higher potency than the commercial standard Taxotere\textsuperscript{®} in vitro. PRINT has also been used to generate better medicines and specific nanoparticle theranostics, which have been reviewed [53, 90].

Paclitaxel has been encapsulated in BTM nanoemulsions with ca. 200 nm size and approximately 6% drug loading (drug/oil). These lipid-based, paclitaxel-loaded BTM nanoparticles showed long-term stability at 4°C and 37°C in PBS with slow release of cargo, lack of initial burst release, and potential for lyophilization without the need for cryoprotectants [45]. These particles were targeted to A431 tumors in vivo using epidermal growth factor receptor-binding Z-domain [46]. Delivery of paclitaxel-loaded nanoparticles in vivo provided anticancer efficacy in nude mice bearing resistant NCI/ADR-RES tumors. Furthermore, BTM nanoparticles overcame P-glycoprotein-mediated multidrug resistance by depleting ATP and inhibiting P-glycoprotein [91]. The delivery and efficacy of taxanes using lipid-based nanoparticles have been extensively reviewed elsewhere [92].

siRNA has been encapsulated in PRINT hydrogel nanoparticles [93]. This was accomplished through noncovalent and covalent means. Noncovalent complexation and physical entrapment enabled gene silencing; however, to retain siRNA during systemic administration or particle modification, a covalent approach was pursued. For reversible covalent incorporation, a polymerizable siRNA macromonomer with a degradable disulfide linkage was polymerized into PEG-based hydrogel

![Fig. 13 Structure and mechanism of degradation for silyl ether prodrugs](image-url)
nanoparticles (Fig. 14). A functional amine monomer was utilized for cellular internalization and endosomal escape. The content of this amine was screened to optimize cytocompatibility and gene silencing efficiency. This system allowed for stable incorporation in serum, triggered release of siRNA under intracellular reducing conditions, and effective gene silencing in vitro.

A double emulsion solvent evaporation technique has been utilized for the preparation of PLGA nanoparticles in the delivery of siRNA [94]. In this study, PLGA nanoparticles loaded with siRNA were administered in a single dose to the mouse reproductive track for effective and continual gene silencing in the vaginal mucosa. Another example of PLGA nanoparticle delivery of siRNA utilized a film-stretching approach to fabricate nanoneedles [95]. Gene knockdown was enhanced by nanoneedle-shaped PLGA nanoparticles compared to their spherical counterparts, attributed to cell permeabilization and induced cytoplasmic delivery. Furthermore, toxicity was minimized by using shape-shifting nanoparticles that lose the sharp, cell-penetrating nanoneedle edges. siRNA has also been encapsulated in PLGA PRINT nanoparticles [96]. In this study, 80\%C2320 nm nanoparticles were synthesized with high encapsulation of siRNA and then surface-modified with cationic, transfecting lipids. These nanoparticles were internalized by multiple cancer cell lines and elicited knockdown of therapeutically relevant gene expression for the treatment of prostate cancer.

Nanoprecipitation has been used for the preparation of cisplatin prodrug PLGA-PEG nanoparticles [97]. These nanoparticles were decorated with prostate-specific membrane antigen (PSMA) targeting aptamer to deliver cisplatin to prostate cancer cells. The cisplatin prodrug was prepared by installing hexanoic acid groups in the axial positions to afford hydrophobicity and affinity for PLGA. The nanoparticles had a drug loading of approximately 6\% and size ca. 140 nm. The targeting ligand was conjugated to nanoparticles post-precipitation through amine-carboxylic acid coupling using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide. Targeting ligand-bearing nanoparticles was internalized through endocytosis by PSMA-positive LNCaP prostate cancer cells. Furthermore, the aptamer-functionalized cisplatin prodrug nanoparticles were more potent than cisplatin or nontargeted nanoparticles in LNCaP cells. This work established a potential method for systemic delivery of platinum drugs targeted to prostate cancer cells.

Poly(beta-amino ester)s (PBAEs) are a member of the poly(ester) class of materials for nanoparticle DNA delivery. A combinatorial library of PBAEs was synthesized, to understand the structure-property relationships for polymeric gene delivery [98]. These polymers condensed DNA to form nanoparticles that effectively
delivered genes to cells with low cytotoxicity. Effective delivery of DNA was achieved with linear polymers of ca. 10 kDa, hydroxyl side chains, primary amine end groups, and tertiary amines. Therefore, the structure, functional groups, and molecular weight of the polymer influenced the gene delivery efficiency. These large-scale studies may be utilized in the design of biomedical polymers for drug delivery.

Similar to the combinatorial library approach for PBAEs, another class of polymers was synthesized through a high-throughput approach. Core-shell nanoparticles were synthesized via a combinatorial method for intracellular delivery [99]. Block copolymers of PEG and epoxides were crosslinked with a library of different amines (Fig. 15). These core-shell nanoparticles were able to effectively deliver plasmid DNA and small interfering RNA to cells in vitro. Some highlighted properties of efficacious particles include tertiary amines with one to two reactive sites, amines with buffering capacity, and thin hydrophilic shells. Covalent attachment of cholesterol allowed for gene silencing in the liver in vivo. Again, the polymer structure and functional groups played a huge role in determining drug delivery efficacy.

Delivery of DNA and siRNA has been accomplished using CD-based polycations. The beta-CD polymers contain amidine groups in the backbone as the cation. When the polycation was mixed with nucleic acid, nanoparticle formation occurred and gene delivery was achieved. To avoid undesirable interactions after intravenous administration, functionalization with PEG was pursued. The optimal approach involved use of adamantane-terminated PEG, which formed an inclusion complex with the hydrophobic pocket of beta-CD [100]. Further functionalization of these polycations with imidazole enhanced gene delivery efficiency [101] (Fig. 16). The pH-buffering properties provided by imidazole facilitated endosomal escape and
gene delivery. Furthermore, the imidazole polycation was shown to have weaker binding with DNA and faster release under intracellular conditions. Here, the introduction of imidazole groups at the ends of the polymer notably altered the structure, properties, and efficacy of the polyplex. Further development of this work led to the first targeted delivery of siRNA to elicit gene knockdown in humans with melanoma [102].

5.2 Imaging and Tracking Biomedical Polymers In Vivo

To understand the behavior of biomedical polymers in vivo and improve the efficacy of drug delivery, imaging modalities are of crucial importance. Imaging polymers can elucidate the role of polymer size, composition, and architecture on in vivo behavior. To analyze the in vivo behavior of polymers, conventional approaches use fluorescent dyes, multiple blood draws, and sacrifice of numerous mice with extensive analysis of collected tissue. Noninvasive imaging modalities include gamma camera imaging (scintigraphy), magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and positron-emission tomography (PET). These modalities are powerful and may provide sensitive, quantitative, and noninvasive analysis of tracers in real time. Here we will cover the use of select modalities to image or track biomedical polymers with linear, dendritic, and nanoparticle architectures.

5.2.1 Linear Polymers

HPMA copolymer-doxorubicin was the first linear polymeric conjugate to be evaluated clinically in 1994 [103]. The copolymer was composed of HPMA and a peptide linker side chain conjugated with doxorubicin. The peptide linker was Gly(D,L)Phe-Leu-Gly, which is cleavable by the lysosomal enzyme cathepsin B. This copolymer had a molecular weight of ca. 30 kDa and a doxorubicin content of 8.5 wt%. Since the copolymer contained doxorubicin, it formed small micelles in solution of approximately 6 nm in diameter. In Phase I trials, the copolymer was labeled with $^{131}$I through simple mixing in an iodogen reaction vial and purified by G-25 Sephadex column for imaging by gamma camera (scintigraphy) and quantitative analysis [104]. Pharmacokinetic analysis showed the labeled copolymer had a distribution half-life of 1.8 h and an elimination half-life of 93 h. Moreover, imaging studies showed that the copolymer is taken up by some tumors.

In Phase II studies, the HPMA copolymer-doxorubicin conjugate was evaluated in breast, lung, and colorectal cancers [105]. Here, an imaging analogue that contained 1 mol% methacryloyltyrosinamide was utilized for radioiodination using $^{123}$I for scintigraphy and SPECT analysis. The polymer showed bi-exponential kinetics with a distribution half-life of 3 h and elimination half-life of 41.2 h and area under the curve (AUC) of 5 mM·h. Radioimaging in one of the patients with breast cancer showed 5.9% of the injected dose after 24 h. The tumor of this patient was one of the major sites of the body with radioactivity present after 48 h.
These results illustrated that therapeutics conjugated to polymers have altered and enhanced pharmacokinetics and anticancer activity.

5.2.2 Dendrimers

Nano-sized macromolecules with specific sizes can be useful as contrast agents. Given that the body may process macromolecules distinctly based on nanometer differences, dendrimers find great utility for elucidating the effect of nanometer sizes on biodistribution. These can be utilized with Gd(III), which enabled sufficient contrast by MRI with sub-millimolar concentrations at 1% of the concentration of iodine required as a contrast agent. Two common chelators for Gd(III) are diethylenetriamine pentaacetic acid (DPTA) and 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA). PAMAM and polypropyleneimine dianinobutane (DAB) dendrimers are water-soluble and provide functional handles for conjugation with chelators. A difference in a few nanometers and internal core (PAMAM vs. DAB) was shown to significantly alter the pharmacokinetics [106, 107]. For example, DAB dendrimers showed notably more liver accumulation and were more rapidly excreted than PAMAM dendrimers. Dendrimers of 10–14 nm showed minimal renal excretion, while 3–6 nm dendrimers were rapidly excreted by the kidney. Furthermore, 6 nm dendrimers were found to leak out of tumor vessels much quicker than 10 or 14 nm dendrimers. This study demonstrated the potential for additional applications of dendrimers with particular physicochemical properties as contrast agents in imaging functional anatomy such as tumor blood vessels and the lymphatic system.

The biodistribution profiles of aliphatic polyester dendrimers have been determined through radiolabeling with Tc(I) and Re(I) [108] using SPECT. Specifically the core of poly(2,2-bis(hydroxymethyl)propanoic acid) dendrimers with different generations was modified with a chelator. Radiolabeling with 99mTc allowed for real-time in vivo monitoring biodistribution; these dendrimers were rapidly cleared from blood by the kidneys and not retained in any organs after injection in rats. Another set of poly(ester)-based dendrimers functionalized with PEG chains was synthesized in a “bow-tie” structure for elucidating biodistribution and tumor uptake [109]. The effect of molecular weight and number of PEG chains for these bow-tie dendrimers was explored. Due to the polyester composition, these dendrimers were demonstrated to be degradable. Dendrimers were labeled with 125I and evaluated for biodistribution. Higher molecular weight bow-tie dendrimers with molecular weight greater than 40 kDa demonstrate prolonged circulation half-life and high tumor accumulation with 109 kDa provided the longest half-life and 52 kDa providing the highest tumor accumulation.

5.2.3 Nanoparticles

An interesting imaging approach involved an amphiphilic core-shell nanoparticles that were prepared from diblock copolymers of poly(acrylic acid) and poly(styrene) or poly(methyl acrylate) [110]. Particles were crosslinked with a diamine to yield shell-crosslinked nanoparticles, and acid groups were functionalized with a macrocyclic chelator (TETA derivative) for chelation of copper-64 (Fig. 17) and imaging by PET. Nanoparticles with different sizes (18–37 nm), core composition, and
surface PEGylation were studied for their biodistribution. It was found that 18 nm NPs with a rigid polystyrene core showed the longest blood retention time and lowest liver accumulation. This study showed the importance of polymer and particle design parameters (size, flexibility, and surface chemistry) on biological behavior in vivo.

To optimize the radiolabeling of shell-crosslinked nanoparticles, the spacer length, crosslinking extent, and charge density were investigated [111]. The block lengths of PAA-block-PS were varied from 30 to 136 repeat units. A nine-atom spacer (DOTAamine) with three carboxylates was compared to a five-atom spacer (DOTAlysine) with four carboxylates. The longer spacer DOTAamine provided enhanced coupling due to steric hindrance factors and electrostatics based on the negatively charged nanoparticle. Twenty percent crosslinked nanoparticles enabled significantly increased amount of $^{64}$Cu-accessible DOTAs compared to 50% crosslinked nanoparticles. This can be attributed to more functional acrylic acid units present on the surface for the lower degree of crosslinking. The specific activity was greatest for the block copolymer with the highest PAA content, which provided small particle size and minimal aggregation. Here, we see that in preparing radiolabeled nanoparticles, several factors must be considered to maximize specific activity for imaging.

5.3 Scaffolds for Tissue Engineering

Polymeric materials are widely utilized as scaffolds in tissue engineering to restore lost or damaged tissue. This goal is often accomplished with a combination of polymeric scaffold, cells, and biological factors. The scaffold serves as an artificial extracellular matrix designed to mimic the environment and physical characteristics of the specific tissue. The scaffold materials are designed to elicit desirable biological responses. Some of the most important properties of the scaffold include high porosity, appropriate pore size, biocompatibility and biodegradability, mechanical strength, and surface chemistry. All of these properties are influenced by the type and preparation of polymeric material. Further investigation into scaffold designs has revealed that micro-/nanostructure, geometry, and topology can make a profound impact on function and physiological responses. Controlling the properties and design features of scaffolds for a particular tissue engineering application depends on the identity and physicochemical characteristics of the polymeric material.
Polyesters
Polyesters represent a large class of polymers that are used as tissue regenerative scaffolds. Their mechanical strength, biocompatibility, and tunable biodegradation times make them ideal candidates as scaffolds in tissue engineering. Electrospinning is a common method to fabricate fibers from polymers for biomedical applications. Using electrospinning, a three-dimensional collecting method was employed to fabricate PLGA/PCL fibrous tubes with different interconnected structures, patterned architectures, and macroscopic configurations for potential tissue engineering applications [112]. Electrospun PLGA/PCL micro- and nanofiber scaffolds were synthesized with diameters ranging from 280 nm to 8 μm with small (700 nm) and large (20 μm) pores [113]. These scaffolds were utilized for nerve regeneration in lesioned rats. There were three groups of rats studied: (1) sciatic nerves were transected; (2) a 10 mm gap was left after removal of a 5–7 mm segment of the sciatic nerve; (3) electrospun tubes were implanted post neurotmesis. The multi-scaled structure of these electrospun tubes enabled the repair of a nerve gap of 10 mm in vivo in a rat sciatic nerve, while the sciatic nerves failed to reconnect in control groups.

Polymer hybrid materials generally show enhanced attachment and proliferation of cells. For instance, electrospun PCL was compared to PCL coated with gelatin and calcium phosphate (apatite) [114]. This coated porous scaffold mimicked the bone extracellular matrix and provided higher rate of proliferation of preosteoblastic cells after 1 week culture relative to PCL alone. Another hybrid material that has been studied for bioactivity is PLGA-collagen-hydroxyapatite [115]. Biomineralization was observed to occur preferentially on the hybrid membrane side compared to PLGA alone using human mesenchymal stem cells (hMSCs). This study demonstrated the development of a hybrid material system as a potential new class of biomimetic scaffolds that facilitate bone tissue engineering.

Poly(aniline)
To maintain signaling in the nervous system, muscle contraction, and wound healing, electrical stimulation can play a crucial role. Nerve cell stimulation can be provided by using the conducting polymer, poly(aniline) (PANI) [116]. PANI is biocompatible and has been copolymerized or blended with other biodegradable polymers to provide a range of mechanoelectric properties. For example, blends of poly(lactide-co-caprolactone) (PLCL) and PANI electrospun into fibers provided unique cellular behavior [117]. Specifically, fibroblasts and myoblasts exhibited higher levels of adhesion to PLCL-PANI compared to PLCL alone. Furthermore, the growth of fibroblasts was enhanced by stimulation under direct current flows. Another study developed a mesh of PLGA and PANI as an electrically active scaffold for coordinating the beating of cardiomyocytes [118]. Abnormalities in the functioning of cardiomyocytes are relevant to myocardial infarction. Synchronous beating of all cardiomyocytes was realized via electrical stimulation of the PLGA-PANI fibers to mimic the heart.
(iii) Hyaluronic Acid
Combination of hyaluronic acid (HA) with other biopolymers can enhance biological functions. HA is a key scaffold for tissue engineering. In particular, HA is FDA-approved, biocompatible, and is a native extracellular component. Covalent incorporation of fibronectin through photocrosslinking with HA provided increased viability of cultured endothelial cells compared to fibrinogen adsorbed to HA [119]. Gelatin-containing HA hydrogel particles embedded into a HA network have controlled the adhesion and differentiation of mesenchymal stem cells [120]. HMSCs are readily attached, migrated deeply, and formed an interconnected population in this network, while isolated spheroids were encountered with gelatin-free networks. Furthermore, production of collagen and mineral deposition were noted in gelatin-containing networks, suggesting osteogenic differentiation, while markers for adipogenesis were found in gelatin-free networks.

Another example of a hybrid material for cartilage repair involved hydrogels composed of methacrylated glycol chitosan with HA prepared by photocrosslinking [121]. These polymers were photocrosslinked with a riboflavin initiator using visible light. High cell viability of encapsulated chondrocytes (~80 to 87%) was observed over 21 days. Furthermore, it was found that the incorporation of HA increased cell proliferation and cartilaginous extracellular matrix depositing. From these studies, we see that HA has provided enhanced biological compatibility and function for tissue engineering.

(iv) Gelatin and PEG
Three-dimensional structures, such as those microengineered through projection stereolithography, have been utilized for eliciting specific biological responses. For example, microengineered structures were prepared starting from gelatin functionalized with methacrylic anhydride [122]. The mechanical properties of this crosslinked gelatin were controlled by varying the porosity and concentration of the prepolymer. Cell growth was supported on defined geometries with uniform cell distribution, high cell density, and homogeneity. To maximize live cell fabrication and minimize damage to cellular DNA through UV irradiation, visible light-based projection stereolithography was developed [123]. Here, hydrogel scaffolds were prepared from PEG diacrylate with different shapes and architectures. Adipose stem cells maintained high viability in the scaffolds, and porous scaffold architectures provided higher cell viability and activity. The three-dimensional structure of the scaffold was shown to influence the biological responses.

5.4 Medical Devices: Replacements for Heart Valves, Arteries, and Joints

Advances in tissue engineering enable the production of replacement organs in great demand. This notable shortage of organs is being addressed by developing new methods to provide biological replacements such as heart valves, arteries, and joints.
Conventional approaches to produce biological replacement have substantial drawbacks. These include the potential for thromboembolism, infection, immune responses, and inability to adapt for growth. Conversely, tissue engineering approaches are aimed toward delivering and integrating living and biocompatible material that avoid the drawbacks.

The heart valve is critical in the cardiovascular network for appropriate one-directional blood flow. Heart valve disease poses a significant threat for death. One approach to create heart valves involves the transplant of autologous cells onto a scaffold shaped like a heart valve. Modern approaches are harnessing three-dimensional printing for the production of heart valves. For example, hybrid hydrogels composed of methacrylated HA and gelatin have been synthesized for the encapsulation of aortic valve cells [124]. With increased gelatin content, lower stiffness, higher viscosity, cell spreading, and enhanced maintenance of fibroblast phenotype were realized. High cell viability was observed in addition to the deposition of collagen and glycosaminoglycans. Another three-dimensional printing technique provided living alginate/gelatin hydrogels with region-specific incorporation of two cell types [125]. Viability was maintained for aortic smooth muscle cells and aortic valve leaflet cells, which expressed muscle actin and elevated vimentin, respectively.

Connections among tissues and organs to the heart are provided by blood vessels. Blood vessel diseases represent a significant percentage of deaths due to complications with atherosclerosis and subsequent myocardial infarctions. Engineering of blood vessels may allow for facile surgery and repaired function instead of challenging operations like cardiac bypass surgery. Poly(glactin)/PGA tubular scaffolds have been fabricated to construct tissue-engineered artery conduits [126]. Harvested artery or vein cells were seeded on scaffolds to create a vascular construct that was then used as arteries in lambs. These vascular grafts demonstrated growth and development of endothelial lining and extracellular matrix and behaved well in pulmonary circulation. Another example of a vascular graft involved PCL, which was utilized in a rat abdominal aorta replacement model [127]. PCL micro- and nanofibers were evaluated over 18 months. PCL maintained structural integrity and showed an absence of aneurysmal dilation, thrombosis, and minimal hyperplasia.

Joint replacements were first developed over a century ago and remain important materials for damaged joints. Ultrahigh molecular weight poly(ethylene) (UHMWPE) has been the main chosen material for joint replacement. This may be attributed to the appropriate properties and biocompatibility of UHMWPE. Specifically, UHMMPWE is a semicrystalline material with a molecular weight of 3–6 megadaltons (MDa), yield/tensile strengths on the orders of megapascals (MPa), and elastic moduli around 1 GPa [128]. Although UHMWPE can function well for at least 15 years, there is growing concern about UHMWPE debris generated in vivo [36]. This has led to advances in processing, sterilization, and crosslinking of UHMWPE [129]. For example, vitamin E has been used to stabilize UHMWPE and showed good mechanical, wear, and oxidation properties and improved longevity of joint replacements [130]. These advancements and further iterations may enable increased longevity without the presence of debris for UHMWPE joint replacements in vivo.
5.5 Surgery and Wound Repair Materials

Polymeric materials have found extensive use in surgery and wound repair. Wounds can be defined by a disruption in the skin arising from a physiological nature or external damage. Three main phases constitute wound healing: inflammation, tissue formation (proliferation), and tissue remodeling. This healing process involves a complex interplay among cells, extracellular matrices, and signaling factors. To aid in the wound healing process, polymeric materials have found utility in promoting re-epithelization, accelerating healing, minimizing scar formation, and stimulating cellular activities. The properties of polymeric materials are important to the effect they exert in the wound healing process. Notable properties of surgery and wound repair materials include moisture permeability, tensile strength, elasticity, bioadhesion, biocompatibility, and bioactivity (increasing angiogenesis). Moreover, combining polymers can lead to synergistic and positive biological responses in wound repair.

Chitin and chitosan (deacetylated chitin) have been widely utilized in wound repair materials due to their inherent regenerative properties. For example, chitosan can activate macrophages to signal healing, encourage cell motility and angiogenesis, and exert antimicrobial activity [131]. Furthermore, chitosan is biocompatible and biodegradable, mucoadhesive, and amenable to chemical modification via free primary amine. A formulation involving chitin nanofibrils, chitosan glycolate, and chlorhexidine was prepared as a spray, gel, and gauze to evaluate efficacy in treating traumatic wounds [132]. In all clinical cases, the preparations demonstrated abundantly satisfactory results. Physiological repair was enhanced with the gel, scar formation was absent with the gauze, and bleeding abrasions were treated with the spray as a first-aid tool.

Blends of chitin and chitosan with additional polymeric materials have resulted in stimulated healing-impaired wound repair [133]. Specifically, a hydrogel was prepared from chitin/chitosan, alginate, and fucoidan as wound dressing material. This blended hydrogel elicited advanced granulation tissue and capillary formation in healing-impaired wounds compared to the commercial calcium alginate fiber material Kaltostat®. Another chitosan-based hydrogel was synthesized with layers of chitosan, alginate, and PGA for wound healing in diabetic rats [134]. This hydrogel provided accelerated wound healing compared to alginate hydrogels alone and increased collagen regeneration and epithelialization.

Materials for biologically interactive wound repair have been explored using crosslinked glycosaminoglycans. Specifically chondroitin sulfate, hyaluronan, and PEG were crosslinked into a polymeric network [135]. Increased re-epithelization was observed for a film of the polymeric network combined with the commercial film dressing Tegaderm™ compared to the commercial product alone. Furthermore, there was more collagen regeneration and organization for the polymer network, while the inflammatory response remained the same. Another example for wound healing was with chondroitin sulfate and involved the preparation of bilayer gelatin-chondroitin-6-sulfate-HA biomatrices [136]. This material was applied for wound...
healing in severe combined immunodeficiency mice. The bilayer material served as a skin substitute, which promoted wound healing and had a high take rate of the skin graft.

5.6 Polymeric Biosensors

The detection of chemical and biochemical species is important in monitoring our health and the environment. Polymeric materials offer the capabilities for sensitive analyte detection with amplified signals. Recently, conjugated polymers have been the focus of developing biosensor materials. Conjugated polymers are composed of unsaturated backbones that can provide conductivity and energy transfer. The advantages of conjugated polymers include inherent material processing and mechanical properties combined with electrical and optical properties of semiconductors and metals. The sensing properties of conjugated polymers are influenced by chemical structure, conjugation length, conformation, and packing.

Conjugated polymers are often hydrophobic and water-insoluble. Common structures of conjugated polymers are provided in Fig. 18. For biological applications, water-soluble polymers are typically preferred. Therefore, there has been extensive effort into designing water-soluble conjugated polymers for biosensing through chemical modification and incorporation. The interactions between the conjugated polymer and biochemical agent of interest can be harnessed for increased and specific sensing.

For instance, a water-soluble cationic conjugated polymer, which can complex DNA, has been designed to change emission color based on conformational and aggregation changes [137]. A benzothiadiazole was incorporated into a quaternary amine-functionalized poly(fluorene-co-phenylene). Different colors were obtained for noncomplementary and complementary single-stranded DNA in addition to DNA-free solutions. This opened the door to multicolor biosensors based on tuning electrostatic and optical interactions. Another example utilized cationic poly(fluorene-alt-1,4-phenylene) derivatized with benzothiadiazole for detecting heparin [138].

![Fig. 18 Chemical structures of biomedical polymers used as biosensors](image-url)
This polymer allowed for multicolor detection and quantification of heparin by harnessing the electrostatic attraction between the polymer and heparin. Detection was observable by the naked eye from orange emission induced by fluorescence resonance energy transfer.

The detection of proteases has been realized with water-soluble poly(phenylene ethynylene)s functionalized with carboxylates through oligo(ethylene glycol) spacers [139]. Covalent incorporation of a fluorescence-quenching peptide yielded a nonemissive substrate. The polymer regained fluorescence after exposure to trypsin, which cleaved the peptide sequence incorporated into the polymer. A small molecule analogue of this system highlighted the effect of polymer signal amplification in biosensing. Another example for the detection of proteins involved a sulfonate-derivatized poly(phenylene vinylene) as a biosensor for cytochrome c [140]. At neutral pH, cytochrome c is positively charged and can form a complex with this conjugated polymer, resulting in a quenching of its fluorescence. The efficient quenching was attributed to the combined effect of electron transfer between cytochrome c combined with complex formation between cationic and anionic polyelectrolytes, which opened the door for improving poly(phenylene vinylene) biosensors.

Enzymatic cleavage and oxidative damage to DNA has been detected using a cationic conjugated polymer [141]. A quaternary ammonium-functionalized polythiophene allowed for real-time, label-free monitoring of DNA cleavage by harnessing the interpolyelectrolyte complex formed between the conjugated polymer and DNA. In complex formation, a planar conformation was assumed with an associated red shift in absorption. After DNA damage, the complex could not form and exhibited a distinct absorption. In addition, the DNA damage could be observed visually by color change. DNA has also been utilized indirectly for biosensing. For example, human thrombin was detected with high sensitivity using single-stranded DNA (aptamer)-conjugated polymer complexes [142]. A specific DNA aptamer changes conformation after binding human thrombin. This change in conformation was detected using a cationic poly(3-alkoxy-4-methylthiophene) derivative to provide a label-free optical signal. This approach provided a foundation for rapid detection and identification of proteins and high-throughput screening in drug discovery.

6 Perspectives and Future Trends

From the biomedical applications covered herein, we have seen unique relationships among polymer identity, structure, properties, and function. These relationships may provide blueprints for the design of a polymer to reach a specific biomedical goal. The approach to designing each biomedical polymer and its structure involves fine-tuning the chemical composition. The chemical composition may be selected based on the breadth of choices covered herein. Distinct structures and compositions may be accessed through novel or defined synthetic approaches. The range of accessible structures and forms of biomedical polymers spans from linear native polymers to
self-assembled systems to synthetic hydrogels. Certain structures provide advantages for a given application. Moreover, we show there are key characteristics and advantages of polymer structures and composition that are similar within each application.

In drug delivery and imaging, key characteristics of biomedical polymers include biocompatibility, stability, chemical functionality, and unique biological behavior. Current and future directions include high-throughput synthesis and screening, enhanced stability of the material and cargo, and specific cargo release mechanisms and rates. For example, enhanced stability of the cargo may be realized by covalent conjugation and protection from degradation by polymer shielding. Cargo release may be tuned via labile linkages, disassembly mechanisms, or pore size of nanoparticles. The stability and biodegradation rate of the polymer can be tweaked based on the chemical composition, structure, and morphology such as increasing crystallinity. Additional focus directions involve controlling the structure, size, shape, and composition of the material. For instance, the size and shape of self-assembled systems can be determined by the molecular weight and hydrophobic-hydrophilic stoichiometry of polymers. Furthermore, top-down fabrication methods allow for versatile control over the composition, shape, size, and surface chemistry of nanoparticles. The repertoire of information presented on drug delivery and imaging may allow for better designs, development, and testing of biomedical polymers for therapeutic and diagnostic purposes.

In tissue engineering, key characteristics of biomedical polymers include mechanical strength, hydrophilicity/hydrophobicity, biodegradability, stability, conformation, and biofunctionality. Select biomedical polymers and combinations thereof have been shown to exert biological interactions and elicit particular biological responses. Furthermore, polymers functionalized with distinct chemical moieties have provided additional bio-interactions. The nano- and microscale features of polymeric materials substantially influence biological behavior. Forthcoming work for these applications involves the design and synthesis of novel polymers and combinations thereof, further chemical modification, and improved or new techniques for processing and production. For example, polymers may be produced by methods including electrospinning, lithographic processes, and three-dimensional printing. The future of biomedical polymers is open to creative polymer designs and material production. New designs and production methods may increase the breadth of unique structures and continually realize improved function and efficacy in biomedical applications.

7 Conclusions

In summary, we have covered different classes, structures, and properties of biomedical polymers with relevant biomedical applications. The structure-property-function relationships of biomedical polymers reveal design criteria that may be harnessed to realize their potential in the biomedical arena. Tailoring the structure and chemical composition of biomedical polymers to match the application may
enable desirable performance. For example, specifying the method of drug incorporation and controlled release can allow for fine-tuned drug delivery. Similarly, controlling the physicochemical and mechanical properties of polymers can provide particular extracellular matrix-like environments for tissue engineering. The range of polymer structures covered herein provides a toolbox of crucial design features. Selecting and building upon these polymer designs may lead to the development of unique properties and improved performance. One of the forefront goals in designing biomedical polymers is ultimately aimed at transforming human health and improving the quality of life. We hope that this book chapter has provided the necessary information to better design and develop biomedical polymers that may advance medicine and healthcare.

References

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Hydrogel Synthesis and Design

Michael J. Majcher and Todd Hoare

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Abstract
The capacity to exploit the many possible applications of hydrogels is strongly tied to our capacity to synthesize hydrogels with well-defined chemistries and structures. Herein, we review the major strategies used for the synthesis of hydrogels, focusing on the key choices to be made in terms of the chemical and structural properties of the backbone polymer, the nature of the crosslinking strategy used (in terms of both the mechanism and the permanence of network formation), and the length scale at which network formation is conducted. The impacts of these various choices on the ultimate properties of the hydrogels generated are emphasized in the context of the rational design of hydrogel compositions and structures for target applications.

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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AIBME</td>
<td>Dimethyl 2,2′-azobis(2-methylpropionate)</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>CD</td>
<td>Cyclodextrin</td>
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<tr>
<td>CNCs</td>
<td>Cellulose nanocrystals</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper(I)-catalyzed alkyne-azide click reaction</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variance</td>
</tr>
<tr>
<td>DHs</td>
<td>Dendrimer-based hydrogels</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally recognized as safe (FDA)</td>
</tr>
<tr>
<td>GelMA</td>
<td>Methacrylated gelatin</td>
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<tr>
<td>HA</td>
<td>Hyaluronic acid, HA</td>
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<tr>
<td>HPMC</td>
<td>Hydroxypropyl methylcellulose</td>
</tr>
<tr>
<td>IPN</td>
<td>Interpenetrating polymer network</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(acrylic acid)</td>
</tr>
<tr>
<td>PAAm</td>
<td>Poly(acrylamide)</td>
</tr>
<tr>
<td>PAH</td>
<td>Poly(allylamine hydrochloride)</td>
</tr>
<tr>
<td>PAMAM</td>
<td>Poly(amidoamine)</td>
</tr>
<tr>
<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
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<tr>
<td>PDADMAC</td>
<td>Poly(diallyldimethylammonium chloride)</td>
</tr>
<tr>
<td>PDLA</td>
<td>Poly(δ-lactic acid)</td>
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<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
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<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
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<tr>
<td>PHEMA</td>
<td>Poly(hydroxyethyl methacrylate)</td>
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<td>PLA</td>
<td>Poly(lactic acid)</td>
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<tr>
<td>PLL</td>
<td>Poly-L-lysine</td>
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<td>PLLA</td>
<td>Poly(1-lactic acid)</td>
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<tr>
<td>PMMA</td>
<td>Poly(methacrylic acid)</td>
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<tr>
<td>PNIPAM</td>
<td>Poly(N-isopropylacrylamide)</td>
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<tr>
<td>POEGMA</td>
<td>Poly(oligoethylene glycol methacrylate)</td>
</tr>
<tr>
<td>POLAMA</td>
<td>Poly(oligolactic acid methacrylate)</td>
</tr>
<tr>
<td>PPF</td>
<td>Poly(propylene fumarate)</td>
</tr>
<tr>
<td>PRINT</td>
<td>Particle replication in non-wetting templates</td>
</tr>
<tr>
<td>PSS</td>
<td>Poly(styrene sulfonate)</td>
</tr>
<tr>
<td>PU</td>
<td>Poly(urethane)</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly(vinylpyrrolidone)</td>
</tr>
<tr>
<td>QDs</td>
<td>Quantum dots</td>
</tr>
<tr>
<td>SPAAC</td>
<td>Strain-promoted alkyne-azide click reactions</td>
</tr>
<tr>
<td>STMP</td>
<td>Sodium trimetaphosphate</td>
</tr>
<tr>
<td>STPP</td>
<td>Sodium tripolyphosphate</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEMED</td>
<td>Tetramethylethlenediamine</td>
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1 Definitions and Introduction

Hydrogels are networks of water-soluble polymers swollen in water. This affinity for water differentiates hydrogels from organogels (that swell in organic solvents) or gels (a more general term that encompasses any solvent-swellable network, including but not limited to hydrogels).

While there is no formal minimum water content associated with differentiating a hydrogel from other types of polymer networks, most functional hydrogels used in applications contain at least 50% by weight water, with some applications of hydrogels (e.g., superabsorbents) containing >99.9% by weight water. A clearer definition of what is or is not a hydrogel can be given based on an understanding of the nature of water binding to a hydrogel network. Figure 1 shows a schematic diagram of the various interactions of water with a hydrogel network.

While the nature or existence of “semi-bound” (or interfacial) water is still a matter of some debate [2], the inherent hydrophilicity of the polymers crosslinked to form a hydrogel results in a fraction of water being “bound” inside the network by hydrogen bonding/dipole interactions with hydrogen bond donors/acceptors or polar functional groups on the polymer backbone; this bound water is difficult to remove from the hydrogel even upon drying. The remaining water is “free” in that it is present inside the hydrogel phase due to the osmotic pressure gradient between the bulk (low polymer concentration) and gel (high polymer concentration) phases, leading to the uptake of water and thus hydrogel swelling. On this basis, instead of defining a hydrogel solely based on its overall water content, the following three criteria best define whether or not a material should be considered to be a hydrogel.

1. Although the polymer chains comprising a hydrogel have an affinity for water (and would themselves dissolve in water if they were not networked), hydrogels will not fully dissolve no matter how much water is added. This criterion distinguishes hydrogels from viscous water-soluble polymer solutions that may have similar bulk properties at higher polymer concentrations but simply dissolve upon dilution. Note that this criterion does not exclude that hydrogels can degrade; indeed degradation can be specifically designed into the building

Fig. 1 States of water inside a hydrogel. (Adapted with permission from [1])
block or crosslinking chemistry of a hydrogel as desired. However, degradation of the hydrogel into soluble components must involve the breaking or dissociation of some kind of physical or chemical bond.

2. Water is fully contained inside the volume of a hydrogel. This criterion distinguishes hydrogels from many types of coacervates or aggregates which expel the majority of the solvent upon forming intermolecular interactions/crosslinks.

3. Hydrogels contain a mixture of bound water and free water. This criterion distinguishes hydrogels from other types of micro-/nanoporous scaffolds or sponges comprised of materials that do not themselves have a specific affinity to swell in water but can absorb large quantities of water due to their porous nature (e.g., cellulose sponges).

In this chapter, we will outline the options for fabricating hydrogels using various building blocks and crosslinking strategies on different length scales.

2 Hydrogel Classifications

Hydrogels are conventionally defined by the nature of the polymers comprising their chains (Sect. 2.1), the mechanism and resulting organization of network assembly (Sects. 2.2 and 2.3), and the length scale of the assembled network (Sect. 2.4). Note that the literature examples and research studies selected for inclusion in these sections are not meant to be exhaustive but are rather selected in order to give the reader insight with respect to the major engineering and design principles governing hydrogels designed for unique applications.

2.1 Types of Building Blocks

2.1.1 Natural

Hydrogels derived from natural or biological sources have inherent advantages such as low cost, non-toxicity, renewability, and (typically at least) degradability [3] relative to synthetic polymers. In addition, as naturally occurring polymers, their degradation products are typically considered to be safe since they are natively used by the body for subsequent cellular reactions [4]. For instance, when starch degrades, the resulting products are amylose (α 1 → 4 glycosidic linkages) and amylopectin (α 1 → 6 glycosidic linkages), both of which naturally occur in the human body [5, 6]. As such, starch and other natural polymers that have natural metabolic by-products have thus been deemed as GRAS (i.e., “generally regarded as safe”) by the Food and Drug Administration (FDA) [7]. Furthermore, in some biomedical applications in which a particular cell response to a hydrogel is targeted, the use of natural polymers that naturally induce such responses is directly beneficial; for example, hyaluronic acid (HA)-based hydrogels have been shown to promote cell spreading based on the native
biological function of HA [8]. Despite these advantages, natural polymers do have limitations in hydrogel design. Batch-to-batch variation is a particularly challenging problem given the varying biological sources of such polymers and the fact they are typically extracted from complex mixtures of natural polymers, an expensive and often inefficient processing step that must be customized for each target natural polymer. For instance, the extraction of lignin and similar phenolic constituents from wood requires a two-step process involving pre-extraction of hemicellulose followed by lignin extraction using one of the many commonly implemented methods (i.e., deep eutectic solvent, organosolv, soda/AQ, hydro trope) [9], while the conversion of chitosan from shellfish chitin requires acid treatment in order to dissolve the calcium carbonate in the shell followed by an alkaline extraction in order to solubilize the proteins present [8]. The often limited number of functional groups amenable to crosslinking reactions can also result in many natural polymer-based hydrogels exhibiting relatively weak mechanical properties, although this is not a universal problem with such hydrogels.

The most heavily studied natural polymers for hydrogel synthesis are carbohydrates such as hyaluronic acid [8, 10], chitin/chitosan [8], water-soluble cellulose derivatives (i.e., ethyl cellulose) and nanoscale cellulose (i.e., cellulose nanocrystals or CNCs) [11, 12], dextran [13], starch [14, 15], alginate [16, 17], fibrin [18], and agarose [19]. Each of these polymers has their own unique chemical compositions and resulting properties. Other biological macromolecules can also be used, with proteins such as gelatin [20], collagen [21], and fibrinogen [22] being most widely used. The diversity of proteins with respect to their number/types of amino acids and overall properties can also be used to control the physical and biological properties of the resulting hydrogels [23], with both natural and synthetic amino acids having been used to direct gel properties. The same advantages are accessible when peptides, short amino acid oligomers, are used as gel building blocks. By tuning the amino acid sequence of the peptide, the capacity of the peptide to form self-assembled fibers, tapes, sheets, wires, or ribbons that can subsequently assemble into fibrillar networks and ultimately hydrogel structures can be tuned [24]. The sequence of the amino acids determines the assembly into β-sheet, β-hairpin, α-helix, and/or coiled-coil structural motifs, with elastin-like polypeptides based on VPGXG pentapeptide repeats being particularly well-studied due to their capacity to spontaneously form fibers and, in some cases, hydrogels upon heating [25]. However, peptides as short as 1–2 repeat units (particularly those containing protecting groups from the solid-phase synthesis process) can also spontaneously form fibers and thus gels under the correct conditions [26]. Alternately, peptide amphiphiles containing both hydrophilic and hydrophobic domains can self-assemble with tunable physical properties depending on the length and relative hydrophobicity/hydrophilicity of the domains produced [27]. The reader is referred to the excellent review by Dasgupta et al. for a broader perspective on the types of peptides that can form gels [24]. Hydrogels have also been successfully fabricated from polynucleotides, with whole DNA sequences or molecularly imprinted sections of genomes successfully used to prepare gel structures [28].
2.1.2 Synthetic

Synthetic hydrogels are based on chemically derived building blocks prepared from small molecule monomeric units. Relative to natural polymers, synthetic polymers offer significantly more chemical flexibility in terms of introducing crosslinking or functional tags (as facilitated by copolymerization, blending, or other chemical strategies) [29], show more consistent batch-to-batch chemistry (and thus properties), and minimize the risk of introducing biological contaminants into the final hydrogel product [30]. The capacity to add any desired density of crosslinking groups as well as introduce any degree of chain conformational mobility also enables synthetic polymer-based hydrogels to exhibit generally enhanced mechanics relative to natural polymer-based hydrogels. However, the native degradability and bioactivity of natural polymers is sacrificed if synthetic polymers are instead used to fabricate hydrogels. As such, judicious selection of the hydrogel building block is essential to achieve the targeted physicochemical properties depending on the targeted hydrogel application.

While many synthetic polymers have been used to form hydrogels, the most commonly used materials for this purpose are poly(ethylene glycol) (PEG) [31], poly(vinyl alcohol) (PVA) [32, 33], poly(meth) acrylic acid) (PMMA/PA) [34, 35], poly(vinylpyrrolidone) (PVP) [36, 37], and poly(hydroxyethyl methacrylate) (PHEMA) [38, 39]. Copolymerization is typically used to introduce functional groups that enable crosslinking of these polymers via the desired mechanism and at the desired density, a significant benefit relative to natural polymers which tend to have a limited capacity for polymer functionalization.

2.1.3 Hybrid

Hybrid hydrogels are fabricated using a combination of natural and synthetic polymer building blocks, typically aiming to take advantage of the key advantages of each type of material while suppressing the disadvantages. For example, Patenaude et al. [40] demonstrated the fabrication of mixed natural/synthetic hydrogels based on poly(N-isopropylacrylamide) (PNIPAM) and a range of natural polymers that enabled enhanced enzymatic degradation (hyaluronic acid), reduced protein adsorption (dextran), or enhanced pH responsiveness (carboxymethyl cellulose) relative to PNIPAM hydrogels alone. Alternately, Widusha et al. developed hybrid hydrogels based on a mixture of short- and long-chain alginates and polyacrylamide that provided the required stiffness/toughness (modulus ~1 MPa and fracture energy 4–16 kJ/m²) as well as load-bearing capabilities required for mimicking native cartilage (Fig. 2) [41].

2.2 Building Block Compositions

Hydrogel building blocks can be further classified based on their physical structures and chemical compositions. Homopolymers (one monomer type) or copolymers (made from more than one type of monomeric unit) can both be fabricated with different morphologies in which the monomers are arranged in different spatial orientations.
2.2.1 Linear Polymer Hydrogels
Linear homopolymers consist of a single main chain which propagates/grows only along a single spatial plane from a single monomer type. As representative examples across the range of polymers already discussed, cellulose is formed by connecting D-glucose repeating units via $\beta(1\rightarrow 4)$ glycosidic linkages, poly(ethylene glycol) (PEG) is formed by ring-opening polymerization of epoxide monomers, and poly(acrylic acid) (PAA) is formed by free radical polymerization through the C=C double bond.

2.2.2 Copolymer Hydrogels
Copolymerization methods, in which more than one monomer is used to prepare a single polymer chain (provided that any monomer selected for the copolymer can be polymerized using the same step-growth or chain-growth polymerization mechanism), offer significant potential to expand the scope of hydrogel properties achievable. In this manner, monomers with dissimilar properties (e.g., charged and neutral, hydrophobic and hydrophilic, or degradable and nondegradable) can be incorporated into a single polymeric building block. This diversity of potential compositions imparts a diversity of properties into the resulting hydrogel in a manner likely to result in a homogeneous gel structure than simple blending of different precursor polymers that is more prone to phase separation and domain formation.

Copolymer structures, and thus their role in governing hydrogel properties, are dependent on the relative reactivity (e.g., reactivity ratios or $f$ values) of the comonomers. For instance, copolymerization can result in alternating (regularly repeating A- and B- units), periodic (repeating sequences of A- and B-) random/statistical (random distribution of A- and B- following the probabilities of the different monomers finding each other in solution), or block copolymers (two or more homopolymers referred to as A- and B- which are linked by covalent bonds or junction blocks) depending on the reactivity ratios of the monomers (Fig. 3).
Periodic or alternating structures tend to result in hydrogels with relatively uniform distributions of monomer compositions throughout the hydrogel volume, as the covalent bonds along the polymer backbone prevent any kind of phase separation [42]; in contrast, block structures are more prone to phase separation, although typically more on the nanoscale than the microscale (as possible with blend or hybrid hydrogels) given that the A and B phases are covalently connected and thus cannot fully phase separate [43]. Statistical copolymers will exhibit intermediate behavior between these extremes depending on the distribution of monomers most probable in the structure [44, 45].

Prediction of the composition of the copolymer, and thus the nature of the assemblies of such copolymers when networked to form a hydrogel, can be achieved for free radical copolymers based on the reactivity ratios of the comonomers involved in the polymerization. The molar comonomer concentration ratios $f_A$ (for component A) and $f_B$ (for monomer B) are given in Eq. 1:

$$f_A = \frac{[A]}{[A] + [B]}; \quad f_B = \frac{[B]}{[A] + [B]}$$

However, the comonomer compositions defined above are general in the sense that they do not consider the change in comonomer concentrations (A and B) as the reaction proceeds. Therefore, it is much more accurate to calculate an instantaneous copolymer composition $F$, as defined in Eq. 2:

$$F_A = \frac{\left(-\frac{d[A]}{dt}\right)}{\left(-\frac{d[A]}{dt}\right) + \left(-\frac{d[B]}{dt}\right)}; \quad F_B = \frac{\left(-\frac{d[B]}{dt}\right)}{\left(-\frac{d[A]}{dt}\right) + \left(-\frac{d[B]}{dt}\right)}$$

The rates included in Eq. 2 can be defined by considering the possible reactions of a radical of either type with a monomer of each type, each of which has an independent kinetic rate constant $k$ (Eqs. 3, 4, 5, and 6):

$$P_{m,n,1} + A \xrightarrow{k_{p,1}} P_{m+1,n,1}$$

$$P_{m,n,1} + B \xrightarrow{k_{p,2}} P_{m+1,n,2}$$

Fig. 3 Visual representation of the most common copolymer arrangements. Note that the red A and the green B represent two unique monomer units for ease of visualization.
The reactivity ratios $r$ can thus be defined as the ratio between the rate constants associated with the propagation of monomers A and B from each type of radical, associated with the relative probabilities of reaction (and thus incorporation into the polymer) of each monomer as the reaction proceeds (Eqs. 7 and 8):

\[
    r_1 = \frac{k_{p1A}}{k_{p1B}} \\
    r_2 = \frac{k_{p2B}}{k_{p2A}}
\]

The resulting compositions of copolymers can be predicted based on these reactivity ratios, with $r_1 = 0$ and $r_2 = 0$ leading to an alternating copolymer, $r_1 = \infty$ and $r_2 = \infty$ yielding two homopolymers (a rare case), $r_1 = r_2 = 1$ resulting in a random copolymer, $r_1 < 1$ and $r_2 < 1$ resulting in a random alternating copolymer, $r_1 = r_2 > 1$ resulting in a block copolymer, $r_1 = r_2$ resulting in $F_1 = f_1$ throughout the polymerization, and $r_1 = r_2$, resulting in $f_1 = F_1 = 0.5$.

The rate of disappearance of each monomer A and B can also be written based on these possible reaction pathways (Eqs. 9 and 10):

\[
    -\frac{d[A]}{dt} = k_{p1A}[P^+_1][A] + k_{p2A}[P^+_2][A] \\
    -\frac{d[B]}{dt} = k_{p1B}[P^+_1][B] + k_{p2B}[P^+_2][B]
\]

By substituting Eqs. 3, 4, 5, 6, 7, 8, 9, and 10 back into Eq. 2, an experimentally accessible expression for predicting the instantaneous fraction of each monomer in a comonomer at any given conversion (i.e., fraction of monomer present) can be expressed as Eqs. 11 and 12:

\[
    F_A = \frac{r_1f_A^2 + f_A f_B}{r_1 f_A^2 + 2f_A f_B + r_2 f_B^2} \\
    F_B = \frac{r_2f_B^2 + f_A f_B}{r_1 f_A^2 + 2f_A f_B + r_2 f_B^2}
\]

More recently, gradient copolymers in which the monomer composition varies gradually from all A to all B over the length of the chain (rather than block copolymers, in which the two constituent monomers are present only in
homopolymer blocks tethered together) have attracted interest. In this case, monomer B is typically fed into a controlled radical polymerization at a particular rate, enabling control of the comonomer distribution not only by alteration of the kinetic copolymerization ratios but also by dynamically changing the mixture of monomers available to react (i.e., $f$ changes dynamically with time). Such materials can self-assemble similar to block copolymers but with finely tuned strength of self-association not achievable with conventional block copolymers.

Note that these same copolymerization ratio arguments can be used to predict the consumption of crosslinker during the preparation of a hydrogel but considering propagation of the polymerization through both reactive vinylic groups in the monomer. For example, we have previously shown that the reactivity ratios between the monomer(s) and the crosslinker(s) used to prepare nanogel particles via precipitation methods (Sect. 2.5.3) can be used to predict the radial crosslinker distributions within such nanogels, essential to engineer desired swelling responses in these materials [46].

2.2.3 Multipolymer or Terpolymer Hydrogels

Hydrogels based on multiple types of polymers mixed together and networked offer a hybrid/heterogeneous phenotype with respect to material properties and thus expand on the number of possibilities available via copolymerization techniques. Such hydrogels are particularly relevant when two types of functionality are required in a hydrogel that cannot be polymerized via the same mechanism, as is required to make a copolymer building block. Examples of such multipolymer hydrogels include poly(acrylamide) (PAAm)/gelatin and poly(acrylic acid-co-hydroxyethyl methacrylate) (P(AA-co-HEMA)/gelatin hydrogels [47, 48], in which the strength and the high water-binding capacity of PAAm and P(AA-co-HEMA) synthetic polymer component is combined with the degradability and the cell adhesive properties of the gelatin component to create a functional hydrogel material.

2.3 Building Block Chain Orientations

By combining different polymerization principles, more advanced polymer chain orientations may be attained, each having unique properties that again impart unique properties to their constituent hydrogels following crosslinking. Figure 4 shows the structures of some of these more advanced polymer morphologies, with the morphologies most commonly applied for forming hydrogels described below.

2.3.1 Branched and Hyperbranched Polymer Hydrogels

Branched polymers are compact and globular structures with a large amount of available functionalities at their periphery but low viscosities; this combination of properties makes such polymers interesting building blocks for functional hydrogels with stronger mechanics or well-controlled distributions of internal functional groups [49]. Branched polymers can be prepared using the general scheme shown in Fig. 5.
The extent of chain branching can be described by applying statistical principles to Flory’s theory of gelation [50, 51]. Following the general scheme shown below, the instantaneous conversion of a particular monomer $x$ can be expressed based on the initial concentration of that monomer ($A_0$) and the instantaneous concentration of that monomer $A$ (Eq. 13).

$$x = \frac{[A]_0 - [A]}{[A]_0}$$  

(Eq. 13)

For each step of the branching process, a general equation can describe the relative probabilities of conversion. In these equations (Eqs. 14, 15, 16, 17, and 18), $A_3$ is the growing polymer chain, while $AA$ and $BB$ (or $aa$ and $bb$ following polymerization) represent the two unique repeating units that branch off of the linear backbone, each time creating a unique structure with a specific probability of occurrence.

$$A_3 + BB \rightarrow A_2abB$$  

(Eq. 14)

$$A_2abB + AA \rightarrow A_2abbaA$$  

(Eq. 15)
Here, $\alpha$ represents the imbalance ratio between the reactive monomer species AA and BB (Eq. 19), while $\rho$ describes the fraction of $A$ on the polymer backbone ($A_3$) (Eq. 20):

$$\alpha = \frac{2[AA]_0 + 3[A_3]_0}{2[BB]_0} \quad (19)$$

$$\rho = \frac{3[A_3]_0}{2[AA]_0 + 3[A_3]_0} \quad (20)$$

The branching coefficient ($\delta$) of a polymer can subsequently be calculated to predict the extent to which a polymer chain will branch and whether or not there is sufficient interaction of the chains in solution to promote gelation, which occurs when the branching coefficient equals 0.50 (Eq. 21).

$$\delta = \sum_{n=0}^{\infty} \alpha^2 \rho \left[\alpha^2 (1-\rho)\right]^n = \frac{\alpha^2 \rho}{1-\alpha^2 (1-\rho)} \quad (21)$$

The use of branched polymers for the development of hydrogels has been described extensively in the literature for a variety of different polymers such as poly(ethylene glycol), polysaccharides, and polyglycerols, with the latter particularly of interest in biomedical applications due to their derivatizable polyether polyol groups and generally low toxicity in biological applications [52, 53].

The term “hyperbranched” was coined with respect to polymers in the late 1980s by Kim and Webster [54], but the concept was first described in Flory’s work in the 1950s (ABm-random polycondensates). More recent designs have exploited the use of hyperbranched polymers prepared via one-step reactions that have large molecular weights (>1 million g/mol) but relatively broad polydispersities and random overall structures [55–57]. This typically broad polydispersity can be reduced using a slow addition semi-batch protocol for polymerizing vinylic monomers via controlled polymerization mechanisms [58], anionic ring-opening polymerization schemes for polyglycerols and other step-growth polymers [59], and optimized monomer to initiator ratios [60]. Several types of hyperbranched hydrogels based on poly(ethylene glycol) [61], poly(phosphoramidate) [62], poly(ether amines) [63], and poly(glycerols) [64] have been reported, with various advantages relative to linear polymer-based hydrogels including enhanced uptake of dyes and drugs, increased stiffness and toughness, lower degrees of swelling, and prolonged controlled release.
2.3.2 Dendrimer Hydrogels
Branching strategies can also be used to create dendrimers, polymeric structures containing a single focal point from which “dendrons” grow using sequential grafting/deprotection cycles to create a very highly ordered branched structure. Dendrimers differ from hyperbranched polymers in the sense that internal cyclization is not possible; as a result, well-defined, monodispersed structures can be attained. However, based on the sequential reactions and required protecting group chemistry used to prepare dendrimers, synthesis requires multiple steps and purification schemes that limit the practical use of dendrimers in hydrogel systems. Regardless, a variety of dendrimer hydrogels (DHs) designed by Yang and co-workers using G3.0 and G5.0 polyamidoamine (PAMAM) dendrimers tethered to PEG-acrylate chains of varying sizes [65, 66] have been reported, with the resulting gels exhibiting excellent drug delivery capabilities for ophthalmic applications relative to hydrogels prepared from linear polymer building blocks due to their greater loading capacities [67] and tunable macroscopic properties [68].

2.3.3 Star and Comb Polymer Hydrogels
Star polymers in which there is one single point from which all growing polymer chains originate have been particularly widely used for preparing hydrogels, with the most notable example being star polymers based on poly(ethylene glycol) (PEG). Star-PEG building blocks have been demonstrated to create stronger hydrogels than corresponding linear building blocks of the same molecular weight, including structurally homogeneous hydrogels [69–71]. Comb/brush polymers in which polymer chains are grown off a polymer backbone are also common, with hydrogels based on poly(oligoethylene glycol methacrylate) (POEGMA) [72, 73] and/or copolymers of POEGMA and poly(oligolactic acid methacrylate) (POLAMA) (the latter of which can exhibit some degree of internal domain formation due to nanoscale phase separation of the hydrophobic POLAMA side chains) being among the most widely studied [74].

2.3.4 Telechelic Polymer Hydrogels
A telechelic polymer is a polymer whose ends are both capped with a particular active moiety which offers some type of property different than the rest of the polymer chain. While most step-growth polymers are inherently telechelic (corresponding to the end group of the monomeric unit that is in excess in the reaction), telechelic chain-growth polymers have recently become of increasing interest, especially with respect to their role in living/controlled radical polymerizations [75]. The active end group may be chemically reactive (facilitating formation of a covalently crosslinked hydrogel [76]) or physically associative (facilitating the formation of an interconnected micelle-type hydrogel [77]). In some cases, the telechelic group can incorporate both a chemically reactive and physically associative component to facilitate dual physical-covalent gelation. For example, Hamley and Castelletto described the insertion of hydrophobic dipeptides (phenylalanine-phenylalanine or tyrosine-tyrosine) to the ends of 1500 Da PEG chains that can promote self-assembled hydrogelation near the body temperature without the need
for further crosslinking while still presenting terminal functional groups for additional modification and/or crosslinking [78]. Other self-associative/reactive telechelic polymers such as diisocyanate-extended PEG conjugates end-capped with hydrophobic alcohols and alkyl groups, [79–81], aldehyde end-capped poly(vinyl alcohol) [82], and methacrylate end-capped poly(hydroxyethyl acrylamide)-b-poly-(N,N'‐dimethyl acrylamide)-b-poly(dimethylsiloxane)-b-poly-(N,N'‐dimethyl acrylamide)-b-poly(hydroxyethyl acrylamide) block copolymers [83] have also been described to facilitate multimodal gelation. Reversible telechelic polymer-based hydrogels have additionally been reported that can use the free functional group to induce dynamic changes in hydrogel properties with respect to the surrounding pH [84, 85], self-heal [86], and/or immobilize specific biomolecules [87].

2.4 Crosslinking Chemistry

For any given combination of building blocks discussed in Sects. 2.1, 2.2, and 2.3, the chemistry by which those building blocks are subsequently connected into a hydrogel network has significant impacts on the ultimate properties of the hydrogels formed. Crosslinking strategies can be broadly classified as either chemical or physical. Chemical crosslinks form as a result of hetero- or homo-covalent bond-forming reactions between chains, while physical crosslinks arise from typically more transient interactions arising physical interactions such as ionic/electrostatic, stereocomplexation, supramolecular interactions, hydrophobic interactions, and/or simple chain entanglement [30].

2.4.1 Physical Crosslinking

Ionic/Electrostatic Interactions
In ionic crosslinking, two polymers with opposite charges or one polymer and one multivalent ion/small molecule with opposite charges self-associate due to electrostatic attraction. The extent to which ionic association occurs depends on the free energy of the polymer complexation relative to the free energy associated with the original counterion association with the free cationic or anionic polymer (s), a property related to the dielectric constant of the solvent system used. In hydrogels, such polymeric associations are typically highly favorable from an energetic standpoint, as the complexation of two polymer-bound charges releases two highly mobile small counterions to increase the net entropy of the system. Note that while polyelectrolyte hydrogels and charge coacervates are similar in terms of their mechanism of interaction, the capacity of polyelectrolyte hydrogels to retain water within their volume (as opposed to coacervates) is the key differentiating feature.

The most commonly reported ionic crosslinking mechanism involves the complexation of alginate with Ca²⁺ ions, an interaction that has been studied extensively for the controlled release of pharmaceuticals [88] and the entrapment of living cells [16, 89] given that the crosslinking mechanism is mild and can occur at
physiological temperature. The use of higher charge density polycations (i.e., poly(allylamine hydrochloride) (PAH), poly(diallyldimethylammonium chloride) (PDADMAC), or poly-L-lysine (PLL)) and polyanions (i.e., hyaluronic acid (HA), poly(styrene sulfonate) (PSS), and chondroitin sulfate) [90, 91] has also led to the development of unique hydrogel structures using layer-by-layer assemblies of polymersomes [92], capsosomes [93], and polyelectrolyte DNA bridges [94].

**Stereocomplexation**

Polymers with stereoisomeric chiral centers can interact more strongly than two like stereoisomers due to the relative orientations of the two isomers. A notable example of this phenomenon for hydrogel formation involves the stereocomplexation between poly(D-lactic acid) (PDLA) and poly(L-lactic acid) (PLLA), first described in 1987 by Ikada et al. [95, 96]. When oligomers of D- and L-lactic acid are attached to water-soluble polymers such as dextran or PEG, hydrogels can be formed due to the enhanced van der Waals interactions between the two enantiomeric helices that cause the helices to pack into a dense crystalline stereocomplex [97, 98].

**Hydrogen Bonding**

Hydrogen bonding between an electron donor and an electron acceptor is widely used for preparing organogels. However, in water (which is also a strongly hydrogen bonding medium), hydrogen bonds used as crosslinks between polymers are highly labile and can be readily disrupted via changes in temperature, pH, or mechanical agitation (e.g., sonication), making hydrogen bonding less useful as a viable crosslinking strategy for hydrogel formation [99]. That being said, there are some examples of polymer solutions in which hydrogen bonding is sufficient to promote gelation, particularly when polymers exhibiting rheological synergism (i.e., polymers whose structures are inherently oriented to promote hydrogen bonding) are selected. As an example, Hoare et al. reported blends of hyaluronic acid (HA) and hydroxypropyl methylcellulose (HPMC) that exhibited highly shear thinning rheological properties but extended gel-like properties relative to either solution alone as a result of complementary hydrogen bonding interactions between the HA and HPMC chains [100]. Hydrogen bonding can also be used to promote hydrogel formation in the context of supramolecular complexation, for example, crosslinking via complementary base pair sequence interactions of single-stranded DNA [28] or peptide self-assembly (in the latter case often in concert with other physical interactions) [101, 102]. Strong hydrogen bond-forming groups can furthermore be grafted to other polymers to promote supramolecular hydrogel formation. For example, ureido-pyrimidinone (crosslinked by urea) [103], benzenetricarboxamides [104], and guanosines [105] can all be used to facilitate hydrogen bond formation in water that results in a much more stable interaction, although one that can still be disrupted by larger shear forces.

**Host-Guest Interactions**

An increasingly popular method of hydrogel crosslinking is the use of inclusion complexes. Cyclodextrins (CD), cyclic oligosaccharides that contain R-1,4-coupled
D-glucose units, are the most commonly applied, as the orientation of the cyclic structure results in the outside of the CD unit being hydrophilic and the inside being hydrophobic. As such, hydrophobic molecules [106] or poly(ethylene oxide) or PEO [107, 108] can complex with these hydrophobic internal cavities; if the complexing CD and hydrophobic units are grafted to water-soluble polymer backbones, this interaction results in the formation of crosslinks. The benefits of such supramolecular interactions are that, while the crosslink formed is relatively strong, it can also be disrupted by the addition of a competitive complexing agent (e.g., for CDs, something more hydrophobic or with a size that better matches the internal cavity size of the CD ring such as adamantane groups) [109]. As such, environmentally responsive degradation responses and/or self-healing responses following shearing can be engineered into CD-crosslinked hydrogels. While CDs are the most commonly used complexing agent for this purpose, a variety of other supramolecular complexing agents such as cucurbit[n]uril [110] (which can uniquely bind two separate guest molecules simultaneously and equally within its cavity, allowing for specific tuning of both the strength and directionality of the resulting bond) or silsesquioxane (which has an organic outer shell and a silicone internal core, allowing for assembly via silicone grafts) can also be used.

Hydrophobic Interactions

Self-association of hydrophobic moieties in aqueous solution, driven by the maximization of the entropy of water when the contact area between a hydrophobic entity and water is minimized, is one of the most commonly applied strategies for physical hydrogel formation [111]. The hydrophobic group can be incorporated into the hydrogel precursor polymers in several ways, including hydrophobic-hydrophilic block copolymers [112], hydrophobically grafted copolymers [113, 114], or copolymers of hydrophilic and hydrophobic comonomers [115]. Such self-associative polymers are widely used as rheological modifiers for aqueous commercial products [116]. Commonly used hydrophobic grafts include cholesteryl (cholesterol) groups [117], long-chain hydrocarbon alkyl chains [118], or even whole lipids and sphingolipids [119]. Comb or graft copolymers containing hydrophobic blocks, including poly(propylene oxide), poly(lactide-co-glycolic acid), poly(N-isopropylacrylamide), poly(propylene fumarate) (PPF), poly(caprolactone) (PCL), poly(urethane) (PU), and poly(organo phosphazene), can also be used as the gelators for hydrophobic self-association [120]; some of these (most notably poly(N-isopropylacrylamide) are hydrophobic only in a temperature-responsive manner to allow for temperature-triggered reversible gelation. Pluronics (poly(ethylene oxide))-block-poly(propylene oxide)-block-poly(ethylene oxide) copolymers are the most notable example of such thermogelling systems, with self-association of the poly(propylene oxide) block creating hydrogels that have been applied for a variety of drug delivery and tissue engineering applications [121, 122]; however, a variety of other ABA-type hydrogels in the A block which can be either hydrophilic [123] or hydrophobic [124] have been successfully used to fabricate self-associative polymers. The strength and duration of the self-associated gel can be controlled based on the physical and chemical properties of the hydrophobic segments.
(e.g., length, degree of hydrophobicity, the presence of any supporting interactions beyond simple hydrophobic interactions) as well as the polymer chains to which the hydrophobes are grafted (molecular weight, hygroscopicity).

**Crystalline Domain Formation and Freeze-Thawing**

The promotion of crystalline domains through freeze-thaw cycles or other physical treatments creates strong intermolecular interactions that can be applied as hydrogel crosslinks. Freeze-thawing using a mixture of dry ice or liquid nitrogen with various organic solvents such as butyl acetate, isopropanol, and acetone is most commonly used, with subsequent lyophilization to sublime the microscale ice lattices resulting in the promotion of intermolecular interactions (most commonly via hydrogen bonding) that can persist upon rehydration due to the lack of solvent accessibility into the crystalline self-assembled domains. The most common example of freeze-thaw-induced gelation, or “cryogelation,” involves poly(vinyl alcohol) (PVA) [125], with the strong hydrogen bond donor/acceptor hydroxyl groups on the polymer side chain promoting crystallization over subsequent freeze-thaw cycles. Effective hydrogels based on other carbohydrates such as xanthan gum [126] have also been reported.

**Pi-pi Stacking**

The interaction of aromatic double bond π orbitals, also known as pi-pi stacking, has been successfully used to create a variety of hydrogels. It should be noted that the terms “pi-stacking” and “pi-pi interactions” are controversial within the scientific community since they do not accurately describe the fundamental phenomena occurring among aromatic groups [127]; furthermore, most systems that promote pi-pi stacking also exhibit other physical interactions such as hydrogen bonding and/or hydrophobic interactions that can enhance the crosslinking achievable with pi-pi stacking alone [128, 129]. However, pi-pi interactions at least strengthen other types of physical interactions. Among the most common pi-pi stacking groups used for hydrogel assembly, N-(fluorenly-9-methoxycarbonyl) or Fmoc, a typical protecting group used during solid-phase peptide synthesis, forms antiparallel β-sheets with alternating positions of the fluorenly groups [130] and ultimately interlocks to form a cylindrical structure that forms a hydrogel from functionalized polymers [131, 132] or small molecule hydrogelators [133]. 3-hydroxyflavone (3-HF) fluorophores [134], porphyrins [135], and Fmoc compounds [136] are also effective via a similar mechanism.

**Metal-Ligand Coordination**

Metal-ligand coordination chemistry between specific ligands and a metal ion with a bond order greater than one can also be used to form hydrogels crosslinked by coordinate covalent bonds/dipolar bonds [137, 138]. The most common coordination centers are transition metals such as ruthenium (Ru), zinc (Zn), nickel (Ni), manganese (Mn), platinum (Pt), palladium (Pd), iron (Fe), and cobalt (Co) [139], although any multivalent ion (particularly with an electron-deficient outer shell) can be used. The most common pi-donor ligands include oxide (O⁻²), nitride (N⁻³), imide (RN⁻²), alkoxide (RO⁻), amide (R₂N⁻), and fluoride (F⁻), while carbine
(alkylidene), amido, alkoxide, imido, carbyne (alkylidyne), oxo, and nitride groups also have the capacity to form metal-ligand multiple bond complexes. One of the earliest examples of such hydrogels was the work of Chujo et al., in which poly (oxazoline) polymer chains functionalized with bipyridine groups underwent coordinate crosslinking with Co(II) [140] and Fe(II) [141]. Similar approaches were used to fabricate coordinative hydrogels with PEG [142], Pluronics [143], and PEG-PLA copolymers [144] containing end-functionalized ligands like terpyridine or bipyridine that can interact with transition metal ions like Mn(II) and Ni(II) to promote gelation. Newer technologies have described the synthesis and fabrication of metallohydrogels containing unique coordination centers such as cobalt [145], copper [146], platinum [147], nickel [148], silver [149], and lead [150]. Composite metallohydrogels using metallic nanoparticles as crosslinking sites have similarly been reported. For example, CdSe quantum dots (QDs) have been encapsulated into cetyltrimethylammonium bromide (CTAB) micelles without the need for any surface modification with ligands to create self-assembled photoluminescent composite metallohydrogels [151].

2.4.2 Chemical

Relative to physical crosslinking approaches that are all at least on some level inherently reversible, chemical crosslinking can offer unique properties in terms of degradability and stability, both in terms of permanence as well as the capacity for rational tuning of gel degradation times. Broadly, chemical crosslinking can be conducted during the synthesis stage (i.e., from monomers) or to link functionalized prepolymers together (i.e., from polymers), both of which are described in the following section.

Assembly from Monomer Units

Crosslinking from monomer units requires the addition of a multifunctional monomer into the reaction that can react at two or more sites and thus form bridges between the growing polymer chains. For step-growth polymers, this requires introducing at least one monomer with at least three functional groups that can participate in the step-growth reaction (typically condensation), allowing for the typical growth of a chain via the first two reactive groups and the formation of a crosslink between chains with the third (or higher number) functional group. In such a scenario, gelation will occur at a conversion directly predictable by both the percentage and the degree of functional group content of the crosslinking monomer. Alternately, for chain-growth polymers prepared typically via free radical polymerization, including at minimum a difunctional monomer will induce crosslinking. The production of radicals via the thermal decomposition of peroxides or azo-containing compounds (i.e., azobisisobutyronitrile (AIBN) or dimethyl 2,2’-azobis(2-methylpropionate (AIBME)), radiation (short wave UV at 254 nm, long wave UV at 365 nm, gamma, or electron beam), and/or chemical treatments (i.e., oxidation) will induce polymerization through the vinyl, (meth) acrylate, and/or allylic C=C unsaturated sites in both the monomers and the crosslinkers to result in bulk gel formation. It should be emphasized that the prediction or measurement of crosslinking density as a result of chemical crosslinking from
monomers is challenging, as both step-growth and (in particular) chain-growth crosslinking mechanisms can be relatively inefficient due to steric inhibition as a result of network formation. As such, relative to the desired crosslink density, it is common to add slightly more than the theoretically calculated amount of crosslinker to account for unreacted end groups (step-growth) or unpolymerized/terminated chain ends (chain-growth).

Additives can be used to control the properties of the chains produced between the crosslinks even when networking is done simultaneous to polymerization. In step-growth polymers, doping of monofunctional monomers will result in chain ends that can alter the swelling, degradation, mechanical, and chemical properties of the resulting hydrogels [152]. Alternately, in chain-growth polymers, chain transfer agents or controlled radical polymerization initiators/agents [153] can be added during the networking process to control the molecular weight of the polymer chains between crosslinks, yielding the same net effects in addition to (if degradable chemistries are integrated into the crosslinker) promoting the degradability of the resulting network [153]. Accelerators such as tetramethylethylenediamine (TEMED) are commonly also used in chain-growth systems to stabilize the free radicals and thus reduce termination/chain transfer events that compete with polymerization and crosslinking, typically resulting in faster generation of networked structures [154].

Assembly from Prepolymers

Rather than assembling hydrogels through the growth of monomeric units, an alternative approach is to chemically modify prepolymers with reactive functional groups in order to promote chain crosslinking and network reinforcement. While the most common chemistries will be briefly reviewed below, the interested reader is encouraged to consult critical reviews on covalent crosslinking mechanisms for hydrogel production for additional details [30, 155].

Bi- or Multifunctional Small Molecule Crosslinkers

Bifunctional or multifunctional small molecule crosslinkers have been widely explored for crosslinking polymers into hydrogels. While the exact nature of the crosslinker required depends on the chemistry of the chains to be crosslinked, as a representative example, linear starch chains and related polysaccharides have been successfully crosslinked with small molecules such as epichlorohydrin [156, 157], formaldehyde, glutaraldehyde [158–160], phosphoryl chloride (POCl₃) [161, 162], sodium tripolyphosphate (STPP) [163], and sodium trimetaphosphate (STMP) [164–166], among others. While all of these crosslinkers use unique chemistries, the commonality is their capacity to react with hydroxyl groups on native starch to promote network formation without requiring additional modification of the building block polymers [167]. The interested reader is referred to Hermanson’s excellent *Bioconjugate Techniques* book for several more examples of commercially available multifunctional small molecule crosslinkers for crosslinking these and other types of hydrogel building blocks [168]. It should be noted that the use of small molecule crosslinkers typically results in residual unreacted crosslinker being entrapped within the hydrogel formed. If purification (e.g., by dialysis) is an option for the
application, this is not problematic; however, in other applications (e.g., in vivo injectable hydrogels), such residual chemicals are not typically desirable.

Polymer-Polymer Crosslinking
As an alternative to small molecules, two polymers with complementary functionalities can be crosslinked via an appropriate mechanism.

Free Radical Crosslinking
The most common example of such crosslinking is the UV or thermally induced polymerization of polymers functionalized with methacrylate groups that can undergo free radical crosslinking to create a hydrogel [169]. Methacrylated gelatin (GelMA) has attracted particular interest in this regard in the biomedical literature in terms of its capacity to support cell adhesion and growth for tissue engineering applications, although a range of other methacrylated natural polymers such as hyaluronic acid [170], starch [171], and chitosan [172] has also been demonstrated in the context of both homopolymer gels and blends that can be stabilized by the covalent crosslinking between the chains (i.e., methacrylated chitosan + PNIPAM hydrogels [173]). Relative to the small molecule crosslinkers, this approach typically induces less cytotoxicity, making it more amenable to biological applications. However, such approaches still require UV light or heat as a stimulus to induce gelation.

Functional Group Crosslinking
As an alternative to the use of UV crosslinking, covalent bond-forming reactions between two complementary functional groups on polymers can be used to induce crosslinking. Any type of bond (most commonly esters, amides, urethanes, anhydrides, or ureas in the context of hydrogels) can be formed if the precursor polymers are functionalized with appropriate functional groups for making these bonds [174]. Chemistries that can proceed in water and at or near room temperature are of particular recent interest. For example, carbodiimide-mediated crosslinking between amine (or, with appropriate base activation, hydroxyl groups) [168, 175] and carboxylic acid groups [168, 176] has been extensively used to form hydrogels from polymeric starting materials. However, such reactions typically require either heating, anhydrous conditions, or the use of additives (such as carbodiimides) to lower the activation energies associated with bond formation, which may be problematic in a biological context.

In Situ Gelling Crosslinking
In response, more recently, a range of in situ gelling (or click) chemistries has been intensively investigated for preparing hydrogels under conditions that can be performed at room temperature in water without the need for an external stimulus or additional additives (Fig. 6).

Depending on the chemistry used, both the rate at which gelation occurs and the stability of the resulting bond (and thus the degradation time of the hydrogel network) can be controlled. In addition, the rate of reaction of many of these
Fig. 6  In situ gelling approach and chemistries
Chemistries make them amenable to their use as injectable hydrogel formulations, in which low-viscosity precursor polymers can be easily injected and rapidly crosslink (ideally orthogonally to native body chemistry) to form a hydrogel following injection in vivo. Such properties eliminate the need for surgical implantation of hydrogels used in biomedical contexts.

Michael-Type Addition

A Michael-type addition describes the 1,4-addition of a nucleophile (Michael donor) to the β position of an α,β-unsaturated carbonyl group (Michael acceptor). Within the biomedical domain, the most commonly exploited carbonyl containing compounds or Michael acceptors are aldehydes, ketones, acrylates/acyryloyls [177], and methacrylates [178–180], with (meth)acrylates the most commonly applied to produce injectable hydrogels for tissue engineering [181, 182] and drug delivery [183, 184]. Maleimides [185] or vinyl sulfones [186], both of which are better electrophiles and/or better in stabilizing the reactive intermediate than the functional groups listed above, have also been used in contexts where faster gelation is desired. Potential nucleophiles for Michael addition chemistry include halide ions, cyanide ions, thiols, alcohols, and amines, with thiols (-SH) and both primary (-NH2) and secondary (-NHR) amines [187–189] most commonly used (although halides and cyanides are also highly effective in applications where there is no concern about toxicity of residual reagents) [190]. Thiols are typically preferred over amines for injectable applications since they have a lower pKa (~8) and thus stronger nucleophilicity in mild fabrication conditions [191]. A Michael-type addition crosslinking mechanism can spontaneously occur under physiological conditions to create a nondegradable bond on the time frame of a few minutes to tens of minutes at physiological pH [192]. The addition of slightly basic solutions or the use of phosphine-based catalysts can accelerate the reaction, although at the cost of adding a small molecule [193]. Michael addition chemistry can optionally be combined with other physical gelation strategies in order to reduce the time required for a gel network to be formed. For example, Vermonden’s group demonstrated crosslinking of (meth)acrylate bearing triblock copolymers in which a poly(ethylene glycol) middle block was flanked by thermosensitive blocks of random N-isopropylacrylamide (PNIPAM)/N-(2-hydroxypropyl) methacrylamide dilactate that underwent rapid thermogelation at the body temperature; the Michael-type addition of thiolated hyaluronic acid was then performed to cure the hydrogels to form a structurally stable hydrogel [180].

Disulfides

Disulfide bridges (S-S bonds) are formed in oxidizing environments, including many areas of the body (e.g., endoplasmic reticulum and the extracellular space). Oxygen alone is typically sufficient to drive dithiol-crosslinking [194], although the reaction can be accelerated by adding additional oxidizing agents such as peroxides in order to accelerate the reaction. For instance, Vercruysse and co-workers made hydrogels based on hyaluronic acid (HA) and polyvalent hydrazide crosslinkers using atmospheric oxygen and 0.3% hydrogen peroxide which can gel in 15 min and degrade in...
the presence of 1,4-dithiothreitol (DTT) [195]. Residual thiol groups left after gelation typically have minimal impact on cytotoxicity and immunological response [196]; however, if such free thiols are problematic in a given application, disulfide exchange reactions can be used to end-cap residual thiols and/or use disulfides themselves as crosslinkers, essentially using disulfides as protecting groups for thiols [197, 198]. Disulfide chemistry generally has relatively long gelation times at physiological pH (tens of minutes to hours), although the combined use of thiol chemistry with a faster gelation chemistry to form a double-network hydrogel [199] can be used to maintain the advantages of disulfides (reversibility, biological compatibility) but maintain injectability.

**Imines**

Imines or Schiff bases (general structure $R_2C=NR'$, where $R'$ is not H) can be generally described as either secondary ketimines or secondary aldimines, depending on if the connectivity of the molecule is in aldehyde or ketone form. Imines form rapidly between amines and aldehydes/ketones but are typically highly labile in water, allowing for rapid formation but also relatively rapid dilution of the hydrogel network formed. Manipulation of the hydrophobicity of the groups around the imine bond, and thus the accessibility of water to the imine bond for hydrolysis, can be used to at least influence the lifetime of the crosslink formed [200, 201]. Double-network approaches in which the imine chemistry is used as the fast-reacting chemistry can also be successfully applied (e.g., combining imines with disulfides) [199]. Imines can also be reduced via sodium cyanoborohydride addition to yield stable secondary amine linkages if a nondegradable crosslink is desired, although this does constitute an additional reaction step using a biologically incompatible small molecule reagent.

**Hydrazones**

A more stable variety of a Schiff base is a hydrazone bond, formed by the nucleophilic addition of a nitrogen from a hydrazine group or a related derivative to a carbonyl group (typically an aldehyde or ketone). Due to the moderate to high toxicity of hydrazine to biological systems [202], structurally similar hydrazide groups are typically used as the nucleophile for hydrogel fabrication. In comparison to imines, hydrazone bonds form relatively faster (on the timescale of seconds) [40, 203, 204] and yield a more hydrolytically stable bond due to the increased nucleophilicity of the lone-pair-bearing amines through the alpha effect [205], with slow degradation (over weeks to months) observed at neutral pH and faster degradation in acidic conditions [206, 207]. Inductive effects as a result of incorporating aromatic groups on either side of the hydrazone bond can also tune bond degradation [208], in some cases creating functionally nondegradable bonds at neutral pH. While hydrazone-based hydrogels have been extensively and successfully demonstrated for biological applications in the literature [40, 204], the storage stability and potential cross-reactivity of aldehyde groups with native tissues can pose challenges. Replacing aldehydes with ketones slows down the gelation but can also form
crosslinks with somewhat slower degradation rates and fully biologically orthogonal chemistry [209].

**Oximes**

Oxime bonds can be rapidly formed between an aldehyde or a ketone and a hydroxylamine under physiological conditions. While the oxime bond is also hydrolytically labile, the rate of hydrolysis is significantly less than imines or even hydrazones under physiological conditions [210, 211]. A potential drawback of utilizing this chemistry is that an acid catalyst is typically required in order to yield realistic reaction rates of crosslinking since the adjacent oxygen has electron withdrawing capabilities which affect crosslinking kinetics [210]. Because of these properties, many researchers have applied oxime click chemistry for crosslinking in regions of interest with a lowered pH (<7.4), although titrating the area of injection with an acidic buffering agent either during or following injection can also be effective [212].

**[2 + 4] (Diels-Alder) Cycloaddition**

The Diels-Alder reaction, in which a conjugated diene reacts with an activated double bond (dienophile) in a single step, is one of the only pericyclic chemistries reported in the literature which has been described in relation to the crosslinking of hydrogels [213]. While the resulting crosslink is widely considered to be nondegradable, retro Diels-Alder reactions have been achieved at higher temperatures (>80 °C) or in alternate solvents to facilitate hydrogel degradation if desired [214]. Maleimide and furan groups are the most common Diels-Alder pair used for hydrogel crosslinking, copolymerization of these functionalities tethered to monomers also possible. Relative to other chemistries discussed, Diels-Alder chemistry is highly specific, and the functional groups involved are relatively unreactive in physiological conditions [215–217]. However, Diels-Alder chemistry typically offers relatively low gelation times on the timescale of tens of minutes to hours, although the combination of the Diels-Alder reaction with other physical crosslinking mechanisms can be achieved in order to reduce the gelation time if desired [218].

**Alkyne-Azide “Click” Reaction**

Cycloaddition between an azide and a terminal alkyne to yield a nondegradable triazole linkage (the first reported “click” chemistry) was first described by Huisgen et al. [219] but made more amenable to use for hydrogel crosslinking under physiological conditions following the development of the copper(I)-catalyzed alkyne-azide click (CuAAC) reaction developed by Sharpless’ group in 2003 [220]. Ossipov and co-workers first applied the chemistry for hydrogel crosslinking [221], with functional hydrogels having since been made based on this chemistry using synthetic polymers [222], biopolymers [223–225], and peptides/proteins [226]. The high selectivity, biorthogonality, and relatively fast gelation kinetics make this a very attractive candidate for in situ forming hydrogels. However, the potential toxicity of any unremoved copper(I) from CuAAC reactions can limit the use of this technique for biomedical applications [223]. Cyclic strained alkynes with lower activation energies
(strain-promoted alkyne-azide click reactions or SPAAC) can be used to perform “copper free” cycloadditions, reactions that have been used to prepare hydrogels spontaneously upon mixing functionalized prepolymer without the need for additives [227].

2.5 Length Scale

The physical size of the hydrogel formed represents another key factor determining the properties and thus potential utility of hydrogels in applications.

2.5.1 Bulk Hydrogels

The majority of examples previously cited are bulk hydrogels with macroscopic dimensions (typically defined as >1 cm) and a shape defined either by (1) adding the hydrogel building blocks/crosslinker(s) into a mold with particular dimensions prior to gelation or (2) punching/cutting a sample of a defined size out of a preformed gel. Injectable bulk hydrogels on the bulk scale are typically formed using a double barrel syringe in which the reactive precursor polymers are loaded into separate barrels and then co-extruded into a mold through a mixing baffle to initiate the in situ gelation reaction.

2.5.2 Gel Microparticles

Gel microparticles or microbeads on the tens to hundreds of microns length scale are widely used for separations and cell encapsulation applications. The most commonly fabricated gel microbead is based on the ionic complexation of alginate and calcium (Ca$^{2+}$), barium (Ba$^{2+}$), and/or strontium (Sr$^{2+}$) ions [228], although PEG-DA-based microbeads have also been commonly fabricated via UV photopolymerization [229].

Microbeads may be fabricated in many ways. First, extrusion dipping can be used in which an alginate solution is extruded through a nozzle, allowed to break into droplets under gravitational or mechanical force (e.g., air-induced shear), and dropped into the counterion solution to ionically crosslink [230]. While most commonly used among all methods due to its simplicity, the size of beads attained is commonly greater than 1000 μm without air jetting and >200 μm with air jetting, albeit with a relatively small coefficient of variance (CV) less than 15% [231]. This method is not amenable to use with slower crosslinking chemistries since the aqueous polymer droplet would dissolve in the aqueous receiving media unless crosslinking is very fast. However, methods such as adding an oil layer between the droplets and the bath [232] and/or using a fast crosslinking reaction (like alginate-calcium) to confine a secondary crosslinking reaction within a preformed microbead [233] have enabled the use of this method with slower chemistries. Second, an inverse emulsion in which the alginate (or another polymer or monomer) dissolved in water is the dispersed phase and an organic solvent is the continuous phase can be used to template the size of the microbeads [234]. Depending on the shear applied, particle sizes from the tens of microns scale down to the nanoscale (Sect. 2.5.3) can be achieved; furthermore, given that the resulting droplets are stabilized at least kinetically, slower crosslinking reactions (e.g., Diels-Alder) can
be undertaken relative to the extrusion process without destabilizing the droplets. In addition, the confinement of the prepolymers in droplets allows for a variety of bulk polymerization techniques (e.g., photopolymerization or heat-induced free radical initiation) to be used to prepare the networks inside the droplets. However, this method requires organic solvent use and typically results in broad particle size distributions. The use of a microfluidic droplet generator can address the latter problem to significantly reduce the polydispersity of the resulting microbeads, albeit at the cost of fabrication speed [235]. Controlling the relative rates of microfluidic processing versus crosslinking can also lead to the production of non-spherical gels with defined microscale dimensions [236]. High-shear processing of bulk gels has also been used to create gel microparticles [237], a process whose simplicity is tempered by the extremely broad range of particulate shapes/sizes of the microparticle products. Photolithographic methods like particle replication in non-wetting templates (PRINT) [238] and other types of templating techniques can also be used, techniques that trade their high size resolution relative to other techniques for slow production rates.

2.5.3 Microgels and Nanogels

Microgels and nanogels are colloidal hydrogel particles (typically on the length scale <5 μm) consisting of a water-swollen, crosslinked polymer network. The literature is highly ambiguous on the distinction between microgels and nanogels. While the International Union of Pure and Applied Chemistry (IUPAC) defines a nanogel as a particle of gel, of any shape, with an equivalent diameter between 1 nm and 100 nm and a microgel as a gel particle with an equivalent diameter in the range of 100 nm–100 μm, the terms are largely used interchangeably in the broad literature for any colloidally stable gel particle.

A variety of fabrication techniques have been developed to fabricate microgels/nanogels. Inverse emulsion techniques described in Sect. 2.5.2 (but using higher shear to break up smaller droplets) are the most commonly used approach, with gelation inside the droplets stimulated by UV photopolymerization, heat, or the addition of an external crosslinker [239]. Membrane emulsification in which a preformed inverse emulsion is forced through a membrane containing pores of equal sizes can assist in reducing the polydispersity typically achieved with inverse emulsion approaches [240]. For thermoresponsive polymer microgels (e.g., based on poly(N-isopropylacrylamide), poly(oligoethylene glycol methacrylate), or poly(vinyl caprolactam)), a precipitation technique developed by Pelton and co-workers [241] can be used in which the growing polymers in solution aggregate on to growing particle seeds once a critical molecular weight (and thus transition temperature) is reached. This technique typically results in highly monodisperse microgels/nanogels on the hundreds of nanometer size scale. Coacervation techniques in which changes in solvent composition, changes in salt concentration, and/or changes in pH [242] can drive similar aggregative assembly from polymer solutions can also be applied to create micro-/nanogels, albeit typically with somewhat broader polydispersities than achievable with the thermal precipitation method. Ionic complexation of polyelectrolytes or a polyelectrolyte with a multivalent ion
or hydrophobically driven self-assembly (e.g., of cholesterol-grafted soluble polymers) performed in dilute solutions under stirring can also in some cases result in micro-/nanogel formation, although again typically with relatively broad particle size distributions. The addition of surfactant to these solution complexation/coacervation preparation techniques can assist in stabilizing the resulting particles and minimizing the resulting particle size distribution.

Hydrogels can also be formed in other, non-spherical diameters at the micro- or nanoscales. Chief among those are micro-/nanofibrous hydrogels that can be formed via 3D printing or electrospinning processes. Hydrogel fibers prepared via 3D printing or other additive manufacturing processes can be formed via templated or free-form methodologies. In templated systems, the gel is deposited around a preprinted sacrificial template or printed as a liquid into a sacrificial gel template, with template removal resulting in the desired structure. Alternately, free-form printing directly prints a 3D gel structure in a single processing step using one of the four approaches: (a) extrusion of preformed hydrogel tubes, (b) printing and simultaneous rapid photocrosslinking of (meth)acrylated pre-polymer solutions to convert the liquid-like prepolymer into a gel, (c) printing a polyelectrolyte into a counterion solution (e.g., sodium alginate into a calcium ion bath) to facilitate near-instantaneous ionotropic gelation, or (d) extrusion of thermoresponsive gelling pairs (e.g., sodium alginate/gelatin) on a cooled or heated support that induces gelation on contact. It should be noted however that existing printing technologies are limited to printing such features on the microscale, with nanoscale resolution not yet achievable. Electrospinning, in which a potential difference is applied to stretch a polymer solution at the outlet of a nozzle, can be used to access nanoscale fibers, typically by either rapidly (photo) polymerizing a functionalized polymer solution as it is electrospun and/or mixing in situ gelling functionalized precursor polymer solutions just before the nozzle such that they can crosslink prior to contacting the collector (Fig. 7).

![Fig. 7](image)

**Fig. 7** Reactive electrospinning of hydrogel nanofibers by rapid reaction of hydrazide and aldehyde-functionalized precursor polymers during the electrospinning process. (Reproduced with permission from [249])
3 Conclusions

The diversity of functional building blocks, crosslinking strategies, and length scales with which hydrogels can be prepared is key to enabling the diverse applications of hydrogels across multiple fields. In particular, judicious selection of the chemistry of the gel building blocks (i.e., is a natural or synthetic polymer backbone most appropriate, what residual functionality is required in the network) linked with a crosslinking strategy that is most beneficial for the application (i.e., is in situ gelation required, is dynamic crosslinking beneficial, and over what time period – if at all – should the network degrade) is essential to design a hydrogel that works appropriately in a targeted application. Emerging strategies to control both the uniformity and microstructure of hydrogel building blocks (e.g., controlled radical polymerization techniques, telechelic polymers, self-immolative polymers) as well as fabricate hydrogels on well-defined length scales particularly within the nanoscale and microscale size regimes (e.g., microfluidics, 3D printing, electrospinning, and other techniques) are anticipated to further enable the production of hydrogels with novel properties and thus new potential applications.

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Advanced Hydrogel Structures

Michael J. Majcher and Todd Hoare

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Abstract

Conventional hydrogels have been successfully used in a range of different applications. However, the weak mechanics, inhomogeneous network structures, and poorly controlled responses of conventional hydrogels to changes in their environment have limited the applications of conventional hydrogels across a broad range of potential uses. Herein, we describe several newer types of hydrogel structures and morphologies that have been developed to address these key limitations of conventional hydrogels, including environmentally responsive hydrogels, homogeneous network hydrogels, interpenetrating network hydrogels,
Double network hydrogels, nanocomposite hydrogels, self-healing/self-adhesive hydrogels, and shape memory hydrogels. The fundamentals of both the design and structure of such materials as well as the key application areas in which such materials have been used to solve key technical challenges are highlighted.

**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CD</td>
<td>Cyclodextrin</td>
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<td>CNCs</td>
<td>Cellulose nanocrystals</td>
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<td>IPN</td>
<td>Interpenetrating network</td>
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<td>IR</td>
<td>Infrared</td>
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<td>LCST</td>
<td>Lower critical solution temperature</td>
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<td>LDHs</td>
<td>Layered double hydroxides</td>
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<td>l-Dopa</td>
<td>l-3, 4-dihydroxyphenylalanine</td>
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<td>NPs</td>
<td>Nanoparticles</td>
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<td>PAA</td>
<td>Poly(acrylic acid)</td>
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<td>PAAm</td>
<td>Poly(acrylamide)</td>
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<td>PAMPS</td>
<td>Poly(2-acrylamido-2-methylpropanesulfonic acid)</td>
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<td>PCL</td>
<td>Poly(ε-caprolactone)</td>
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<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
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<td>Quantum dots</td>
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<td>SMPs</td>
<td>Shape memory polymers</td>
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<td>Superporous hydrogels</td>
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<td>Superparamagnetic iron oxide nanoparticles</td>
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<td>Surface plasmon resonance</td>
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<td>UCST</td>
<td>Upper critical solution temperature</td>
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<td>Ultraviolet</td>
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<td>VPT</td>
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1 **Introduction**

The unique properties of hydrogels (e.g., their high water content, their soft mechanics, and their tunable internal porosities) have led to the successful application of hydrogels across a range of applications in medicine, personal care products, agriculture, separations, and other fields [1]. However, the inherent properties of conventional hydrogels also offer notable drawbacks in terms of their potential performance in a range of other applications. First and foremost, the generally weaker mechanics of hydrogels (on the Pa – kPa modulus scale) significantly limits the use of hydrogels in any application in which any load-bearing stress is involved and/or any deformation of the material over time would be...
disadvantageous. Such weak mechanics are a product of the combination of the high water content in conventional hydrogels, the inherent brittleness of crosslinked polymer networks, and the statistical nature of most hydrogel preparation chemistries that typically leads to inhomogeneous network structures and thus inherent weak spots at which fractures can be initiated and begin to propagate [2]. Second, while all hydrogels are sensitive to some degree to changes in their solvent conditions, effectively controlling the type and magnitude of those responses to specific stimuli is significantly more challenging and demands close attention to both the backbone chemistry of the hydrogel and the manner in which the hydrogel building blocks are assembled into a network structure. Third, as an effective thermoset material, conventional hydrogel networks are difficult or impossible to dynamically break and reform, at least not in a reversible way that can lead to full recovery of the original properties of the hydrogel. Finding novel synthetic and/or fabrication solutions to overcome these challenges has the potential to significantly expand the applications of hydrogels, a fact that has motivated extensive effort (particularly over the last two decades) focused on the creation of new functional hydrogel chemistries and morphologies. In this chapter, we outline recent innovations in the design of hydrogels that overcome the drawbacks of conventional hydrogel materials, with an emphasis on the mechanisms by which the new chemistries and structures developed enable the improved performance of these novel materials.

2 Environmentally Responsive Hydrogels

Environmentally responsive, or “smart,” hydrogels undergo a physicochemical and typically reversible swelling transition when exposed to an external stimulus. Given that the properties of any hydrogel are governed by the interaction between the polymeric hydrogel building blocks and the solvent, any hydrogel is in a way environmentally responsive as a change in solvent will induce a change in swelling. However, many other types of hydrogels have been specifically designed to exhibit responses to other physical cues (e.g., temperature, pH, magnetic fields, or electric fields) or chemical cues (i.e., changes in the concentration of a specific chemical) [3]. The mechanisms of the most common transitions will be described in the following sections. Note that combining the principles of these various designs into a single material can lead to multi-responsive hydrogels that can simultaneously respond to multiple stimuli [4]. Such materials are potentially beneficial to activate the response only under a specific combination of environmental conditions as well as enhance the magnitude of a targeted transition [5] (Fig. 1).

2.1 Temperature-Responsive Hydrogels

Thermoresponsive hydrogels have attracted broad research interest since hydrogel volume phase transition (VPT) behavior was first reported by Tanaka in 1979 [7].
The most common temperature-responsive hydrogel is based on the networking of polymers with a lower critical solution temperature (LCST), below which the polymer is soluble but above which the polymer is insoluble. Following crosslinking of these polymers into a network, this transition is manifested by a discontinuous deswelling response as the hydrogel is heated. Hydrogels (first reported by Tanaka [7]) and micro/nanogels (first reported by Pelton [8]) based on poly(N-isopropylacrylamide) (PNIPAM) are by far the most common materials within this category, as such materials exhibit a VPT temperature at ~32 °C between room temperature and body temperature. Thermoresponsive behavior is related to the balance between the (hydrophobic) isopropyl groups and the (hydrophilic) amide linkage between the isopropyl group and the main chain; as the material is heated, the entropic contribution (hydrophobic interactions) more effectively competes with the enthalpic interactions (hydrogen bonding) until a spontaneous phase transition occurs as predicted by the Gibbs’s free energy relationship \( \Delta G = \Delta H + T \Delta S \). Other poly(N-alkyl acrylate) polymers can similarly be used, allowing for modification of the VPT temperature according to the relative hydrophobicity of the alkyl groups [9]. Increasing research interest has more recently been devoted to poly(oligoethylene glycol methacrylate) (POEGMA) given that its VPT temperature can be tuned precisely by controlling the number of ethylene glycol repeat units included on the side chains in these materials [10]. Gels based on poly(vinylcaprolactam) (PCL) [11], block copolymers of poly(ethylene oxide)-co-poly(propylene oxide) (e.g., Pluronics) [12], methylcellulose (Fig. 2) [13], and elastin-like polypeptides [14] have also been reported with similar beneficial properties. Alternately, if a hydrogel is fabricated from a polymer exhibiting an upper critical solution temperature (UCST), heating the hydrogel will induce a swelling volume phase transition, typically a result of competing hydrogen bonding interactions within and between the comonomers in the hydrogels as the temperature is increased. There are relatively fewer examples of UCST polymers, with poly(acrylamide-co-acrylic acid) [15] or homopolymer networks of poly(N-acryloyl glycinamide) [16] among the most common.
2.2 pH-Responsive Hydrogels

pH-responsive hydrogels can be fabricated by crosslinking polyelectrolytes comprised entirely or partially of monomers with a pKₐ value within an accessible pH range for a given application. Ionization of these functional groups drives hydrogel or micro/nanogel swelling according to Donnan equilibrium-based swelling that is reversible upon neutralization [17]. Poly((meth)acrylic acid)-based hydrogels are the most common example of these materials, although a range of other polyacids (e.g., carboxymethyl cellulose, poly(methacrylic acid), or hyaluronic acid) and polybases (e.g., chitosan, poly(diethylaminoethyl (meth)acrylate), poly(vinylamine), poly(vinyl pyridine), or poly(vinyl imidazole)) have also been reported [3]. Note also that any pH-responsive hydrogel is also inherently ionic strength-responsive, as the charges generated upon ionization can be screened by increased ionic strength to drive gel deswelling via double layer effects.

2.3 Light-Responsive Hydrogels

Two approaches can be used for creating light-responsive hydrogels: photo-isomerization or photocleavage. In the former approach, chemical units that can reversibly switch conformation upon light exposure are used as crosslinkers, with the most common examples being molecules that undergo reversible cis-trans isomerization (e.g., azobenzenes or stilbenes) [18] and molecules that undergo reversible ring-opening/ring-closing isomerization (e.g., diarylethenes, spiropyans, or fulgimides) [19]. The reversible structural and electronic changes in the crosslinker upon isomerization (e.g., changes in geometry, hydrophobicity/hydrophilicity, or redox potential) induce changes in the degree of gel swelling, with ring closure and cis isomerization typically driving hydrogel deswelling responses. In the
latter approach, molecules with the capacity to reversibly photodimerize are grafted to the hydrogel precursor polymers, with crosslinking triggered or de-triggered according to exposure to various wavelengths of light to drive deswelling/swelling according to changes in the elastic osmotic gradient [17]. Cinnamoyl, anthracene, and coumarin (and related derivatives) are most commonly used for this purpose [20], although, in comparison to the photoisomerizing groups, the wavelengths required for decrosslinking are very low (<254 nm) and the decrosslinking reactions are relatively inefficient.

### 2.4 Chemoresponsive Hydrogels

Chemoresponsive hydrogels have attracted particular interest in on-demand drug delivery applications in which therapy can be triggered when the need is identified and biosensing in which the gel phase transition can be used as a (semi)-quantitative readout of analyte concentration. By far the most studied example in this area is glucose-responsive hydrogels, which have obvious appeal for both sensing and drug delivery for diabetes patients. Phenylboronic acids grafted on or copolymerized within the hydrogel precursor polymers are the most popular glucose-responsive units [21]. Boronic acids switch from a trigonal geometry in the neutral state (with relatively low affinity for glucose) to a tetrahedral geometry in the ionized state (with a high affinity for cis-diol containing carbohydrates like glucose). Since glucose has two such cis-diol units, depending on the distribution and quantity of boronic acid groups grafted to the polymer, glucose can either bind twice to act as a crosslinker (reducing gel swelling at high glucose concentrations due to elastic contributions) [22] or (more commonly) once to shift the ionization equilibrium and generate more charged boronic acid groups in the hydrogel (increasing gel swelling at high glucose concentrations due to Donnan contributions) [22]. While this ionization equilibrium is active only around the pKₐ of boronic acids (pH~9), this active pH can be lowered by changing the position of the boronic acid on the aromatic ring, substituting electron-withdrawing substituents on the ring, and/or attaching the groups to the polymer backbone via an amine linkage that inductively lowers the pKₐ of ring substituents [23]. Such groups have been incorporated in a range of bulk gels and micro/nanogels [24], with the overall charge of the gels manipulated based on the remaining gel components to drive desirable swelling responses at targeted pH values (Fig. 3). Given that the boronic acid interactions are not exclusive to glucose but can occur with any cis-diol containing carbohydrate (in particular fructose), more specific enzymatic systems incorporating glucose oxidase have also been developed. In the presence of hydrogen peroxide and catalase, glucose oxidase enzyme activity lowers the pH to drive a pH-responsive deswelling event in acid-functionalized gels or a swelling event in base-functionalized gels [25]. Alternately, the lectin concanavalin A can be co-grafted to the hydrogel as an alternative affinity agent to boronic acids, with the addition of free glucose inducing dissociation of the concanavalin A/polymer-bound glucose intermolecular crosslinks and thus hydrogel swelling [26].
Other chemoresponsive hydrogels have also been developed. Antigen-responsive hydrogels have been designed using a similar approach to the concanavalin A/glu-
cose system described above, with both the antigen and the corresponding antibody grafted to the gel building blocks; gel swelling is induced when free antigen is added that can competitively bind with polymer-bound antibody to break the polymer-
polymer antibody-antigen crosslinks [27]. Antibody-responsive hydrogels can be designed using the same approach. Molecularly imprinted hydrogels, typically polymerized in the presence of the target chemical using one or more comonomers that can associate in some way with the target chemical [28], have also been developed to bind and detect a range of different chemical targets including choles-
terol, various drugs, and allergens. Reassociation of the target molecule with the binding pocket defined by associating monomer units polymerized in the presence of the target molecule (and thus, when the templating target molecule is removed, aligned inside the resulting void to maximize the intermolecular interactions with the target molecule) tends to increase the crosslink density of the hydrogel and thus restrict swelling [29]. However, the speed and the degree of swelling achieved for all these targets (including glucose) are still limiting toward ultimate applications of these materials.

3 Homogeneous Network Hydrogels

Despite their highly networked structure, conventional hydrogels typically exhibit weak mechanical properties. This observation is most commonly related to the inherent inhomogeneities present during the random crosslink process
(and significantly magnified following hydrogel swelling) that reduces the local capacity of the gel to cooperatively respond to applied stresses. As a result, the gel will fracture at the weakest point. To instead create homogeneous network structures that do not have such weak points and could cooperatively respond to a given stress, two approaches have been taken: (1) convert the crosslinking strategy from static to dynamic such that the crosslinker distribution can be dynamically adjusted upon the application of a stress to maximize the cooperativity of the hydrogel response or (2) create a structurally perfect static network that does not have inhomogeneities and thus no mechanically weaker points where stress can focus to cause fracture. Schematic diagrams of both of these approaches are shown in Fig. 4.

![Schematic diagrams of approaches to create homogenous network hydrogels: (a) slide-ring hydrogels. (Reproduced with permission from [30]); (b) tetra-PEG hydrogels. (Reproduced with permission from [2])](image)

**Fig. 4** Schematic diagrams of approaches to create homogenous network hydrogels: (a) slide-ring hydrogels. (Reproduced with permission from [30]); (b) tetra-PEG hydrogels. (Reproduced with permission from [2])
3.1 Slide-Ring Hydrogels

Among dynamic hydrogels approaches (also referred to as topological gels), slide-ring (or slip-ring) hydrogels [31] created based on supramolecular interactions, most commonly PEG-cyclodextrin (CD) [32] or other polyrotaxanes (e.g., cucurbit[n]uril or silsesquioxane [33]), have been the most widely studied. Upon the application of a stress, the CD groups can dynamically move up and down the poly(ethylene glycol) or PEG chains to uniformly disperse an applied stress throughout the polymeric network akin to the action of a pulley [34], resulting in an extremely soft hydrogel with high tensile strength and potential for elongation. This general approach of using slide-ring crosslinkers has also been used to create high-strength networks based on other polymers, including stimulus-responsive hydrogels [35]. The use of other types of cyclic slide-ring crosslinkers including pillararenes, cucurbiturils, and calixarenes can impart different properties to the gels depending on the strength of the interaction between each inclusion agent and the polymer (or polymer graft) with which it interacts [30].

3.2 Structurally Homogeneous Hydrogels

Alternately, the use of hydrogel building blocks with structurally perfect geometries, low conformational mobility, and fixed functional group positioning can avoid the loops and defects arising from using less regular and constrained building blocks that introduce structural inhomogeneities in gel networks. In particular, tetrahedron-oriented star macromonomers based on poly(ethylene glycol) (tetra-PEG) with telechelic crosslinkable functional groups have been used to create highly homogeneous network structures. The consistent internal correlation length of two PEG arms between each “inhomogeneity” (i.e., star-PEG building block) within the hydrogel enables such hydrogels to support compressive moduli on the MPa range [2]. While the original example was crosslinked by mixing amine and N-hydroxysuccinimide ester-terminated tetra-PEGs of the same length [36], other chemistries (e.g., triazoles, oximes, urethanes, disulfides, and others [37]) have since been used to control the degradability and gelation time of the resulting hydrogels.

4 Interpenetrating Network Hydrogels

An interpenetrating network (IPN) hydrogel is characterized by the interlocking of two independently crosslinked but chemically noninteracting hydrogel networks [38]. Specifically, an IPN hydrogel contains at least two networks that are not covalently linked but are mechanically interlocked such that the networks cannot be separated without breaking covalent bonds. In this sense, IPNs are frequently referred to as gel alloys in the sense that two independent components are intimately mixed together into a single structure. IPNs were first reported in 1914 [39], with the
pioneering scientific studies on the structure of IPNs conducted by Millar, Frisch, Sperling, and Friedman in the 1960s and 1970s [40, 41].

IPNs can be characterized by structure or chemistry (Fig. 5). In terms of structure, full IPNs consist of two or more interlocked networks, while semi-IPNs consist of at least one linear polymer entrapped inside a bulk hydrogel. While it is not necessary for the networks to be chemically different (indeed, homo-IPNs in which two identical

Fig. 5 Schematic diagrams of the synthesis pathways of (a) simultaneous full and semi-IPN hydrogels and (b) sequential full and semi-IPN hydrogels
chemistries are used for the two gel phases are among the earliest commercialized hydrogel IPN structures) [42], most IPNs feature two chemically distinct networks. In terms of chemistry, IPNs can be made in one of two ways: sequential or simultaneous [43]. In a simultaneous IPN, both networks are formed at the same time via different independent crosslinking chemistries; in a sequential IPN, one network is preformed, after which the monomers/precursor polymers/crosslinkers used to prepare the second network are swollen into the pre-existing gel network and the second network is polymerized in situ. While the simultaneous route is faster, it is also more likely to produce more phase separated and thus less interpenetrating structures since both networks are mobile at the start of the IPN formation. Furthermore, for the simultaneous route to work, the chemistry of the two crosslinking mechanisms must be both fully independent and fully compatible in terms of the crosslinking conditions required to fabricate each network. As such, when IPN hydrogels of multiple chain growth polymers are made, the reaction must be done sequentially to avoid chemical reactions between the phases; in contrast, for chain/step growth polymer IPN hydrogels or step/step growth polymer IPN hydrogels in which the step growth mechanisms are chemically orthogonal, sequential or simultaneous IPNs may be achievable [44]. As an example of a simultaneous step/step growth polymer IPN hydrogel that is also in situ gelling, we have reported on injectable IPN hydrogels formed by mixing hydrazide/thiol (nucleophilic) and aldehyde/maleimide (electrophilic) prepolymer via co-extrusion from a double barrel syringe [45]. Hydrazone and thiosuccinimide bond-forming reactions are strongly kinetically favored relative to any competing reaction pairs, enabling the fabrication of an IPN via a simultaneous reaction pathway.

IPNs are typically formed for one of three reasons: (1) the mechanical interlocking of the two networks can significantly enhance the mechanics of the hydrogel relative to a single network gel [46]; (2) two polymers that are otherwise incompatible (i.e., would phase separate in solution) can be mixed and maintained as mixed due to the kinetic freezing of polymer chain diffusion upon crosslinking [47]; and (3) two types of polymers that cannot be chemically formed into a single network (i.e., they crosslink via a different mechanism) can be combined into a single hydrogel structure. The balance between the rate of crosslinking and the rate of phase separation during IPN formation determines the homogeneity of the IPN network. Either homogeneous or heterogeneous IPNs may be beneficial depending on the application. For example, in drug delivery, phase separation during the fabrication of IPNs can be used to regulate pore size/morphology (facilitating control over diffusion properties) [48] as well as facilitate the creation of a network of segregated domains enriched with one of the constituent polymer networks (enabling, e.g., enhanced uptake and control over release of drugs with specific properties [49]).

5 Double Network Hydrogels

First reported by Gong et al. in 2003 [50], double network hydrogels represent a special case of an IPN hydrogel that exhibits the combination of both high water content and high mechanical strength/toughness. Such properties, typically not achievable with
conventional IPNs, are accessed when two types of polymeric building blocks with strongly asymmetric structures are used. Specifically, a double network hydrogel must satisfy three key properties: (1) the first network is comprised of a rigid and brittle polymer (most commonly a polyelectrolyte), while the second network is comprised of a soft and ductile polymer (most commonly a neutral, high molecular weight polymer); (2) the molar concentration of the second (soft) network is 20–30 times higher than that of the first (rigid) network; and (3) the first (rigid) network is tightly crosslinked, while the second (soft) network is loosely crosslinked (at levels as low as 0.01 mol%) [51]. The result of this combination of inputs is a hydrogel with >90% water content, high hardness (elastic modulus >100 kPa), high strength (failure tensile and compressive stresses on the MPa range and failure strains of >20-fold extension/compression), and high toughness (>100 J/m²), placing these hydrogels on par with rubbers in terms of their mechanical properties despite their high water contents.

The gel mechanics are improved compared to conventional heterogeneously crosslinked IPNs as well as homogeneous single network hydrogels based on two factors. First, the beneficial mixture of the (majority) ductile network with the (minority) brittle network allows for local yielding of a propagating crack to prevent failure [52]. Mechanical analysis suggests a high hysteresis in the first loading cycle [53] consistent with fragmentation/fracture of the brittle network into clusters that subsequently serve as crosslinking points for the majority ductile network. Given that this ductile network is not chemically attached to the stiff brittle network, it can subsequently slide to reduce the number of defects in the hydrogel akin to slide-ring hydrogels described in Sect. 3.1 [54]. Second, residual unreacted vinyl groups in the first network can also react during the polymerization of the second network (via a sequential mechanism) to create a connected network structure that is not a true IPN but provides additional mechanical enhancement [51].

The first double network hydrogel was prepared by UV photopolymerizing a poly (2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) hydrogel as the first network, swelling the network in acrylamide monomer, and then UV copolymerizing the poly (acrylamide) second network [50]. The high degree of swelling of the first network is essential to create stretched chains in the brittle network in order to promote the stress-absorbing mechanism outlined above. Most reported double network hydrogels have been prepared in the same way with similar monomers [55], although newer approaches have also been developed to prepare double network hydrogels using a one-pot method using a combination of a physically crosslinked first network (e.g., thermally gelling agar or calcium-crosslinked alginate) [56] and chemically crosslinked second network [57]. For additional details, refer to Chen et al.’s comprehensive review of double network hydrogel chemistry and physics for other polymer combinations found to be useful for double network hydrogel formation [55].

6 Nanocomposite Hydrogels

A nanocomposite hydrogel is defined as a hydrogel that contains nanoparticles (NPs) or nanoscale structures of a different composition to the continuous phase [58]. The creation of such hydrogels has been motivated by the distinctive optical,
mechanical [59, 60], stimulus-responsive [61, 62], and biological [63] properties of NPs relative to those same materials in the bulk.

Depending on the targeted application of the nanocomposite hydrogel, NPs may be attached through either chemical or physical interactions to the surrounding hydrogel or simply physically encapsulated into the hydrogel. However, in either case, for the nanocomposite hydrogel to exhibit the desired properties, it is essential to disperse the nanoparticles evenly throughout the hydrogel structure. Five main approaches have been identified to create a uniform distribution of NPs within a gel matrix (Fig. 6) [64]: (1) using an aqueous NP suspension as the solvent to prepare the hydrogel [65–67], (2) physically embedding NPs into the hydrogel after gelation [68–70], (3) fabricating NPs in situ within a preformed gel from NP precursors [62, 71–73], (4) crosslinking functional groups on the surface of NPs to use NPs themselves as a building block for the overall hydrogel [74–76], and (5) hydrogel formation by entrapping polymers and NPs inside hydrogels formed by small molecule gelators [77]. The choice of NP as well as the method by which it is incorporated (primarily the degree to which it is dispersed and interacts with the surrounding hydrogel phase) will govern the mechanics and functionality of the resulting nanocomposite hydrogel. Some common examples will be discussed in this section, but for a more in-depth review of particular NP/polymer combinations, the reader is directed to recent critical reviews that put an emphasis on material selection and properties [78, 79].

The most common examples of hard nanocomposites include those made from metals [80, 81], metal oxides and magnetic particles [82, 83], and layered silicates [84, 85]. Among metals, silver (Ag) nanoparticles have been particularly widely exploited for their antimicrobial properties in biomedical applications [86], their enhancement of surface plasmon resonance (SPR) signals for biosensing [83], and their electrical conductivity for conductive coatings [87, 88]. Both synthetic and natural polymers such as poly(acrylamide) (PAm) [89], poly(acrylic acid) (PAA) [84], poly(vinyl alcohol) (PVA) [80], chitosan [90], dextran [91], and gelatin [92] have been used to prepare silver NP hydrogel composites for these various applications. Gold (Au) nanoparticles have similarly been used in biosensing and antibacterial applications [93, 94]. In addition, anisotropic gold nanoparticles (e.g., gold nanoshells or nanorods) have been used as actuators to induce remote-controlled heating in thermoresponsive hydrogels upon exposure to near-infrared irradiation as a result of surface plasmon heating effects [95]. Platinum [96], cobalt [97], nickel [98], and copper [99] NP-based nanocomposite hydrogels have also been reported for various applications.

The incorporation of metal-oxide NPs into a nanocomposite hydrogel can offer ferromagnetic and semiconducting properties. The most common example is the incorporation of iron oxide NPs to create “ferrogels” [100] in combination with hydrogels based on poly(acrylamide-co-maleic acid) [101], PNIPAM [102], 4-vinylpyridine [103], chitosan and PAA [104], and methacrylate copolymers, the latter of which can subsequently absorb toxic ions like lead, chromium, and arsenic [105]. Superparamagnetic iron oxide nanoparticle (SPION)-impregnated hydrogels have the potential to generate heat in situ via hysteresis heating when placed in an oscillating magnetic field operating on the hundreds of kHz frequency range [106];
when coupled with a PNIPAM or other thermoresponsive hydrogel, such localized heating induces a phase transition to trigger remote-controlled deswelling of the hydrogel [107] to enable remote on/off gating applications as well as on-demand drug delivery. As such, ferrogels can be useful for recovering a gel (and in particular micro/nanoscale gels) from complex mixtures by applying a constant magnetic field or remotely triggering a phase transition in a thermoresponsive gel by applying an oscillating magnetic field. The combination of near-infrared (IR) or thermally sensitive nanoparticles along with supramolecular chemistry has also been used to create self-healing nanocomposite hydrogels in which heating can be remotely induced to re-heal the gel following damage [108]. Other examples of metal-oxide nanoparticles used to prepare nanocomposite hydrogels include titanium oxide (enabling ultraviolet or UV-induced photocatalytic sterilization localized to the hydrogel) and zinc oxide [109].

Fig. 6 Schematic overview of the five methods for creating nanoparticle-hydrogel composites: (1) hydrogel formation in a nanoparticle suspension, (2) physically embedding the nanoparticles into hydrogel after gelation, (3) reactive nanoparticle formation within a preformed gel, (4) crosslinking using nanoparticles themselves to form the overall hydrogel, and (5) hydrogel formation using nanoparticles, polymers, and physical gelators. (Reproduced with permission from [64])
Nonmetallic hard nanoparticle-impregnated hydrogels involving carbon-based nanomaterials (e.g., graphene, nanotubes, or nanowires), quantum dots (CdSe/ZnS and CdSe/CdS), or silica have been described in the literature. With carbon-based materials, of which carbon nanotubes are the most prominent [110], the objective of nanoparticle incorporation is to control the electrical and photothermal properties of hydrogels. Graphene, a planar monolayer of sp²-hybridized carbon atoms held in a 2D honeycomb lattice, has attracted increasing attention for the preparation of nanocomposite hydrogels due to its high electron mobility at room temperature, very high thermal and electrical conductivity, high Young’s modulus, and high tensile strength [93, 111, 112]. Quantum dots (QDs) are typically added for their semiconductor properties and their ability to serve as fluorescence biomarkers for biomedical applications [113, 114]. Silica NPs are typically used to increase mechanical properties [115] or, if mesoporous silica NPs are used that contain aligned pores with dimensions in the few nm range, increase the loading and control the release of small molecule drugs that can diffuse into the pores [116, 117].

Anisotropic nanoparticle composites have also attracted interest. Nanocomposite hydrogels based on 2D nanoplatelet layered double hydroxides (LDHs) or mineral clays (primarily montmorillonite and laponite) have attracted considerable attention given the capacity of such nanocomposites to substantially improve the mechanical, thermal, and optical properties of hydrogels [118]. The incorporation of clay has been demonstrated in many cases to enhance the swellability of hydrogels due to the role of the 2D clay additive in enhancing the porosity and ion exchange capacity of the network, resulting in the use of smectic clay-based nanocomposite hydrogels as superabsorbent hydrogels [119] and sorbents for heavy metals or dyes [120]. Cellulose nanocrystals (CNCs), rod-like nanoparticles derived from acid hydrolysis of cellulose, have also attracted significant recent attention as a nanoscale additive for hydrogels given their diamagnetic properties (enabling alignment in a magnetic field), their very high mechanical strength, and their facile surface functionalizability that promotes their uniform distribution within a gel matrix [121]. We have recently shown the potential to use the diamagnetic alignment of CNCs to direct the alignment of myotubules, which grow parallel to the direction of the anisotropic NP alignment [122].

Soft nanocomposite hydrogels may include those with nonmetal nanoparticles [123] or polymeric nanoparticles including micelles [124], nanogels [125, 126], core-shell particles [127], dendrimers [128], hyperbranched polymers [129], and liposomes [130]. All these types of nanoparticles have been used to prepare hydrogel composites, primarily exploiting their potential benefits in prolonging drug delivery or maintaining drug delivery at a specific site in the body rather than the mechanical or optoelectronic motivations of most of the hard nanoparticles previously described. However, polymeric nanoparticles can also be used as alternatives to hard nanoparticles in those applications, with polystyrene (PS) often used as a mechanical filler [131] and polypyrrole (PPy) applied due to its semiconducting properties for optoelectronics [132].
7 Self-Healing/Self-Adhesive Hydrogels

Self-healing or self-adhesive hydrogels are a unique classification of materials which can autonomously and intrinsically heal defects in the hydrogel network to prevent the propagation of cracks through the hydrogel and thus maintain the integrity of a hydrogel even under high loads [133]. Self-healing or self-adhesive hydrogels are primarily designed around dynamic chemistries that can spontaneously reform following breaking of a crosslink. Such properties are highly relevant in applications such as soft robotics, 3D printing, and tissue engineering in which it is essential that material properties can be restored without any external intervention by the user (Fig. 7) [134].

Constitutional dynamic chemistries (CDCs) are most typically used to formulate self-healing/self-adhesive hydrogels, a term that describes any chemistry that is both dynamic and reversible (i.e., broken bonds may spontaneously reform). CDC may be either covalent or non-covalent in nature. Physical self-healing gels have been fabricated using hydrophobic interactions [135–139], host-guest interactions [140–142], hydrogen bonding [143, 144], crystallization [145–147], and polymer-nanocomposite interactions [148–150], including combinations of these techniques [151, 152]. Chemical self-healing gels have employed boron-oxygen bonds (phenylboronate ester) [153–156], sulfur-sulfur bonds (disulfides) [157, 158], carbon-nitrogen bonds (imines [159–161] and hydrazones [162–164]), carbon-carbon/sulfur bonds (reversible radical reaction) [165–168], and reversible Diels-Alder cycloadditions [169–171].

Several self-healing hydrogels have also been designed around the biomimetic chemistry of blue mussels, which are known to offer longer-lasting adhesion in comparison to synthetic glues or adhesives [172]. Blue mussels are able to attach themselves to solid substrates through the use of disc-tipped acellular threads called byssi that contain proteins high in L-Dopa (L-3, 4-dihydroxyphenylalanine).

![Fig. 7](image)

**Fig. 7** A schematic illustration of self-healing mechanisms for hydrogels which using dynamic chemistry. For instance, a gel can be cut with a knife (a) in order to yield two unique pieces with a damaged region (b). When the two pieces are placed back into contact with one another (c), the cut may be healed by dynamic association and dissociation until equilibrium is reached. (Reproduced with permission from [134])
While the role of L-Dopa in attachment has not yet been fully elucidated, self-healing hydrogels containing functionalities related to the oxidized O-quinone and O-catechol forms of L-Dopa have been successfully applied to create self-healing and self-adhesive hydrogels from a variety of polymer backbones. For specific information on catechol and quinone analogues used in self-adhesive hydrogel designs, readers are referred to Haag’s work [173–175] and recent comprehensive reviews [172].

8 Shape Memory Hydrogels

Shape memory polymers (SMPs) are materials that utilize an external stimulus such as electricity, water, temperature, magnetism, or light in order to recover their original shape post-deformation [176]. While conventional polymer composite and blends have been more commonly reported, shape-adaptable hydrogels with various time scales and degrees of shape recovery have more recently been investigated. Early work with SMP-based hydrogels was focused on making “water-triggered” systems in which water is allowed to diffuse into the gel and then exposed air to evaporate. The constituent polymer chains will be drawn close to each other via capillary forces upon drying, introducing intermolecular interactions to lock the structure into a confined, but transient, morphology [177]. However, hydrogels are inherently challenging in terms of effective design of shape memory materials. Conventional hydrogels are not sufficiently stiff to withstand most physical deformations of relevance to shape memory; as a result, more recent examples employ double network or nanocomposite hydrogels with high tensile strengths in the MPa regime [178]. Moreover, the ability for shape memory hydrogels to break and recover their original shape is strongly correlated with the concentration and interchain strength of the collective polymeric constituents. While the glass transition is typically used for this purpose in conventional shape memory materials, the high water content of hydrogels sharply limits the utility of the glass transition to regulate shape memory in hydrogels [178, 179].

Environmentally responsive hydrogels sensitive to temperature [180], pH, and/or ionic strength [181] (Sect. 2) have been most widely used to overcome the aforementioned barriers in aqueous systems to create functional shape memory hydrogels. Heat or ionization/protonation is used to dissociate weak physical bonds between crosslinks that will lower the strength of the bulk material and allow chains to interact and form a transient shape; subsequent cooling or ionization/re-protonation drives the re-formation of physical crosslinks. Other physical self-assembly strategies exist such as supramolecular interactions (e.g., metal-ligand and host-guest) [182], chemically responsive hydrogel transitions (e.g., glucose-responsive materials) [183], and external stimuli such as redox reactions [184] or ultrasonic frequencies [185] that can all be used to trigger the shape memory response, with multi-responsive materials also having been designed that are capable of showing shape memory behavior in response to multiple variables [186, 187]. Self-healing hydrogels can also be used as shape memory hydrogels in terms of their capacity to dynamically promote the
formation of new bonds as old ones are broken (Sect. 6), enabling “locking in” of the gel structure after a deformation.

9 Conclusions

Rational manipulation of both the chemistry (backbone type and crosslinking strategy) and the morphology (network structure) of hydrogels enables the production of hydrogels with multiple unique and well-defined properties. Specifically, environmentally responsive hydrogels, self-healing hydrogels, and shape memory hydrogels all exhibit unique and controllable dynamic behavior upon the application of various stimuli, while homogeneous network, interpenetrating network, double network, and nanocomposite hydrogels all facilitate the fabrication of stronger hydrogels with the potential to be used in load-bearing or high-shear applications. These properties collectively address many of the key barriers to using hydrogels in multiple applications in which stronger mechanics and tunable dynamic responses are desirable.

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Cell Surface Engineering

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Abstract
Recent preclinical studies demonstrated the use of engineered cells as a potential way to treat many diseases and disorders. Tailoring the cell’s function and interactions using surface engineering methods is a very promising approach in developing novel cell-based therapeutics. For instance, cell surface modification has been used for the development of universal blood donor cells. In another example, it has been shown that surface modification of stem cells is a doable approach to regulate the fate of cells into specific phenotypes, which is necessary to regain function in specific environment such as different injury sites. Cell surface engineering using macromolecules/polymers could provide desired properties and functions to cells for applications in targeted delivery, biosensing, transfection, imaging techniques, and in the regulation of cell fate. This chapter will review the recent advancements in polymer-based cell surface engineering approaches for various applications. In terms of the cell types, we have chosen to focus, specifically, on red blood cells, lymphocytes, splenocytes, stem cells (multipotent and pluripotent), islet cells, endothelial cells, and hepatocytes as they offer the most promise in generating cell-based therapeutics. In terms of modification approaches, we mainly highlighted the literature associated with the use synthetic polymers via covalent conjugation and non-covalent bonding. We also discuss the future of such cell surface engineering methods for their potential clinical utility.

Keywords
Cell surface engineering · Polymers · Bioconjugation · Red blood cells · Stem cells · Islet cells · Endothelial cells

Abbreviations
AL Alginate
BAEC Bovine aortic endothelial cells
BNHS Biotin N-hydroxysuccinimidyl
CAM Cell adhesion molecules
CH-PC Chitosan-graft-phosphorylcholine
CNS Central nervous system
CP Choline phosphate
DMPE 1,2-Dimyristoyl-sn-glycerol-3-phosphatidylethanolamine
EC Endothelial cells
ECM Extracellular matrix
ESCs Embryonic stem cells
FGF2 Fibroblast growth factor 2
GAGs Glycosaminoglycans
HPGs Hyperbranched polyglycerols
HS Heparan sulfate
HSCs Hematopoietic stem cells
HTPs HaloTag proteins
ICAM-1 Intercellular cell adhesion molecule-1
Cell surface is a highly heterogeneous environment with distinct types of proteins, glycans, and lipids [1–8]. Cell surface has a critical role in governing the fate of cells as it would regulate cell-cell interaction, cell-niche communication, and intracellular signaling pathways [2, 6–10]. Although the cell surface is highly dynamic and complex in nature, several ways have been developed for manipulating the cell surface in order to alter their functionalities/properties for variety of applications (Fig. 1). Controlling the biochemical and cellular functions of cells by resurfacing the cell membrane with biomaterials alongside inhabitant functionalities allows new opportunities in drug delivery, cell-based therapeutics, transfusion, and tissue engineering [2–4, 9, 11–15]. Most common cell surface engineering applications include bioimaging, manipulating cell biology, tissue engineering, cell therapies, and targeting cells to the desired sites of the body [2, 8, 9, 15]. For instance, by systemic infusion of engineered hematopoietic stem cells (HSCs) with cell homing ligands onto their surface, it is possible to home such cells to the bone marrow to have a safe and efficient cell targeting for transplantation applications [16]. Although proposed approaches in this field have been promising, many challenges remain. Such issues include the development of cell-friendly modification methods that suit highly heterogeneous and super dynamic nature of the cell surface to have a significant
effect on cellular functions such as adhesion, proliferation, and differentiation without compromising the viability.

In this chapter, we will discuss cell surface engineering approaches using synthetic polymers for various applications. It will also address how chemical approaches including covalent and non-covalent methods are used to manipulate the cell surface effectively to enhance their therapeutic potential and other cellular functions. These include covalent conjugation of polymers to primary amine groups on cell surface proteins, incorporation of amphiphilic polymers into lipid membrane of cells via hydrophobic interaction, electrostatic binding between cationic macromolecules and negatively charged cell surface, and grafting from strategies and modifications through nonnative functional groups. In addition, this chapter also highlights a huge body of work on the engineering the surface of cells, including red blood cells (RBCs), white blood cells, multipotent and pluripotent stem cells, islet cells, endothelial cells, and hepatocytes for transplantation and transfusion applications. These cells are selected due to their promising therapeutic potential for various diseases. We review the advantages and challenges associated with these methods and how these approaches can be applied to improve the therapeutic applications of cells. Finally, conclusions about the current state of the field and insight into the future directions are given.

2 Methods for Cell Surface Engineering Using Polymers

Cell surface engineering has a pivotal role in tuning the cell function by controlling their biochemical interactions with their environments [2–4, 15, 18]. The availability of different functional groups on the cell surface will be an excellent opportunity for cell surface modification (Fig. 1). However, surface modification is quite challenging due to the fact that cell surface is not static, and also the modification should only have minimal effect on biological function of the cells [5, 7, 19, 20]. In the past two or three decades, researchers have focused on developing various tools for engineering the surface of cells [2, 9, 19, 21, 22]. A variety of functional groups and bioactive substances have been introduced onto cell surface by different biological transformations and physicochemical methods [9, 14, 21, 22]. Here, our intention is to focus on methods that are commonly used and utilize hydrophobic, electrostatic, covalent interactions and enzymatic approaches for cell surface modification.

2.1 Hydrophobic Insertion into the Cell Membrane

Hydrophobic interactions are frequently exploited for cell surface engineering, especially poly(ethylene glycol) (PEG) conjugated phospholipid amphiphilic polymers are known for their ability to attach on the cell membrane [23, 24]. Tatsumi et al. have developed a versatile method to engineer the surface of hepatocytes by immobilizing polymers on the cell membrane [25]. They used a PEG-phospholipids conjugate bearing FITC (FITC-PEG-lipid, in particular, 1,2-dimyristoyl-sn-glycerol-3-phosphatidylethanolamine (DMPE)) to intercalate into the hepatocyte lipid
membrane. This modification process did not alter either functional parameters in vitro or engraftment potential of the cell in vivo. Moreover, Iwata and co-workers highlighted the importance of hydrophobic modification of the cell surface using a layer-by-layer method [26]. They used a combination of PEG-conjugated phospholipid derivatives and poly(vinyl alcohol) (PVA) bearing different anchoring units. When PEG-phospholipid conjugates bearing maleimide groups were incubated with islet suspension, a thin layer of PEGs on islet surface formed spontaneously by a hydrophobic insertion mechanism. Then, the first PVA layer was introduced via a maleimide/thiol reaction between maleimide group of the PEG layer and thiol groups on modified PVA. The PVA layer was further enforced using a layer-by-layer method which utilized thiol/disulfide exchange reactions [27, 28]. Figure 2 showed how such approach is used to engineer the surface of islet cells.

Recently, Temura et al. reported another surface engineering approach using PEG-lipid derivatives and DNA hybridization instead of PVAs for cell-cell interactions [29]. In this approach, a single-stranded ss-DNA (polyA20 and polyT20 with the thiol group) was conjugated with PEG-phospholipid (Mal-PEG-lipid), and the resulting polymeric conjugate was inserted onto the cell membrane [30]. In another study, Won et al. optimized the mesenchymal stem cell (MSC) surface by incorporating recombinant CXCR4 (rCXCR4) protein on the membrane of MSC to improve the homing of MSCs. They incubated a PEG-phospholipid which was conjugated to a rCXCR4 with MSCs at room temperature to bind the stromal-derived factor-1 (SDF-1) on MSC’s surface [21]. Another eloquent study was on the use of lipid-modified hyperbranched polyglycerol (HPG) for generating a coating on stem cell to deliver them to target tissues (Fig. 3) [16]. Bioactive HPGs conjugated with octadecyl chains and vasculature-binding peptides (VBP) were used for directing MSCs to vascular endothelium.
Fig. 2 A schematic view of surface engineering of the islet cells by a multilayered PVA membrane approach [Adapted from reference [26], with the kind permission of Elsevier Ltd.]
2.2 Electrostatic Interactions

By exploiting the charged nature of cell membrane, various electrostatic methods were explored to engineer the cell surface. Over the two past decades, layer-by-layer assembly of polyelectrolytes to form polyelectrolyte multilayer (PEM) film, which is formed by alternate assembly of polycations and polyanions, represents a renowned approach for engineering the cell surfaces at the molecular level. Ionic polymers, such as chitosan, poly(allylamine hydrochloride), poly(styrene) sulfate, poly-L-lysine (PLL), and poly(ethyleneimine) (PEI), strongly interact with negatively charged mammalian cell surface and have been widely used for this approach [31]. However, the cytotoxicity of the polycations is one of the main limitations of this approach. To circumvent this issue, several groups have used a spacer between a polycation and the cell surface to avoid the direct contact between the cationic polymer and cell surface [32–34]. Recently, Wilson and co-workers reported a PEG-modified cationic polymers by taking advantage of PEG-dependent conformational changes and biocompatibility (Fig. 4) [22].
In another eloquent work, Brooks and Kizhakkedathu groups reported that neutral polymers can be utilized for cell surface modification. They have decorated hyperbranched polyglycerols (HPGs) with choline phosphate (CP) (neutral zwitter-ionic polymers) to modify the cell surface by electrostatically interacting CP groups with the phosphatidylcholine end group of the cell surface lipids on cell membrane (Fig. 5) [35]. Various groups adopted this methodology to modify the cell surface for different applications [22, 31, 36]. The encapsulation of live cells in polymeric coatings is a versatile approach to modulate or control the response of cells to their environment. The layer-by-layer assembly of non-immunogenic polyelectrolytes is employed here to attenuate or suppress the binding of antibodies to red blood cells (RBCs) and, consequently, decrease their inherent immunogenicity in vitro. The optimized shell was composed of four bilayers of alginate (AL) and chitosan-graft-phosphorylcholine (CH-PC) surrounded by two bilayers of AL and poly-L-lysine-g-polyethylene glycol (PLL-g-PEG).

2.3 Covalent Modification

Although a variety of functional groups are readily available on the cell surface, only a few functional moieties can be used to covalently modify the cell surface proteins due to the extreme complexity and heterogeneity of cell surface. This strategy is involved in a direct chemical reaction of functional groups on the cell surface such as amines, thiols, and carboxylic acids with proteins, polymers, nanoparticles, and
Fig. 5: An illustration represents the structure of polyvalent choline phosphate molecules contained hyperbranched polyglycerol (HPG-CP) and schematic representation of adsorption of HPG-CP on cell membrane. Adapted from reference [35] with the kind permission of Springer Nature.
other small molecules. The most commonly used functionality for cell surface modification is the amine groups originating from the lysine side chains present on cell surface proteins. The most easily and extensively used strategy is the treatment of amine groups on the cell surface with N-hydroxysuccinimide (NHS)-activated macromolecules at nearly physiological conditions. Although cell surface carbohydrates were used for covalent conjugation of small molecules on cell surface [37a], there is limited information available on such methods used for polymer conjugation.

Wilson et al. masked the pancreatic islet surface covalently with functionalized thrombomodulin in order to reduce donor cell-mediated procoagulant and pro-inflammatory responses [37a]. The bifunctional PEG-based linker, with a triphenylphosphine derivative at one end and an NHS-activated carboxylic acid at the other end, was attached to the amine groups of the pancreatic islet surface by an amide coupling strategy. Subsequently, an azido-functionalized thrombomodulin was attached to PEG linker chemoselectively using Staudinger ligation [22]. The obtained surface modification was very effective in increasing the production of activated protein C with a reduction in islet-mediated thrombogenicity [37a]. Recently, Kizhakkedathu and co-workers have used a similar strategy to modify the RBC surface with HPGs and PEGs in order to camouflage the antigens on the RBC surface. The carboxylic acid functionalized HPG molecules are activated with NHS followed by incubation with RBC in phosphate-buffered saline (PBS) (Fig. 6) [37b]. This RBC surface modification provided significantly higher levels of CD47 self-protein accessibility and greater protection of certain antigens on the RBC surface without changing native properties of RBC. These functional RBCs have greater potential for therapeutic delivery applications. Later on, Hsiao et al. used a

![Fig. 6](image-url) Pictorial representation of covalent modification of cell surface with HPG via NHS ester-amine coupling. Reactions were performed under physiological conditions. (Adapted from [37b] with the kind permission of Elsevier Ltd.)
bifunctional PEG molecule to couple the mammalian cell surface with biopolymers such as ss-DNA [38]. They attached maleimide group on one side of the PEG chain, and the other end group of the PEG chain is modified with NHS ester [38]. The activated carboxyl group reacts with amine groups on the cell surface, whereas maleimide group reacts with thiol group on the ss-DNA. This rapid technique is very effective in the modification of different cell lines, such as RBCs, primary T cells, and cardiac myoblasts. This new protocol greatly expands the applicability of DNA adhesion strategies for different applications [38]. Using a similar strategy, Cheng et al. covalently conjugated peptides, with cysteine C-terminus, on MSC’s surface without affecting cell functions [39]. The engineered MSCs exhibited rolling on E-selectin.

The covalent conjugation of cell surface amines with cyanuric acid containing polymers is another commonly used strategy for various cell surface modifications, in particular RBCs and T cells [40–45]. Hashemi-Najafabadi et al. developed a cell surface modification technique to mask the RBCs surface via covalent attachment of m-PEG to the cell membrane [41]. They optimized PEGylation conditions in order to achieve the attenuated immunorecognition of RBCs in both organ transplantation and blood transfusion applications. The cyanuric acid derivative of m-PEG-OH was first synthesized under inert conditions; then, the Rh positive RBCs were suspended with cyanuric acid-PEG derivatives for different time intervals at different pH, temperature, and polymer concentrations. PEGylation, with linear PEG of molecular mass 5 kDa, of RBCs through this approach was quite successful at pH 8.7, temperature 14 °C, and reaction time 30 min. The polymer concentration was varied with molecular weight. The morphology of m-PEG-RBCs was intact, and it was further confirmed by light microscopy and scanning electron microscopy. Using similar approach, Scott and co-workers has shown that the grafting of m-PEG on peripheral blood mononuclear cell surface decreased the antibody recognition of different surface receptors involved in essential cell communication [46].

Connecting cell surface amines with aldehyde containing moieties (macromolecules, drugs) through Schiff base formation is another useful strategy. Tucaresol, a molecule bearing an aldehyde group, is an investigational drug as an immunopotentiator in chronic hepatitis B virus and HIV infections. Chen et al. conjugated the Tucaresol with T-cell surface amines via Schiff base formation to understand its immunoresponse mechanism. However, this method is not widely explored [47–49].

It is known that mammalian cell surface is abundant with free thiol groups from cysteine residues, at least 15 cell surface proteins have thiol groups in either oxidized (disulfide bridges) or reduced form (free thiols). The balance between oxidized and reduced forms of thiol group is dependent on the redox environment of cells. Various research groups have made use of these thiol groups to engineer the cell surface for various biomedical applications, for instance, controlling immune functionality and cell signaling [27, 50]. A significant advantage of this strategy is the plethora of commercially available reagents and linkers. In addition, the fact that covalent linkage between the reduced form of the disulfide group and targeted moieties can be easily tuned by altering the reaction conditions [51, 52]. Maleimide functionalized probes are one of the best option as they are stable, efficient, light insensitive, and show high chemoselectivity.
to cysteine thiol groups [53]. The free thiol groups on cell surface can be chemoselectively conjugated with maleimide-containing nanoparticles, biopolymers, and dyes at neutral conditions (pH 6.5–7.5) to form very stable thioether bridges. This thiolation-directed maleimide chemical approach is notable even bigger target molecules, in size of 100–300 nm [54]. Stephan et al. conjugated the maleimide-containing nanoparticles to thiol groups present on the surface of T cells and HSCs to promote them as promising vectors for targeted cell-mediated drug delivery [54] (Fig. 7).

These strategies are very useful to enhance drug loading on the cell surface [55]. In another report, Nacharaju et al. conjugated PEG-maleimides to the RBC surface to camouflage the RBC surface antigens from antibodies. This methodology worked well for different molecular weights of PEG, and also this linkage is stable at in vivo conditions [56].

The process of biotinylation is a covalent attachment of biotin molecule, a watersoluble vitamin B7, to cell surfaces, biomaterials, small molecules, and macromolecules. Biotinylation reagents are commercially available for chemoselective targeting of specific functional groups including amines, thiols, carboxylic acids, carbohydrates, and carbonyl groups. Researchers took advantage of extremely high-affinity binding between biotin and its binding partners such as avidin, streptavidin, and NeutrAvidin to probe the cell surface or for cell surface modification. In addition, this binding is highly resistant to heat, pH, organic solvents, and other

![Fig. 7 Schematic illustration of covalent attachment of phosphorous lipid maleimide-functionalized nanoparticles with the free thiol group which is linked to the cell membrane proteins at physiological pH. (Adapted from [54] with the kind permission of Springer Nature)](image-url)
denaturing environments. The biotin-avidin interaction ($K_d = 10^{-15}$ M) is widely explored for different biotechnological and biomedical fields [57–59]. Biotinylation of cell surfaces is achieved through a reaction of primary amine groups present on the cell surface with amine-reactive biotin, such as NHS biotin derivatives. Once the cell is labeled with biotin, it can be readily functionalized with a wide range of small molecules, microspheres, polymers, and proteins through a streptavidin bridge [60, 61]. Building on this approach, recently, Dou et al. reported a cell-matrix interaction protocol using diverse types of cells (osteoblastic, human endothelial, and human hepatoma cells) and hydrogels to enhance the cellular adhesion in three-dimensional extracellular matrix which is very important for avoiding cell death. To this end, three-dimensional nanofibrous hydrogel matrix was generated by co-assembly of 1,4-benzyldicarboxamide-based supramolecular gelators and their biotinylated versions [62]. In parallel, the used cells were modified with avidin; the avidin-modified cells were well encapsulated in three-dimensional matrix using the well-known, highly specific avidin-biotin bridge. The adhesive cells showed high cell proliferation rates, and it was confirmed by reverse transcription polymerase chain reactions. The expected cell adhesion was achieved in great amount in comparison with arginylglycylaspartic acid-based adsorption techniques. This approach is appealing more universal and might have potential to expand to other cell lines too (Fig. 8).

**Fig. 8** (a) Generation of molecular structures of three-dimensional nanofibrous networks from co-assembly of supramolecular gelators and biotinylated gelators. (b) Schematic representation of avidin-modified cells. (c) Cell adhesion was observed in three-dimensional networks through the expected avidin-biotin interaction between and fibrous nanomatrix and avidin-modified cells. (Adapted from [62] with the kind permission of American chemical society)
Although the biotinylation strategy is very versatile and widely used for different applications, it has few limitations, for instance, when natural cell surface functionalities are targeted with highly reactive molecules, the cell surface might have over flooded with biotinylated products; this could generate some toxicity [63]. In addition, the protein component of these techniques is of bacterial origin (e.g., streptavidin) which could generate immune reactions. This will be harmful especially for cell surface engineering for in vivo applications. Although covalent immobilizations/linkages of polymers or macromolecules to cell membranes were expected to be stable for chemical degradation for a long time, in few cases these non-covalent modifications disappeared from the cell surface over the time [14].

### 2.4 Modifications Through Nonnative Cell Surface Functional Groups

The other cell modification approach involves the generation of cell surface functional groups that are not normally present on cell surface and utilization of these functional groups for covalent grafting. The ligation of nonnative functional groups on cell surface with exogenous materials is used for different applications such as live cell imaging, cell separation process, and cell-based sensors [64–66]. In the past few years, various attempts have been employed to generate the different functional groups, in particular, carbonyl, azides, and thiol on the cell surface to chemoselectively modify the cell surface [67–70]. Carbonyl groups can easily react with amines and alkoxyamines, resulting in stable bonds at ambient conditions. However, the availability of carbonyl groups on the cell surface is very limited. For many years, aldehyde or ketone groups on cell surface are generated with high specificity and turn out by direct chemical modification or enzymatic treatment or by the metabolic incorporation of desired functional moieties [71]. Sialic acid moieties have a crucial role in cell aggregation and recognition and are found on cell surface glycoproteins, linked to either galactose or N-acetylgalactosamine at the non-reducing terminal positions [69, 72]. Gahmberg et al. used galactose oxidase to selectively oxidize the diol units of the sialic acid on the penultimate sugar molecules of the cell membrane glycoproteins in order to generate aldehyde groups (Fig. 9) [73, 74]. Since these diol units are available on the penultimate sugar molecules of the glycoproteins, another selective chemical or enzymatic treatment is necessary. In this case, the enzyme neuraminidase is used to cleave glycoside linkage between the last two terminal sugar units; it provides better access to the diol groups for the subsequent reaction. Sodium periodate-mediated selective oxidation of terminal diol

Fig. 9 Selective modification of diol units of glycoproteins of cell membrane. (a) A mild sodium periodate oxidation of cell surface proteins generates the aldehyde groups at C-9 positions of the N-acetyl galactose. (b) Neuraminidase enzyme cleaves seductively terminal glycosidic linkages of protein, flowed by galactose oxidase treatment yields aldehydes at C-6 positions of the sugar molecules. (Adapted from [17] with the kind permission of Royal chemical society)
units into aldehydes was known for small molecules, oligosaccharides, and proteins. De Bank et al. demonstrated the generation of aldehyde groups through a mild oxidation of 1,2-diol units of sialic acid residues of living L6 myoblast cells with sodium periodate to induce cell aggregations. This approach showed high cell compatibility and did not show any significant effect on cell morphology [75].

In 2010, Holden et al. also used a similar approach to coat the macrophage surface with polyamidoamine dendrimers through sialic acid modification. The geminal diol units of sialic acid on the cell surface are oxidized with sodium periodate to generate aldehydes, and the cells are dispersed with polymers to form a covalent bond between amine groups of polyamidoamine dendrimers and aldehyde groups on the cell surface. Finally, formed Schiff bases were further converted into stable secondary amine groups using sodium cyanoborohydride [76]. However, the main limitation of this approach is that the reactive groups must be generated prior to the covalent grafting of cells with polymers.

The other prominent way of generating carbonyl groups on the cell surface is metabolic approach. Carbohydrate chains on the cell membrane are very important for most of the cell communications and cell functions [77]. Tagging of specific functionalities on carbohydrates or incorporation of unnatural carbohydrates into live cells surface, followed by surface engineering with polymers would be a viable approach to control the cell functions. Bertozzi research group is pioneered in introducing unnatural functional groups on the live cells membrane, for instance, acetyl, levulinoyl, and azidoacetyl groups, through glycosylation method [78, 79]. Taking advantage of this approach, Akiyoshi and co-workers demonstrated a new cell surface engineering strategy by attaching biomimetic polymer on cell surface via cell surface tag method (Fig. 10) [80]. To achieve proper surface masking, they developed a synthetic library of biofouling 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, which were modified later with hydrazide functional groups. Metabolic treatment of human promyelocytic leukemia cells with N-levulinoylmannosamine, an unnatural carbohydrate acts as a cell surface tag, generated ketone-containing carbohydrates abundantly on the surface of cells. The N-levulinoylmannosamine-treated human promyelocytic leukemia cells were attached to hydrazide-modified 2-methacryloyloxyethyl phosphorylcholine polymers by selective recognition of a cell surface tag (ketone groups) within 15 min of incubation. The modified cells retained their morphology and showed high cell viability, which is further confirmed by flow cytometry.

Although various enzymatic treatments and metabolic approaches have been also employed to incorporate different functional groups such as biotin, alkyne, azide, thiol, and ketones into live cells surfaces, these technologies might influence cell physiology in the long run [81, 82].

2.5 Grafting from Strategies

Surface-initiated polymerizations such as atom transfer radical polymerization and reversible addition fragmentation chain-transfer polymerization have intensely been
studied for different applications including generating non-biofouling surfaces, cell-selective adhesiveness, and stimuli-response materials [83–86]. Although cell surface with abundance of different functional groups is very attractive for surface-initiated polymerizations, the main challenge is reaction conditions which are very toxic to live cells. In contrast to traditional cell surface modifications, grafting of synthetic polymers on cell surface also offer a few advantages, especially in terms of changing the physical properties of modified surface and increasing the functional groups for secondary interactions, and provide opportunities in the generation of cell-polymer hybrid structures [87]. Recently Niu et al. modified cell surfaces (human Jurkat cells and yeast cells) by a novel “grafting from approach”; they generated the polymers on the cell surface by utilizing covalently linked -NH₂ groups on the yeast cell surface proteins as initiators for visible light-mediated photoinduced electron transfer-reversible addition fragmentation chain-transfer polymerization (PET-RAFT) [88]. The cell surface amine groups were conjugated with the dibenzocyclooctyl-based activated ester by an amidation reaction. Then, the highly strained cyclooctyl groups on the cell surface were conjugated with azide-containing chain transfer agent,
(2-(butylthiocarbonothioyl) propionic acid), through a copper-free, strain-promoted azide-alkyne cycloaddition. Next, cell surface-initiated PET-RAFT was conducted on yeast cell surface, by adding eosin Y (triethanolamine) as a catalyst and methoxy-PEG acrylamide-1k (PEGA-1k) as a monomer in PBS buffer (pH 7.4) with the aid of a light-emitting diode source (465 nm) to achieve the polymer-grafted yeast cells (Fig. 11).

Cell viability and proliferation assays were confirmed that high cell viability and compatibility of this modification approach and the density of polymer chains on the cell surface was confirmed by different characterization techniques. From a synthetic prospective, this PET-RAFT process is highly controlled and generated very finely distributed polymers on the cell surface and no polymer formation was observed inside of the cells. However, the similar methodology did not work well for mammalian cell surface since mammalian cells lack a cell wall which protects the cells from unwanted cell stress conditions. Therefore, Niu et al. used a slightly modified chain transfer agent bearing a lipid-type molecule to insert the chain transfer agent non-covalently on the Jurkat cell membrane, and then polymerization process took place as described previously (Fig. 12). Overall, these approaches helped in increasing the number of grafted chains directly on the cell surface when compared with traditional polymer grafting strategies and can also be used to incorporate a wide range of functional groups for post-polymerization functionalization of the cell surface. The chemical reaction is completed in less than 5 min.

Surface-initiated atom transfer radical polymerization, for instance, on solid surfaces, nanoparticles, and metallic surfaces, has been extensively studied. Taking advantage of this, recently Kim et al. reported a yeast cell surface modification with synthetic polymers (“grafting from” approach) using highly cytocompatible surface-initiated activator regenerated by electron transfer, atom transfer radical polymerization (SI-ARGET-ATRP) [89]. They coated the cell surface with polydopamine by dispersing the cells in a solution containing dopamine-bearing ATRP initiator. The polydopamine-coated cell surface was treated with ARGET-ATRP solution for different time intervals to optimize the density of polymer chains on the cell surface. Such polymer-live cells hybrid structures have enormous potential for different applications and might serve as cell-based sensors, biomotors, and diagnostics [83, 90, 91].

3 Cell Surface Modification of Different Cell Types and Applications

The reasoning behind the cell surface engineering is to bring new advances in cell therapies which would eventually lead to control the fate and function of therapeutic cells. Engineered cells will be potentially used for their enhanced survival, proliferation, or differentiated function. In the following section, we will detail various cell surface modification methods applied to different cell types and applications associated with. Our main focus will be on RBCs, stem cells (multipotent and pluripotent), islet cells, endothelial cells, lymphocytes and splenocytes, and hepatocytes.
Fig. 11 A scheme showing yeast cell surface modification by “graft from approach.” The chain transfer agents are linked to amine groups on the cell surface followed by polymerization of different monomers by PET-RAFT. (Adapted from [88] with the kind permission of Springer Nature)
Since cell surface engineering has emerged as a promising method for applications such as tissue replacement, regenerative medicine, transfusion, and transplantation medicine, the aforementioned cell types play key roles in these fields. Such approaches often involve the use of combinations of materials and cells to create functional structures that can be used in place of the original tissue. For instance, stem cells have the unique property of pluripotency, the ability to differentiate into any cell type making them particularly useful in tissue engineering and regenerative medicine. Here, we aim to summarize the benefit of cell surface engineering of these cells and report the current challenges associated with their application.

3.1 Antigen Protected Red Blood Cells

To overcome the challenges in preventing the immunological rejection of donor cells and tissue organs, many biochemical approaches have been designed [39]. Considerable amount of work has been undertaken to generate polymer coating on RBC to mask minor and major antigens on RBC surface for the creation of universal or immunocamouflaged antigen-protected blood donor cells [12, 13, 39, 56, 63, 92–94]. In another application, surface-modified RBCs have been used as a natural drug delivery system to carry therapeutic agents in the vasculature [95]. Cell surface engineering approaches would allow introducing different drugs onto the surface of RBCs for such applications. Figure 13 shows the schematic of common methods in engineering the surface of RBCs.

One of the earlier approaches for modification of RBCs is by covalent grafting of PEG on the surface of RBCs to protect or mask the blood group antigens on the surface
Fig. 13  Schematic representation of a method in engineering the surface of RBCs. A macromolecular crowded approach was shown here.
which was pioneered by Scott and co-workers [46, 92, 96]. The polymer coating on the cell surface acts as a shield to prevent the interaction of antibodies and recognition of cell surface antigens. Scott and co-workers have shown that engineering the surface of RBCs using methoxy-PEG (m-PEG) had decreased anti-blood group antibody binding to human RBCs and the importance of polymer size on the antigen camouflage [46, 92, 96] (Fig. 14). Results from these studies have shown that m-PEG-modified sheep RBC which were transfused into mice resulted in improved survival when compared to the

![Fig. 14](image_url) Immunocamouflage of membrane antigens is a function of linker chemistry, polymer size, and polymer surface density. (a) Shown is a graphical representation of the RBC membrane and the topical distribution of the Rh (C/c), Kidd (Jk\(^{a/b}\)), and MNS (S/s) blood group antigens. The PEG exclusion layer is the physical entity which gives rise to the immunocamouflage of the membrane antigens. (b) Influence of polymer size of Rhc antigen camouflage. (c) Influence of polymer size on immunocamouflage of the MNS and Kidd blood group antigens. The Rh antigens are located close to the membrane surface; consequently even relatively short polymers (e.g., 5 kDa) can effectively camouflage these sites, and however, MNS and Kidd blood group antigens extend far from the cell surface needs large molecular weight polymer. (Adapted from [46b] with the kind permission of Elsevier Ltd)
untreated RBC. This immunocamouflaged strategy has shown to drastically reduce the immunogenicity of the foreign cells and tissues. For instance, Bradley et al. have shown that PEG modification significantly could attenuate RBC immunogenicity while maintaining normal morphology and function. Moreover, PEG-modified RBCs have shown normal in vivo survival in murine models [92]. More importantly, Scott’s group has shown that engineering the surface of RBCs with Cm-PEG (cyanuric chloride-activated methoxy-PEG) has the potential to prevent alloimmunization in chronically transfusion diseases such as sickle cell anemia and thalassemia [46b]. In addition to these reports, Nacharaju et al. have introduced PEG onto the surface of RBCs by thiolation-mediated chemistry with maleimidophenyl-PEG (Mal-Phe-PEG) with different molecular weights [56]. Using this approach, they could successfully mask the antigenicity of group A Rh(D)+ and B Rh(D)+ to universal blood donor cells (group O Rh(D)-), confirming the masking of these antigens [56].

Due to the issues related to the antibody generation associated with PEG [46, 92, 96], our group has extensively explored the RBC surface engineering using a novel branched polymer and hyperbranched polyglycerol (HPG) [12, 13, 37, 63, 93, 97], which has shown similar or better biocompatibility than PEG. Chapanian et al. developed antigen-protected RBCs by grafting succinimidyl succinate (SS) group modified onto the surface of RBCs [63]. HPG modification resulted in significant reduction in binding of blood group antibodies to cell surface of engineered RBCs compared to control RBCs [63]. In another study, Chapanian et al. investigated the in vivo circulation of grafted RBCs with HPG in mice and showed a normal circulation behavior for RBCs modified with HPG of selected molecular weights and graft concentration (Fig. 15) [12]. The molecular weight of HPG and grafting concentrations are two important parameters that influenced both camouflage of red cell antigens and the viability of modified cells [98]. A comparison of grafting PEG and HPG has shown that HPG-modified cells gave promising results compared to PEG-grafted RBCs due to the compact nature of HPG [94, 98]. Recent studies also highlight the fact the HPG modification on RBC surface can be utilized to modulate the innate immune response to surface-modified cells. Leung et al. have shown that the complement activation on cell surface-modified HPG-dependent on the molecular weight and graft density. Recent studies also tested polymers such as polyethyloxazoline as replacement to PEG [97]; however, the immunocamouflage was lower, but this polymer showed improved RBC morphology. Overall, cell surface engineering of RBCs using covalent grafting of polymers has shown promising results in production of antigen-protected RBCs toward universal blood donor cells. In addition, these methods provide a general working principle for cell surface engineering using simple non-nucleated cells that can be adapted for other type of complex cell types.

3.2 Lymphocytes and Splenocytes

Due to the complexity of human immune system and antigenic diversity of the human cells, rejection of biomaterial, foreign tissues, and donor organs is still a great
Fig. 15 Blood circulation of HPG-grafted RBCs in mice at different graft concentrations. (a) HPG 20 kDa, (b) HPG 60 kDa. (Adapted from [13] with the kind permission of Elsevier Ltd). Polymer concentration played a significant role in tuning the blood circulation times of grafted RBCs.
challenge in translational medicine. This questions the long-term compatibility of transplanted tissues and quality of the recipient’s life. Especially, graft-versus-host disease causes significant morbidity and mortality among the transplanted patients. Although few pharmacologic agents, for instance, azathioprine and methotrexate, have been successfully used to inhibit T-cell activation, these drugs are highly toxic to the kidney, liver, and gastrointestinal glands [99–101]. Surface engineering of the cells with biomaterials may provide a viable solution to reduce the rejection of allografts. Scott and co-workers examined the covalently m-PEG (5 kDa)-grafted allogeneic lymphocytes in minimizing of allore cognition necessary for T-cell activation and graft-versus-host disease both in vitro and in vivo in murine models. The masking of cell surface dramatically attenuated allore cognition of cells, and it was evident by dramatic differences in T-cell proliferation between unmodified and m-PEG-modified versions in both one- and two-way mixed lymphocyte reactions and flow cytometric analysis. Further, the in vivo murine models, graft-versus-host disease is induced in immunocompetent as well as immunocompromised mice via transfusion of allogeneic splenocytes which are collected from murine major histocompatibility complex disparate mice, further validate the effect of m-PEG derivatization in attenuation of allore cognition and subsequent reduction of the risk of graft-versus-host disease in mice [40].

The grafting density and depth of m-PEG brushes on cell membrane are vital in controlling the efficacy of immunocamouflage of the grafted cells [21]. The density of polymer brush border on the cell membrane is highly dependent on linker chemistry, molecular weight, and the concentration of m-PEG derivatives that are being used. In order to achieve the optimum immunological efficacy of polymer grafting, Chen et al. elaborated their studies to understand the role of linker in m-PEG grafting on murine splenocytes. Three different modifying chemistries and PEG derivatives (cyanuric chloride m-PEG (Cm-PEG), m-PEG-1-benzotriazolyl carbonate (BTCm-PEG) and tresylchloride m-PEG (Tm-PEG)) were used, and the murine splenocytes were modified with the 0–5 mM m-PEGs at pH 8.0 (Table 1) [102]. Flow cytometric analysis of leukocyte markers and mixed lymphocyte reactions demonstrated that both Cm-PEG and BTCm-PEG were highly efficient at camouflaging cell surface markers, while Tm-PEG was ineffective. Furthermore, Cm-PEG and BTCm-PEG significantly blocked mixed lymphocyte reactions allore cognition and cellular proliferation. The length of the polymer chain is highly detrimental in these studies; immunocamouflage of cells with BTCm-PEG-2 (20 kDa) was very effective than other counter parts.

Immunoquiescence, a state of low baseline immune activation, is another parameter to evaluate the efficiency of immunocamouflaged cell surface. Wang et al. studied whether m-PEG-modified allogeneic human peripheral blood mononuclear cells (PBMC) or murine splenocytes can produce immunoquiescence or tolerance in both in vitro and murine in vivo models [103]. Lymphocyte proliferation, differentiation, and cytokine production was verified in mixed lymphocyte reactions and conditioned media experiments. The studies demonstrated that PEG grafting does not have any significant effect on cell viability and immunomodulatory response and cytokine production, whereas the controls demonstrated significant ($p < 0.001$)
effect on pro-proliferative potential and enhancing interleukin-2, tumor necrosis factor alpha, and interferon gamma levels.

*m*-PEG-grafted donor murine splenocytes showed significant in vivo immuno-suppressive effects in H2-disparate mice. In contrast to unmodified to allogeneic splenocytes, PEG-modified allogeneic splenocytes showed significant increment in Tregs and baseline levels of Th17 lymphocytes. And also, this effect was seen in at least 30 days post challenge and was not reversed by unmodified allogeneic cells (Fig. 16). These studies conformed that the PEGylation of allogeneic lymphocytes induced an immunoquiescent state both in vitro and in vivo in murine models [104].

Although various reports provided the detailed understanding of late events in T-cell activation of allografts such as T-cell proliferation and cytokine secretion, a better understanding of initial triggering events/molecular mechanisms is limited. Scott research group, in their subsequent studies, monitored the initial triggering events by examining the effect of PEGylation of cells in initial cell-cell interactions, changes to activation pathways, and apoptosis [101, 104, 105]. The role of these events in minimizing proliferative response is observed in modified cells during mixed lymphocyte reactions. The *m*-PEG-engineered cells exhibited significant global immunocamouflage of surface proteins of lymphocytes and also minimized interactions with antigen-presenting cells and other intracellular signaling process. And also, the reported PEG approach is nontoxic. Due to the global immunocamouflage of this approach, it overcomes the biological redundancy inherent to surface adhesion, costimulatory, and growth receptors and shows no evidence of systemic toxicity.

<table>
<thead>
<tr>
<th>m-PEG derivatives</th>
<th>Molecular weight (Da)</th>
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<tr>
<td>RCl</td>
<td>5000</td>
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<tr>
<td>Cm-PEG</td>
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</tr>
<tr>
<td>BTCm-PEG-1, BTCm-PEG-2</td>
<td>20,000 (BTCm-PEG-2)</td>
</tr>
<tr>
<td>Tm-PEG</td>
<td>5000</td>
</tr>
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**Table 1** The efficiency of induced immunocamouflage of grafted murine splenocytes was examined using three different modified PEGs and covalent linkages. Two different molecular weights of PEG were used [102].
3.3 Modification of Stem Cells

Stem cells are currently known as one of the most promising candidates for developing novel and clinically translatable cell therapy [106–109]. In particular, focuses on multipotent and pluripotent stem cells (PSCs) have been significantly increasing [108, 109]. PSCs are characterized by immortality – the ability to continuously self-renew – and pluripotency, the ability to differentiate into all somatic cell types [108]. PSCs include both embryonic stem cells (ESCs) and induced PSCs (iPSCs). Both pluripotent and multipotent stem cells can generate the necessary quantities of cells required for transplantation due to their ability to continuously divide. These cells can then be differentiated into desired phenotypes for therapeutic applications. HSC’s transplantation is also known as one of the most commonly used cells for clinical trials [18, 39, 110–112].

Cell surface engineering approaches have been used to modify the cells to provide them with a desired property. Stephan et al. have proposed the conjugation of drug-loaded liposome nanoparticles onto the surface of HSCs [113]. Not only such approach did compensate normal HSC’s function, but they also increased the

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**Fig. 16** The PEGylated splenocytes showed long-term immunomodulatory effects; inhibited the changes in T regulatory (Treg) and Th17 (pro-inflammatory and allorejection) levels consequent to rechallenge with unmodified allogenic cells. As shown in this figure, Treg levels persistently elevated, while Th17 levels remain the same or decreased from that of naive mice even after 30 days transfusion of modified allogenic cells. (Adapted from [103] with the kind permission of Elsevier Ltd)
self-renewal durability of such cells. In addition to self-renewal and differentiation capability of stem cells, one of the current importance of stem cell therapy is the safe and efficient delivery of such cells into the desired tissue without losing their prominent properties such as proliferation or differentiation. For the delivery of stem cells into their desired tissues, the cell surface can be engineered to enhance the homing properties. In particular for MSCs, due to insufficient expression of surface markers, these cells would not present efficient homing properties. Consequently, cell surface modification approaches can play a key role in enhancing and presenting surface ligands onto the cell surface in order to address such issues. For instance, Sarkar et al. presented a promising modification technique in which they modified MSCs with a nanometer-scale polymer containing SLeX which has been found to be present on the surface of leukocytes and regulate the cell rolling of MSCs [107].

Figure 17 presents the schematic of such engineering strategy for enhancing the rolling of MSCs. It has been reported that the modified MSCs not only showed a great homing in vivo but also the MSC phenotypic properties including multi-lineage differentiation have been conserved as well. In another study, Cheng et al. have used a specific peptide conjugation strategy based on peptide-selectin interaction to improve adhesion of such cells onto blood vessels [39]. Zhao et al. have also shown that engineered P- or L-selectin binding nucleic acid onto MSCs made them engaged to inflamed endothelial cells and leukocytes [6]. Their in vitro results showed that such engineered MSCs can be directly captured from the flow stream by selectin surfaces or selectin-expressing cells under flow conditions [39]. Joeng et al. have used HPG to covalently conjugate oligopeptide containing the VHSPNKK sequence into polymer to create a bioactive HPG [16]. The bioactive HPG can be then associated with the surface of MSCs by hydrophobic insertion to further increase their homing properties. Finally, they have shown that the coating MSCs surface with such bioactive polymer significantly enhanced the cellular affinity for the vascular endothelial adhesion molecule which is overexpressed by inflamed blood vessels (Fig. 18) [16].
In another study, improving the homing transplantation of MSCs were carried out by using engineered mRNA-transfected MSCs which highly expressed homing ligands, such as P-selectin glycoprotein ligand-1 (PSGL-1) and sialyl-LewisX (SLeX), leading eventually to enhancing homing of such cells into mouse’s inflamed ear vascular endothelium. Lecy et al. have shown that engineered MSCs with the homing ligands PSGL-1/SLeX via mRNA transfection significantly improved their homing to the mouse bone marrow [110]. Their results confirmed that mRNA-transfected MSCs have enhanced homing to inflamed ear 2 h after injection by 30% compared to native MSCs.

So far, we have explored most studies that investigated the effects of cell membrane modification on regulating the fate of stem cells. Glycans are considered as one of the
most important cellular component of stem cells where they are in charge of cell signaling communications to their exterior environment. Therefore, the glycan engineered using different techniques to stimulate the fate of stem cells into their desired properties accordingly. Such modulation would be mainly controlled by the signaling molecule transmission such as fibroblast growth factor 2 (FGF2), Wnt, and Notch and other lineage-specific signatures such as the stage-specific embryonic antigens (Lewis X, stage-specific embryonic antigens-1, stage-specific embryonic antigens 3 and 4). For instance, Huang et al. have shown that by using synthetic neoproteoglycans (neoPGs) they were successful in engineering the surface of mouse ESCs to further enhance their affinity to bind to FGF2 [114]. FGF2 is widely used for culturing many stem cell types including ESCs and PSCs. Using such synthetic approach, these researchers could remodel the glycocalyx of mouse ESCs which eventually lead to enhance the neural differentiation of such cells. In another study on engineering the glycans of stem cells, Pulisipher et al. have focused on how to regulate the fate of ESCs into neural phenotypes [112]. They have used HaloTag proteins (HTPs) to present heparin sulfate glycosaminoglycan (HS-GAG) anchor onto the membrane of mouse ESCs. It has been shown that remodeling the glycocalyx of ESCs with such strategy could accelerate the self-renewal exit and eventually promote neural lineage commitment and their differentiation into mature neuronal cells. Pulisipher et al. showed that, consistent with an accelerated loss of pluripotency, transcription factor NANOG levels in heparin sulfate chloroalkane-treated cells was decreased which then accompanied by a corresponding increase in the neuroectoderm-specific marker SOX1. Overall, bioengineering approaches to regulate the fate of stem cells toward their desired application is becoming very promising for preclinical and clinical researchers in the field.

3.4 Surface Modification of Islets

Pancreatic islets are responsible for regulating the sugar levels in the blood [115–118]. They are a cluster of pancreatic cells that are made up of different cells. Many research groups have published reports highlighting the importance of islet transplantation as an extremely promising therapy for diabetic patients [14, 26, 115–119]. Despite of their clinical promises, minimizing the risk of immune rejection of such cells is one the challenging problems. To address these challenges, cell surface modification has been adopted. Researchers have used amphiphilic PEG-lipid and the biotin/streptavidin reactions to immobilize human embryonic kidney cells 293 (HEK293) on the surface of islets, as shown in Fig. 19 [14, 26]. In another work, Golab et al. have shown that by using biotin polyethylene glycol-N-hydroxysuccinimide (biotin-PEG-NHS), they could successfully attach T cells onto the surface of islet cells without any loss of islets viability and function [115]. Although this technique is very promising, major limitation is that streptavidin is derived from bacteria and is a potent antigen in humans. Therefore other strategies were investigated. Specific DNA pairing with its complementary sequence can be used to control cell-cell interaction. Inserted single-stranded DNA-polyethylene glycol (PEG)-lipid (poly A) on the cell membrane showed a
specific attachment to the complementary DNA-coated cell (poly T) or a glass surface to further improve the function of islet cells.

Teramura and Iwata have reviewed various cell surface engineering methods which can be applied to provide non-recognizable surfaces against the immune system. Such methods have been challenged for immune evasion: PEG23, multi-layered PVA-PEG-lipid24, hyperbranched alginate-poly(amidoamine) dendrimer complex, hyperbranched polyglycerol, complement receptor 1-heparin layer-by-layer assembly, factor H-binding peptide, and apyrase [14, 26].

3.5 Endothelial Cell Engineering

Another important class of cells are endothelial cells which protect the vasculature and provide antithrombotic and anti-inflammatory properties [120]. Surface modification of endothelial cells has been used as a tool to manipulate the properties so that cell adhesion and cell behavior can be altered. One of the earlier studies describing such approaches uses avidin-biotin method (Fig. 20). Studies have shown that high-affinity avidin-biotin binding was successful to bring biotinylated cell surface onto synthetic surfaces and co-adsorbed with avidin and fibronectin [121, 122]. This cell surface modification eventually enhanced the formation of lower affinity integrin-mediated focal adhesions. Engineered biotinylated endothelial cells hold great promise for enhancing the attachment of endothelial surfaces onto the synthetic surfaces especially when the cells are placed in flow conditions. Such methods have been used to enhance the adhesion of intact biochemically viable endothelial cell layer on polymeric vascular grafts [73]. It is reported that the protein-PEG conjugates can be used to prevent the undesirable platelet deposition in the case of
damaged arterial tissues. Deglau et al. further expanded this concept by employing PEG-modified human coronary artery endothelial cells for site-specific targeted delivery in ex vivo conditions [123]. First, the efficiency of targeted delivery to damaged arteries was confirmed using NeutrAvidin-coated polystyrene microspheres. Under arterial shear stress flow conditions, solution of PEG-biotin conjugates was delivered to scrape-damaged bovine carotid arteries that were loaded into the tubular perfusion chamber, followed by NeutrAvidin-coated fluorescently tagged microspheres for 10 min. A very high dense layer of styrene microspheres, almost sixfold higher, was found on NHS-PEG-biotin treated bovine carotid arteries, whereas control arteries showed minimal adhesion. To deliver the endothelial cells, the same experiment was repeated with sequential injections of PEG-biotin, fluorescently tagged NeutrAvidin (as a bridging molecule) and PEG-biotin-modified endothelial cells to scrape-damaged bovine carotid arteries. The damaged endothelium surface was well coated with injected PEG-modified endothelial cells and was confirmed by epi-fluorescence microscopy. Although the demonstrated approach has severe limitations and examined only in vitro conditions, this strategy might ultimately find applications in catheter-based or surgical procedures. Such studies are ongoing and have immense potential for clinical translation. It is worth mentioning that even though avidin-biotin was shown to significantly promote initial endothelial cell adhesion, there is no report on the effects of avidin at longer adhesion times [121].

### 3.6 Hepatocytes Modification

Hepatocyte-based therapies have immense potential to be alternatives to liver transplantations in many liver-related diseases including liver failures and other liver disorders. However, the sufficient grafting of hepatocytes and their viability is highly essential for the success of both hepatocyte-based therapies and liver tissue engineering applications. A detailed understanding of the surface modification/interaction of hepatocytes with biomimetic materials is another crucial factor for the success of this therapeutic approach [25, 125, 126]. To better understand the hepatic cell interactions with biomaterial surface, recently, Kojima et al. coated hepatic cells with poly(lactic acid) through avidin-biotin binding system without losing crucial metabolic functions such as serum protein secretion and metabolic capacity [126]. Initially, hepatic cells were attached with sulfo-NHS-biotin, and these modified cells were grafted on an
avidin-adsorbed flat poly(lactic acid) surface. The adhesion process is completed in less than 10 min. The proliferation of these modified cells was intact and almost comparable with cells cultured in collagen plates.

Hepatic functions of the attached cells including albumin secretion, induction of genes, metabolic capacity, and molecular signaling transfer ability of transmembrane receptor complexes were not compromised. Later, Ohashi and co-workers reported another interesting hepatic cell surface modification approach using PEG-lipid derivatives [25]. Murine primary hepatocyte cell surface was immobilized with different PEG-phospholipid conjugates bearing a fluorophore. All the vital hepatocyte functions such as cell viability, protein secretion ability, gene expression ability, induction of cytochrome P450, and hepatocyte transplantation ability were assessed using different in vitro and in vivo experiments (Fig. 21). This modification process was also tested successfully for engineering of hepatocyte sheets in order to generate ectopic liver tissues for different therapeutic applications. This cell surface modification process might show new avenues to advance hepatocyte-based therapies and drug discovery research.

![Fig. 21](image)

**Fig. 21** In vivo studies of PEG-grafted hepatocytes. (A) Surface engineering of transplanted hepatocytes was assessed by measuring recipient serum human alpha-1 antitrypsin (hA1AT) levels at day 3 and 10. Dotted lines and straight lines indicate the recipient groups that were transplanted with cells via portal vein to liver and under kidney capsules, respectively. FITC-PEG-DMPE-modified hepatocytes were resuspended in Dulbecco’s modified Eagle medium and transplanted into the liver (B) and kidney (C). Histochemical staining of hA1AT of livers (D, E) and kidneys (F, G) were harvested for 10 days after transplantation of control hepatocytes (D, F) and FITC-PEG-DMPE-modified hepatocytes (E, G). (Adapted from reference [25] with the kind permission of Elsevier Ltd)
4 Summary and Future Prospective

The attachment of polymers onto the surface of different cell types opens a new and exciting avenue in the field of cell-based therapy which needs to be further explored in clinical studies. In fact, cell surface engineering enhances the therapeutic potential of cells used for transfusion and transplantation applications. Cell surface engineering explores how manipulation of cell fate can present a dominant innovative technology that will likely find wide applications in cell therapy, tissue engineering, drug delivery, and biosensing/bioimaging.

Here, we have described three major types of modification used for cell surface engineering including hydrophobic insertion, electrostatic, and covalent modification, along with the modifications through nonnative cell surface functional groups, and graft from strategies for most clinically important cells such as RBCs, stem cells, islets, lymphocytes/splenocytes, endothelial cells, and hepatocytes. Although we have highlighted the importance of polymer-based methods, advances in enzyme engineering can enhance the efficiency of cell surface engineering approaches by introducing new biomolecules that are designed for specific targets and applications. In addition to the benefits of enzymatic approaches, metabolic strategies to engineer the cell surface glycans are very promising as well, but considerable progress still needs to be achieved using the various pathways. For instance, unlike enzymatic approaches, which, for example, delete entire saccharides from cell surface proteoglycans [127], metabolic strategies can modify these structures in a manner to engineer the cell surface to eventually regulate the function of cells.

Overall, advances in cell surface engineering approaches, especially in the design of new polymers for modifying the cell surface with specific biological functions combined with either enzymatic approach or metabolic modification, will hold great promises in the fields of bioengineering and transplantation medicine.

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Polymer Scaffolds for Anterior Cruciate Ligament Tissue Engineering

Fiona Serack, Nathaniel Holwell, and Brian G. Amsden

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Abstract

There is a clear clinical need to generate effective tissue engineered anterior cruciate ligaments (ACL) for reconstructive surgery. A variety of polymeric materials, scaffold designs, cell sources, and bioreactor conditions have been explored for this purpose. It is the objective of this chapter to introduce the structure and function of a healthy anterior cruciate ligament, outline the desirable properties of an effective tissue engineering approach, and review the current directions in generating an effective replacement tissue through rationale choice of scaffold design and material, cell source, and culture conditions. While significant progress has been made, and a greater understanding of the challenges involved has been gained, there has yet to be a replacement tissue engineered ACL suitable for clinical translation.

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1 Introduction

The knee is passively stabilized by four ligaments: the lateral collateral, the medial collateral, the anterior cruciate, and the posterior cruciate (Fig. 1). The anterior cruciate ligament (ACL) plays an important role in the stabilization of the knee joint, preventing excessive anterior translation and rotational loads. It is also the most frequently injured ligament of the knee, with an estimated annual incidence of 1 per 1000 Americans [1]. ACL tears are common in athletes, with the highest incidences seen in adolescents playing pivoting sports such as soccer, basketball, volleyball, and skiing; additionally, the incidence rate is 3–5 times higher in women than men [1–5]. Many studies have been undertaken to try and understand the mechanisms involved in ACL injuries, as well as the effects of intrinsic and extrinsic risk factors [2–5]. Several studies have shown that noncontact injuries occur in greater frequency than contact injuries, with noncontact injuries accounting for around 70% of total ACL injuries [4]. Of these noncontact ACL injuries, most occur with the knee approaching full extension, usually in combination with valgus collapse or tibial rotation [4]. While partial tears of the ACL can occur, full ruptures are more common [6, 7]. Unlike the other ligaments of the knee that are capable of self-repair, the ACL does not heal effectively when injury occurs. This lack of healing is believed to be due to the inability of a blood clot to form around the damaged tissue. The ACL resides completely within the synovial capsule of the knee
and is bathed in synovial fluid which prevents clot formation. Without a clot to form a provisional matrix and stabilize the torn ends of the ACL, cells cannot regenerate the damaged tissue [8]. As a result, the afflicted knee joint cannot support the same loads and if left untreated, chondral and meniscal injury occurs and early osteoarthritis sets in [9]. Suture repair of the ACL has been ineffective, with greater than 90% of the patients treated in that manner failing to heal [10, 11]. Surgical reconstruction of the ACL is therefore often required, with an accompanying healthcare cost. Between 100,000–200,000 North Americans annually undergo reconstructive surgery of the ACL, with an associated cost greater than five billion dollars [12].

Conventional reconstruction techniques involve removal of the damaged ACL tissue, and replacement with a tissue capable of withstanding similar loads. Current approaches mainly consist of autografts using the patellar tendon, the hamstring tendon, or the quadriceps tendon [13]. When the patellar tendon is used, portions of the bone are also excised (bone-patellar tendon-bone grafts) to act as insertion sites. Similarly, a quadriceps tendon graft consists of all layers of the tendon, excised with a section of the patella to use as a bone plug [14]. With the hamstring tendon approach, approximately 25 cm is removed from each of the gracilis and semitendinosus tendons, and the tendon segments are folded together to form a strand of quadruple thickness for the replacement graft. Regardless of the graft source, it is typically fixed in place by passing the tissue through holes drilled in the tibia and femur and using suspensory or interference fixation. All three options feature advantages and disadvantages that must be discussed between patient and
surgeon. The bone-patellar tendon-bone allows for bone-to-bone healing which can theoretically result in faster healing, though this method is associated with more donor site morbidity. The hamstring approach minimizes donor site morbidity and the associated anterior knee pain but is recommended for patients that are less active [13]. The quadriceps tendon is less studied as a graft but is comparable to the bone-patellar tendon-bone method with respect to its strengths and weaknesses [15]. Although generally successful, conversion of the tendon autograft to ligamentous tissue is a long process and has the disadvantages of anterior knee pain, decreased range of motion, donor site morbidity, and limited donor tendon availability [9, 16]. Allografts circumvent the disadvantages of donor site morbidity and the need for a secondary surgery, but, could potentially transmit disease, elicit an immunological response, and they lose mechanical strength when sterilized, which may result in a failure to integrate with the host tissue [9, 16]. In the early 1970s, orthopedic surgeons looked for an artificial substitute to mitigate the risks of surgically transmitted infections and diseases from allografts, and donor site morbidity related with autografts [17, 18]. Biomedical companies responded with ACL prostheses made of polymers including polyester, polypropylene, ultra-high molecular weight polyethylene, polyacrylamide, and polytetrafluoroethylene [19]. These implants had disappointing results, with success rates ranging from 30–60%, and many associated problems. A retrospective analysis of various implants demonstrated that the prostheses suffered from abrasion of the textile fibres and that healing inside the synthetic grafts was poorly organized, incomplete, and unpredictable [19].

Limitations with these surgical approaches have led to the exploration of tissue-engineered ligament grafts as a substitute for the conventional replacements. In these approaches, replacement tissue is grown in a bioreactor for subsequent implantation in a patient. A scaffold is used to support the seeding of appropriate cells, and this construct is cultured in a cell-supportive environment that provides the appropriate nutrition and regulatory stimuli such as mechanical forces and growth factors. In response, the cells produce and model the extracellular matrix, and this ideally results in a biomimetic tissue capable of withstanding loads within the knee.

2 Background

2.1 The ACL

To appreciate what is necessary in creating a replacement ACL, knowledge of the structure and composition of a healthy ACL is required. The human ACL ranges in length between 27–32 mm and has a cross-sectional area of 44–57 mm² [20]. It contains blood vessels to supply nutrients and nerves for proprioception. Three distinct tissue regions can be delineated within the ACL: bone, fibrocartilage, and ligament. The bone region is the point of attachment for the ACL at the femoral and tibial condyles; it is semicircular in shape and measures approximately 11–24 mm in diameter. This region is mineralized and inserts into the bone via fibres known as Sharpey fibres. The transition from inflexible bone to the compliant ligament region is mediated by the fibrocartilage region, which acts as a transition zone. The fibrocartilage region is subdivided into two
zones: (i) non-mineralized and (ii) mineralized. The non-mineralized zone is characterized by the presence of chondroblasts and ovoid fibroblasts and has high proteoglycan content, while the mineralized zone consists of hypertrophic fibro-chondrocytes [21–23]. The ligament region contains a sparse population of fibroblasts whose function is to maintain the ECM. The fibroblasts contained in this region are spindle-shaped and make up nearly 20% of the tissue volume. The ECM is composed of 70% w/w water, and the major organic component of the ECM is collagen (~75% dry weight), with <1% (dry weight) elastin, while proteoglycans (e.g., decorin, biglycan), glycoproteins (e.g., tenascin C), and other cells constitute the rest [24]. The principal collagens in the ligament region are types I and III, in a ratio of approximately 9:1, respectively, while tenascin C is present at a ratio of collagen I:tenascin C of 15.7:1 [25]. The collagen molecules are sequentially assembled into microfibrils, subfibrils, and fibrils (1–32 μm in diameter). The fibrils are cross-linked to each other, forming fibre bundles called fascicles (100–250 μm in diameter) (Figs. 2, 3) which in turn group together to form the ligament [24]. The fibrils are aligned along the long axis of the ligament and display a wave-like pattern along their length called crimp. This crimp pattern repeats every 45–60 μm, with an amplitude of 5–10 μm [24]. Crimp plays a biomechanical role at both the cellular and tissue level [12].

2.2 ACL Biomechanics

The ACL is subjected to dynamic cyclic tensile loading in a healthy individual, experiencing tensile loads varying from 67 N to 620 N about 1–2 million times per year, depending on the individual’s degree of activity [26]. The ACL exhibits a characteristic triphasic stress-strain response during uniaxial strain (Fig. 4). At low strains, the unfolding of the crimped fibrils yields a “toe region” of 2–5% (mm/mm) [27, 28]. Larger loads transition to the linear region, where fibrils elongate elastically and the tissue stiffens. As the strain further increases, plastic deformation of the collagen fibrils and ligament damage occur in the failure region, until eventually failure of the bone-ligament-bone complex can occur [12, 29–31]. Typical loads applied to the ACL during daily activities exist within the toe and linear regions [29]. The accepted standard mechanical properties of the human ACL are a Young’s modulus of 65–111 MPa [20, 32], an ultimate tensile strength of 38 MPa [20] and 12.8 N·m for energy absorbed at failure [27].
Tissue engineering combines principles and techniques from the life sciences and engineering to repair or replace damaged and diseased tissues [33]. The development of biomaterials to support tissue repair or growth is an important and significant factor in tissue engineering. Fundamentals within material design, biological
signaling, and bioreactors are combined to create a biomimetic template that promotes tissue repair and/or development similar to healthy tissue. With the design of an engineered ACL, the focus is on material choice and processing methods, scaffold design, cell source, and finally bioreactor conditions. An engineered construct must simultaneously meet design criteria under all these considerations to be successful clinically.

3.1 Cell Source

An important consideration in tissue engineering and biomaterial design is the cell source, as this will ultimately affect the quality and composition of the developed tissue. The ideal cell source should be easily obtainable and in sufficient quantities, proliferate at an appropriate rate, and generate an ECM similar to that of the native ACL.

3.1.1 Fibroblasts

Fibroblasts are the primary cell type in natural ACL tissue, which makes them an obvious starting point for this application. Fibroblasts from different source tissues are phenotypically different and express different levels and types of collagens [21]. Moreover, phenotypic differences of ACL fibroblasts (ACLFs) exist within different regions of the ligament; the proximal and distal portions of the ligament feature ovoid fibroblasts, while the middle region contains spindle-shaped fibroblasts [22, 25]. The use of ACLFs as a cell source for ligament tissue engineering would be ideal, but, they are available in limited quantity and adult ACLFs grow slowly [34]. Nevertheless, a comparison of fibroblasts isolated from the ACL, the medial collateral ligament (MCL), the patellar tendon, and the Achilles tendon of rabbits demonstrated the importance of using ACLFs to generate the appropriate tissue composition; each cell type was seeded onto 3D braided polymer scaffolds and tissue culture polystyrene and cultured in vitro, and while fibroblasts from the patellar and Achilles tendon proliferated faster, the ACLFs had the highest expression of ECM genes specific to the ACL [35].

3.1.2 Stem/Stromal Cells

Adult stem cells, and in particular mesenchymal stem cells (MSCs), have been explored as an alternative cell source for ACL tissue engineering. MSCs can be isolated from a number of soft connective tissues, such as bone marrow, adipose tissue, the periosteum, peripheral blood, muscle, and the synovium [36]. One of the most intensively studied of these is bone marrow-derived MSCs (BMSCs), as they can be readily obtained with minimal potential for long-term tissue damage, they can be expanded rapidly in vitro, produce ECM quickly, and can be differentiated into a fibroblastic phenotype [9, 37]. Of these, the two most intensively studied have been bone marrow-derived MSCs (BMSCs) and adipose-derived stem/stromal cells (ASCs) as they can be readily obtained with minimal potential for long-term tissue damage, they can be expanded rapidly in vitro, produce ECM quickly, and can be
differentiated into a fibroblastic phenotype [9, 37]. However, BMSCs must be harvested under general anesthesia, and low yields of BMSCs are obtained [38]. In contrast, ASCs can be harvested in a much less invasive procedure and in far greater yields [39, 40], can be cultured in vitro for extended periods with stable population doubling, and have a higher proliferation capacity and lower levels of senescence [41]. As such, ASCs have been increasingly examined as a potential cell source for ligament tissue engineering [42].

3.1.3 **Co–Culture**

Nevertheless, neither the use of MSCs nor ACLFs alone have resulted in the generation of an effective replacement ACL tissue. However, recent studies have demonstrated that indirect co-culture of BMSCs with ACLFs increased the expression of collagen I and III and tenascin C by BMSCs, as well as increased their proliferation and total collagen production [43, 44]. Moreover, direct co-culture of BMSCs with other cell types such as endothelial cells [45], tenocytes [46], and chondrocytes [47] can lead to the effective differentiation of the BMSCs towards the co-cultured cell type. This differentiation is considered to be due to the release of regulatory molecules by each cell type at a sequence determined by interactions between the cells themselves. Recently, Canseco et al. reported that direct co-culture of BMSCs with ACLFs on tissue culture polystyrene at a cell ratio of 1:1 resulted in increased expression of the ACL ECM markers collagen I and III and tenascin C [48], demonstrating the potential of this approach for ACL tissue generation. Furthermore, it has recently been shown that ASCs isolated from porcine patellar fat pads directly co-cultured with ACLFs had significantly increased collagen I gene expression, suggesting differentiation of the ASCs towards a fibroblast phenotype [49].

3.2 **Materials**

There are many different biomaterials that can be chosen for tissue engineering applications, but it is important to first consider the criteria for a desirable material. For ACL tissue engineering, the important material characteristics are its biomechanical properties, biocompatibility, the biological activity elicited by the material, and its biodegradability. There are several biomechanical behaviors and properties that must be met. The most commonly analyzed and compared biomechanical properties are the Young’s modulus and maximum load at failure. Values for these properties have been measured in native ACL tissue and are stated in a previous section. These values provide standards against which tissue engineering replacements are measured. The material must provide the scaffold with the resiliency to withstand the dynamic loading conditions that it will experience physiologically. While there are no “bio inert” materials, the influence of the material and its degradation products on the host tissue response when implanted must be minimal. Ideally, the material would also be cell instructive; it should promote cell attachment, proliferation, induce MSC differentiation if necessary, and ensure appropriate cell
morphology and extracellular matrix (ECM) secretion. Many of the scaffolds currently being developed for ACL replacement employ biodegradable polymers. In this regard, the material must degrade in a manner that allows gradual transfer of the applied load from the material to the new tissue being formed as well as cell infiltration into the growing tissue. If material degradation occurs too quickly, the scaffold will not be able to support loading in a bioreactor (vide infra) or in the knee following construct implantation. Alternatively, a material that degrades too slowly will not allow for the ingrowth of newly formed tissue.

Many different polymers have been explored as biomaterials for tissue engineered ACL replacements, including natural (e.g., hyaluronan [37], silk [50, 51], and type I collagen [52–56]) and biodegradable synthetics (e.g., poly(desamino tryrosyl-tryrosine ethyl ester carbonate) [57], poly(L-lactide) (PLL) [35], poly (lactide-co-glycolide) (PLG) [34, 58]). The different polymers have their own respective disadvantages and advantages when compared to the design criteria.

3.2.1 Naturally Derived Polymers

Type I collagen is the primary structural component of the ACL and is therefore a logical material to examine for ACL tissue generation. Type I collagen-based scaffolds have demonstrated excellent cellular adhesion, with ACL fibroblasts adhering and remaining viable both in vitro and in vivo [52, 53]. However, collagen scaffolds show a reduction in mechanical strength during culture and can be completely resorbed in vivo [59]. Collagen scaffolds are therefore crosslinked to increase their mechanical strength, a process that also retards their in vivo degradation. A variety of chemical crosslinkers have been used in this strategy, including glutaraldehyde, epoxides, carbodiimides, azides, and nordihydroguaiaretic acid [60]. Many of these options are not considered viable due to issues with cytotoxicity or reversibility of the crosslinks in vivo [61]. Physical crosslinking techniques such as UV light or dehydrothermal treatment have also been studied; while they have significantly improved mechanical properties, they tend to have deleterious effects on fibroblast interaction with the fibres [62, 63]. Finally, despite the improvement in mechanical properties due to crosslinking, collagen scaffolds have not yet been created that have a modulus equivalent to that of the native ACL.

Naturally derived polysaccharide-based materials, such as hyaluronic acid, chitosan, and alginate, are frequently used as biomaterials for tissue engineering applications. These materials have been shown to promote healing, improve cell-biomaterial interactions due to increased protein adsorption, and promote the secretion of growth factors and ECM proteins [64, 65]. However, they are generally used in the form of hydrogels and are too weak to use as ligament scaffolds on their own. To take advantage of the biological properties of these materials, several groups have used them as coatings on or as components of composites with more mechanically robust materials, demonstrating improved cellular attachment and proliferation, and increased ECM synthesis, when compared to non-coated materials [66–68]. One example that has been used for ligament tissue engineering applications is chitosan. Chitosan displays low immunogenicity, possesses a cationic nature for electrostatic coating onto polymers, and its degradation products do not exhibit any cytotoxicity.
Additionaly, chitosan displays a cell supportive nature as well as reactive amino and hydroxyl groups to allow for post-processing chemical modifications [67, 69]. It may be desirable to prolong or increase the degradation rate of chitosan with a synthetic polymer. One group has shown that the degradation rate of chitosan is dependent on the degree of acetylation and the concentration of the chitosan. They were able to modulate the final degradation from 75% to 55% by changing the acetylation from 98% to 82%, respectively. By coating chitosan or compositing it with a synthetic polymer with a controllable degradation rate, one can endow the synthetic polymer with the cell supportive properties of the natural polymer. Deepthi et al. coated poly(ε-caprolactone) electrospun fibres with hyaluronic acid (HA) and 100–150 kDa, 85% degree of de-acetylation chitosan, reasoning that the coating would initiate the proliferation of the ligament fibroblasts until it degraded, and the underlying fibres would aid in the maturation of the cells. They tested both aligned and random fibre orientations, as well as electrospinning both micron and nano-sized fibres onto the scaffold. The coating had no effect on the mechanical properties of the fibres and exhibited no cytotoxic properties. Upon seeding rabbit fibroblasts onto the aligned fibre scaffolds, the orientation of cell growth matched the alignment of the fibres. However, fewer cells infiltrated the aligned fibre scaffolds. Conversely, more cell infiltration occurred in the randomly oriented fibre scaffold due to the higher porosity. Unfortunately, the randomly oriented fibres failed to achieve the required mechanical properties for ligament tissue engineering. This group concluded that increasing the porosity of the aligned fibre scaffolds would be the best approach [67].

Another naturally derived polymer that has been examined for use in ligament tissue engineering applications is hyaluronic acid. Hyaluronic acid is a mucopolysaccharide found in the interstitial space of joints to bind water. Its main role is to hold cells together in a hydrogel matrix and to serve as a lubricant and shock absorber in joints [70]. It is attractive for ligament tissue engineering since it has been used to increase the rate of rabbit tendon healing and also contributes to reduced inflammation [71]. Wiig et al. investigated whether injecting high molecular weight, 3.6 × 10 [4] Da, hyaluronic acid (HA) would result in repair of the ACL in New Zealand white rabbits. A blind study was performed at 4 and 12 weeks postsurgery to evaluate the healing response of HA compared to saline. Of the 21 rabbits that were injected with HA, 10 displayed a healing response that was graded as completely covered. Conversely, the saline control contained only 1 out of 21 rabbits that was graded as completely covered. Type III collagen levels were significantly higher in the HA-treated rabbits than in the saline-treated controls (13.4 ± 1.1% and 11.0 ± 0.8%, respectively) [64]. This promising preliminary work suggested the use of HA for ACL regeneration as a coating. In a study by Cho et al. [71], a poly (ethylene terephthalate) (PET) artificial ligament was coated with HA and cationic gelatin (CG) to improve the tissue response and cell adhesion. Although PET is a nondegradable polymer, coating it with HA was used in an attempt to prolong the use of the scaffold before ultimate failure. A layer-by-layer polymer deposition approach was used, which utilizes two macromolecules of opposite charges, in this case CG and HA, to form uniform layers on the PET surface. CG has been shown to effectively increase the affinity of cells for polymers [72], therefore making it an
ideal candidate as a component for this layer-by-layer system. However, as PET is a nondegradable polymer and long-term analysis of implanted PET fibres indicated that the fibres began to fray, swell, and separate individually, these changes lead to mechanical abrasion against the bone and eventually a mechanical failure of the PET. Moreover, the collagen deposition onto PET fibres is more akin to randomly oriented scar tissue rather than well-aligned collagen fibrils as seen in native ACL tissue.

Silk has a history of use as a suture material and so has a demonstrated biocompatibility [73]. It is often used in ligament tissue engineering as it is biodegradable and has mechanical properties matching those of the ACL [50]. Originally there was concern about the cytotoxicity of silk from native silkworms, due to the immunological nature of its sericin coating. This issue is mitigated with the use of processed silk, where the coating is removed and the material is considered biocompatible [74]. Silk is a proteolytically degradable material, with a degradation rate dependent on its environment [73, 74]. It degrades very slowly, particularly when compared to hydrolytically degradable synthetic polymers [73]. This rate of degradation may be beneficial or problematic, depending on the rate at which neotissue formation occurs. Silk can be readily modified so as to possess cell recognition sites such as the RGD amino acid sequence to promote cell attachment and proliferation, either chemically [75] or through recombinant techniques [76]. Alternatively, it can be coated with cell supportive proteins such as collagen or gelatin [47, 46, 78, 82]. The eligibility of silk-based scaffolds has been validated by large animal models and clinical trials of silk-based grafts being tested in human knees [77, 78].

3.2.2 Synthetic Polymers

While natural polymers possess the important ability to support cell attachment, growth, and differentiation, they are generally enzymatically degraded with kinetics that are not readily predictable or controlled. Furthermore, they generally possess poor mechanical properties, and there is an inherent variability in these properties. To their advantage, properties of synthetic polymers can be precisely tuned for the desired application. Additionally, the molecular weight of synthetic polymers can be readily controlled during polymerization. Further, synthetic polymers with different characteristics can be blended in different formulations in order to target specific mechanical properties and degradation rates. The poly(α-hydroxy esters) such as poly(L-lactide) (PLL), poly(D,L-lactide) (PDLL), poly(glycolide) (PG), poly(lactide-co-glycolide) (PLG), and poly(ε-caprolactone) (PCL) are hydrolytically degradable polymers that have been widely investigated as tissue engineering scaffolds due to their relative ease of synthesis, history of clinical use, and commercial availability [79]. Their hydrolytic degradation is more readily predictable in vivo than the degradation of natural polymers [80]; however, the acidic degradation products have been implicated in a prolonged inflammatory response following implantation [81]. An inherent disadvantage of these materials is their lack of cell recognition sites. However, cell attachment can be promoted either through covalent attachment of cell adhesive peptides such as RGD or IKVAV [82–84] or through coating with proteins such as fibronectin [85]. Another disadvantage of these
polymers is that, although they can initially have moduli within the range, or greater than that of the ACL, they are readily plasticized by the absorption of water, which occurs within 24 h and causes a notable decrease in modulus [86]. Moreover, they are prone to creep [87] and fatigue failure [88] under dynamic loading. These problems can be addressed by crosslinking the polymer. Crosslinking reduces the rate of water absorption and increases the glass transition temperature of the polymer, reducing the effect of plasticization and increasing resistance to creep and fatigue failure [86]. In this approach, functional groups such as acrylate groups are incorporated into the polymer, either pendant from the backbone or at the ends. A radical initiator is added to the polymer, and crosslinking can be induced either thermally or using UV irradiation.

3.3 Scaffold Design

With respect to ACL regeneration, tissue engineering strategies have invariably utilized polymer scaffolds. In designing these constructs, it is important to not only consider the nature of the material used but also the physical structure of the scaffold. A clinically acceptable engineered ACL tissue graft should possess biological architecture and mechanical properties closely aligned with native ACL to allow for physiologic loading after implantation. However, during the remodeling stage, the tissue formed is disorganized, hypercellular, and has a significantly different crimp pattern than is present in healthy tissue. As a result of this difference, the healing tissue has reduced mechanical strength and is vulnerable to re-injury [89]. The optimal generation of replacement ACL tissues would be achieved if the organization of the collagen fibres in the ECM is maintained through the healing process. Therefore, an effective strategy should provide and maintain the necessary mechanical properties to support daily physiological loading but also needs to facilitate the orientation and alignment of the cells along the direction of applied load. These results can be achieved using a polymer scaffold that has topographical features that regulate the spatial distribution of cell adhesive contacts and the direction of cell spreading. In this context, it is important to produce topographical features at the subcellular level (<10 μm) [90–92]. Most approaches have focused on the preparation of fibrous polymer scaffolds, which provides control over the scaffold topography.

3.3.1 Knit/Braided Scaffolds

In early scaffold designs, polymer scaffolds were either knit or braided into “yarns” that could match the mechanical properties of the ACL; twisted polymer fibres have also been used to make scaffolds, as well as constructs made using a combination of these techniques. Buma et al. [93] worked with a braided poly(dioxanone) scaffold in a goat model that showed promising strength directly after surgery. However, over a period of 6 weeks the polymer scaffold’s strength drastically decreased. Although this scaffold was not appropriate for long-term use as an ACL replacement, the conclusions from its study demonstrated the importance of in vivo efficacy
assessment. A study by Lu et al. [85] looked at a variety of polymers to determine which would be optimal for ACL replacement. The polymers studied consisted of PG (45–50% semi-crystalline), 82:18 (LL:G), 160 kDa PLLG, and 250 kDa PLL. All were coated in fibronectin, to promote cell attachment, and seeded with ACL fibroblasts. The mechanical properties were highly dependent on polymer composition. The PG alone was the strongest material; however, the PLL scaffold possessed mechanical properties most similar to that of native rabbit ACL tissue. The native rabbit ACL tissue has a maximum tensile load of 314 ± 68 N [94] and a tensile modulus of 514 MPa [95] while the PLL scaffold was able to achieve a maximum tensile load of 298 ± 59 N; however, there was no reported tensile modulus. The PLL scaffold also exhibited the least decrease in mechanical properties after 3 days in cell culture media. Furthermore, the PLL and PLLG scaffolds better supported physiological cell morphology after initial cell attachment than did the PG scaffolds. Based on the results with respect to cell attachment morphology, cell proliferation, and overall mechanical and degradation properties, it was determined that out of the polymer compositions studied, PLL was the most suitable for ACL tissue engineering. However, these conclusions were based on observations over a 3 day period, which is not enough to see long-term effects of hydrolytic degradation on the polymer mechanical properties. Surrao et al. investigated the change in mechanical and physical properties of lactide-based polymers over a 6-month period. Their conclusion was that high molecular weight (250 kDa) PLDLA exhibited the least decrease in mechanical properties and hydrolytic degradation over this time period [86]. For ligament tissue engineering, it is clear that material properties must be studied over a longer-time period to ensure their suitability for clinical application.

However, the polymer fibres commonly used in braided scaffolds have diameters ranging from 10s to 100 s of μm, which are greater than that of the ligament fibroblasts (~10 μm). The use of fibre diameters of this scale result in the cells interacting with a planar surface as opposed to a three-dimensional matrix, such as they would in the native tissue. In addition, the use of large fibre diameters and a braided design restricted cell seeding to the outside of the scaffold, which resulted in poor tissue integration into the scaffolds [34, 35, 37, 50]. While these types of scaffolds supported the mechanical loads of the ACL environment, they lacked the cell-supportive microarchitecture necessary to produce an organized ECM. Chen et al. [76] used a scaffold consisting of braided silk fibres with collagen incorporated between the fibres. These scaffolds were seeded with BMSCs, cultured for 5 days, and then implanted into a rabbit medial collateral ligament (MCL) model. At 12 weeks, the newly developed tissue was less organized than the native tissue, significantly weaker, possessing a modulus approximately half of that of native MCL, and had much smaller collagen fibril diameters. Moreover, the MCL model is not indicative of the environment the ACL would experience as the MCL can heal without surgical intervention, while the ACL cannot. In a similar approach, Fan et al. employed a knitted silk mesh seeded with BMSCs which was wrapped around a braided silk cord [77]. The internal silk cord was necessary to provide sufficient mechanical stiffness to the scaffold. BMSCs were seeded onto the construct and cultured overnight prior to implantation in a porcine model. When examined
24 weeks postoperatively, the engineered tissue supported roughly half the load of native ACL, and the modulus was less than half that of native tissue. No quantitative assessment of tissue composition was reported, and the tissue did not possess crimped collagen fibres or a toe region when tested in uniaxial tension.

Freeman et al. combined two scaffold design techniques, fibre twisting and fibre braiding, to achieve biomimetic mechanical properties with a PLLA scaffold [96]. The braiding angle and twisting angle could be adjusted to alter the ultimate tensile strength of the scaffolds, as well as the length of the toe region. Additionally, some combinations of braid and twist angles lead to premature scaffold failure. Optimization of the design resulted in a scaffold with a modulus and ultimate tensile strength that exceeded that of the native tissue, and a toe-region of similar length to native ACL. This design was combined with a PEG diacrylate (PEGDA) hydrogel to improve overall viscoelastic properties. The scaffold supported fibroblast growth but had a significantly higher modulus than native ACL tissue [97]. This supra-physiologic stiffness could cause range of motion difficulties in the knee in vivo use. Further, while the seeded fibroblasts demonstrated cellular activity on the scaffolds, their proliferation did not increase between days 7 and 28, and no analysis of ECM production and composition was performed.

3.3.2 Electrospun Scaffolds
Electrospinning has recently been used to develop scaffolds for ACL tissue engineering. The advantage of electrospinning is that it produces polymer fibres in the nanoscale range. Such nanoscale topography has the potential to improve cellular interactions and infiltration compared to scaffolds with microscale and larger topography. In this technique, the polymer is dissolved in an appropriate solvent system and exposed to a high-voltage field resulting in an electronically charged jet of polymer solution eluting from the syringe. The solvent rapidly evaporates, and small fibres are attracted to the collector which is exposed to either an oppositely charged or grounded electrode. Different types of collectors can be used to influence the architecture of the fibres. Many parameters in the electrospinning process (concentration, voltage, flow rate, distance from needle to collector, etc.) can be optimized to manipulate the physical properties of the resulting fibrous scaffolds, such as porosity and fibre diameter [98]. This process has been demonstrated to work with many different polymer systems and is a very promising technique in ACL tissue engineering.

Electrospun fibres have been combined with other scaffold components to improve mechanical properties and cellular infiltration. Petrigliano et al. developed an electrospun PCL scaffold with laser-cut pores to improve vascularization and cell infiltration into the scaffold [99]. While the introduction of pores improved the cellular response and ECM production in the scaffolds, the load to failure and stiffness of PCL are much lower than native tissue, and it is too weak as a homopolymer to support the physiological loads in the ACL [99]. Thayer et al. developed a modified electrospinning/spraying technique to produce meshes made of PLG or poly(ester urethane) and included PEGDA hydrogel to reduce the hydrophobicity of the scaffold [100]. As cells were directly electrosprayed into the
constructs, cellular infiltration and distribution through the scaffold was improved, but the cells were unable to proliferate and therefore may not be able to produce adequate ECM. Additionally, the inclusion of the hydrogel phase improved the scaffolds’ resilience to cyclic loading but decreased the Young’s modulus far below acceptable values for an ACL replacement. Vaquette et al. developed a scaffold by combining a knitting technique for high tensile strength and aligned electrospun fibres to improve control over cell seeding [101]. Knit sutures were made from either PLG (10:90 L:G) or silk, and the electrospun fibres surrounding the scaffold were composed of PLCL (70:30 L:CL). Promisingly, the composite scaffolds had a Young’s modulus similar in magnitude to that of native ACL, which is generally not seen in materials made solely of electrospun materials. Additionally, BMSCs attached and proliferated on the scaffolds, while secreting collagenous ECM. Nevertheless, the most promising results of this study were only obtained with the scaffolds made of knitted silk sutures. If shorter degradation times are desired for tissue ingrowth, these tests should be repeated using the PLG knit scaffolds as well.

Surrao et al. have recently developed an electrospinning technique that generates nano-/microscale polymer fibres that are uniaxially aligned and that can be induced to crimp along this axis. In this technique, the polymer fibre jet eluted from the needle is collected under tension using a rotating mandrel. Following solvent evaporation, a residual stress remains in the fibres. This stress can be released by either increasing the ambient temperature above that of the glass transition temperature of the polymer or by reducing the glass transition temperature to below ambient temperature using a plasticizer (Fig. 5) [101]. Further, the crimp amplitude and frequency could be tuned by altering the difference between the ambient temperature and the glass transition temperature of the polymer, in both plasticized and non-plasticized fibres (Fig. 6) [101]. Using PLCL fibres, a temperature difference of approximately 10 °C resulted in a crimp amplitude and wavelength of 9 μm and 51 μm, respectively. These values are comparable to reported human native ACL tissue properties, stated above. This technique can theoretically be applied to generate aligned, crimped fibres with any polymer that can be electrospun. To date it has been used with PLCL [102], PLL, PDLL, PLG [86], and a copolymer of lactide and trimethylene carbonate bearing a pendant acrylate group [87]. The crimp pattern was beneficial in generating de novo ACL tissue. Fibroblasts seeded on these scaffolds and cultured under dynamic stretching conditions attached to and aligned along the crimped fibres, proliferated, and synthesized a collagen- and proteoglycan-rich ECM which covered 90% of the fibres by day 14 [102]. The ECM composition was consistent with that of native ACL tissue, and fascicle structures were formed. The improved cellular response with crimped fibres over linear fibres was attributed to generating a maximal cell deformation which triggered appropriate mechanotransduction pathways. Additionally, the Young’s modulus of the resulting constructs more than doubled between weeks 2 and 4 (Fig. 3) [102]. Nevertheless, the modulus was only 33 MPa by week 4, which is less than half that of native ACL, suggesting that longer culture times, or more robust polymer fibres, are necessary to produce a suitable tissue for implantation.
Another promising method to create scaffolds with desired designs is through melt electrowriting. This process is similar to solution electrospinning; however, instead of dissolving the polymer in a solvent, the polymer is melted. One advantage of this process is that it does not involve the use of potentially cytotoxic solvents to dissolve the polymer. Additionally, the whipping action that results from the
destabilization of the Taylor cone is eliminated, thus providing a precisely controlled fibre deposition. Melt electrowriting is similar to traditional polymer additive manufacturing techniques. There is a heated chamber that holds the polymer, which is melted and then extruded through an electrically charged needle head. The needle head possesses an opposite charge to the translating collector plate. As the polymer is extruded from the needle head, a Taylor cone is formed as a result of the electrostatic forces overcoming the surface tension. This process creates a concentrated point of charge, forming fibres that are collected by the oppositely charged collecting plate. Much like 3D printing, the stage and the head can be manipulated in the x, y, and z directions to generate complex patterns. In other additive manufacturing techniques, fibre diameters are determined by the diameter of the extruding head [103], whereas melt electrowriting utilizes electrostatic drawing of the fibre to, in theory, consistently produce a fibre with a finer diameter [104].

As noted above, a high level of control over fibre deposition is achievable with melt electrowriting (MEW), including the possibility of creating sinusoidal patterns that mimic the crimp pattern of collagen fibrils in the ACL. When a viscous fluid, such as for example a melted polymer, falls onto a moving belt, a variety of fibre patterns can be formed, including sinusoidal patterns, depending on the speed of the belt and the velocity at which the falling fluid strikes the belt. Brun et al. investigated the processing parameters influencing pattern design and determined that only the ratio of the speed of the belt to a critical speed was needed to vary the pattern [105]. However, their analysis did not incorporate the electrostatic forces that are present in a MEW setup. A setup that included electrostatic forces was performed by Duan et al. in which polyvinylidene fluoride (M_w = 440,000 Da) was dissolved at a concentration of 18% (w/w) in dimethyl formamide to form a highly viscous fluid. These researchers were able to consistently create a sinusoidal meandering pattern by varying the speed of the collector. The wavelength and amplitude of the sinusoidal pattern was modulated from approximately 650–800 μm and 140–360 μm, respectively [106]. These ranges are, however, insufficient to recapitulate the
wavelength and amplitude of the crimp pattern found in the ACL. Moreover, the polymer used in nondegradable and the presence of the organic solvent is problematic for tissue engineering. Recently, MEW using melted 60 kDa PCL has been used to generate similar sinusoidal patterns provided an appropriate collector speed was used (Figs. 7, 8). The deposited fibres had diameters that were too large for ligament tissue engineering, but, with further study and process optimization, the approach may yield a fabrication process that forms crimped fibre scaffolds with high precision.

### 3.3.3 Composite Designs

A composite of two polymers can possess desirable properties from each of its constituent polymers. One such approach has recently been developed for ligament tissue engineering. In this method, an aligned fibrous component mimicked the fibrous collagen while a hydrogel matrix mimicked the proteoglycan-water component. The components chosen included a base-etched PCLDLL fibrous scaffold and a photo-cross-linked methacrylated glycol chitosan (MGC) hydrogel. The fibrous component provided mechanical support while the hydrogel component allowed an even distribution of cells throughout the scaffold. The composite scaffold was also seeded with primary ligament cells. After 4 weeks of culture, the ligament cells were still viable and displayed ligament specific ECM proteins, such as collagen types I, III, and decorin, important for maintaining a ligamentous phenotype. Another advantage of this strategy was that the hydrogel allowed an even distribution of

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**Fig. 7** Schematic of a melt electrowriting (MEW) process. (1) nitrogen-gas pressure-assisted feeding system; (2) print head including polymer reservoir with electrical heating system; (3) high voltage source electrode with advanced temperature control at the spinneret (T2 > T1); (4) computer-assisted planar movable stage; (5) printing head (image provided by Gernot Hochleitner)
the cells throughout the scaffold, while the fibres acted as contact guides for the cells [108].

The introduction of a hydrogel component has been used in several other studies. For example, Dunn et al. [109] used extruded cross-linked collagen fibres (dry diameter 50–70 μm) surrounded by a PLL matrix for rabbit ACL reconstruction. Neo-tissue was observed after 4 weeks in 88% of the implanted scaffolds, but the authors concluded that PLL was not optimal for this application, given that it did not provide facile distribution of the collagen and there was poor bonding between the collagen and the PLL. Gentleman et al. [110] used cross-linked collagen fibres embedded in a fibroblast-seeded collagen hydrogel to create a scaffold to mimic the mechanical and viscoelastic properties of the ACL. Notably, the resulting scaffolds exhibited a toe region in uniaxial tension and the fibroblasts remained viable and accumulated collagen during in vitro culture over 25 days. However, both these scaffold approaches used fibres with diameters larger than that of collagen fibrils to meet the design criteria of ligament-like mechanical properties. Consequently, the cells were interacting with essentially a planar surface and as such were not receiving
appropriate mechanical stimulation. Furthermore, the cross-linked collagen may cause immunologic issues in vivo.

### 3.3.4 Multiphasic Scaffolds

Many of the tissue-engineering approaches taken to date have focused on the mid-ligament region of the ACL, with fixation following the approach used with hamstring tendon grafts. Due to the advantages of bone-tendon-bone autografts with respect to faster healing and improved load transfer, some researchers are looking towards multiphasic scaffolds that incorporate the ACL-to-bone interface. Spalazzi et al. used poly(lactide-co-glycolide) (PLG) of varying compositions and bioactive glass to prepare a triphasic scaffold with regions to support: (A) fibroblast culture and soft tissue formation; (B) co-culture of fibroblasts and osteoblasts for fibrocartilage development; and (C) osteoblast culture and bone formation [111]. The scaffold was able to support distinct zonal distributions of cells and phase-specific extracellular matrix deposition over time, demonstrating the feasibility of developing multiple heterogeneous tissues on a single scaffold. Additional studies with an optimized material demonstrated the ability of the scaffold to form and support distinct cellular and matrix regions within a single scaffold, in vivo [112]. However, only the compressive modulus of the constructs was investigated, which is an inadequate assessment for the midsubstance region of the ligament. Further, the compressive modulus decreased significantly over time, due to degradation of the material, indicating a potential lack of mechanical stability. He et al. used a hybrid silk scaffold and a trilineage co-culture system for in vitro bone-ligament interface regeneration [113]. A knitted silk scaffold had a section coated in hydroxyapatite to improve the osteoconductivity, in order to mimic the mineral component of the ECM. Silk scaffolds were seeded with fibroblasts, BMSCs, and osteoblasts, cultured separately for 1 week, and then sutured together. BMSCs co-cultured in the region between the fibroblasts and osteoblasts differentiated towards the fibrocartilage lineage, while the fibroblasts and osteoblasts maintained their respective phenotypes. This study demonstrated the potential use of tri-lineage co-culture to induce a gradual transition region at the ligament-bone interface on polymer scaffolds. Although the group stated that knit silk scaffolds can be produced with appropriate tensile properties, the co-culture technique must be evaluated on uniform, non-sutured scaffolds before it can be used to successfully sustain loads in vivo.

### 3.4 Bioreactor Conditions

Dynamic culture conditions are necessary for effective neo-tissue formation. The health and remodeling of native ligament tissue is dependent on the mechanical forces and/or deformations that are imposed during routine daily activities [54, 114–116]. Several ligament tissue-engineering studies have demonstrated that cyclic mechanical stretching results in the desired spindle-shaped fibroblast morphology.
and increased fibroblast proliferation, synthesis of collagen types I and III, and growth factor expression [117–119].

The ACL is subjected to cyclic, uniaxial stretching during daily activities and this dynamic loading is instrumental to the architecture of the ligament tissue. The highly organized structure of the ACL is maintained by fibroblasts and a number of studies have shown that in response to cyclic stretching, ligament fibroblasts demonstrated enhanced expression of the ligament-associated genes for collagen types I and III [120, 121] as well as tenascin C [122]. The mechanical stretching is transmitted to the fibroblasts through the ECM via cell membrane mechanosensors. These mechanosensors (such as focal adhesion complexes, discoidin domain receptors, stretch-activated ion channels) react in response to mechanical stimulation by activating signaling cascades that produce ECM components, enzymes, or signaling molecules such as growth factors and cytokines [85, 123]. This interaction is considered essential for the generation and maintenance of an organized ECM with the appropriate mechanical properties to support physiological loading.

As discussed above, the ACL is characterized by a triphasic stress-strain response that is a result of its crimped and aligned collagen fibril microstructure. Therefore, to generate an effective replacement ACL, it is necessary to create a scaffold possessing the characteristic architecture of native ligament to stimulate the appropriate mechanotransduction pathways. However, many of the scaffold materials examined to date possess few cell recognition sites, and so cell attachment, and thus transmission of mechanical signalling, is poor. Scaffold fibre surface modifications have been used to improve cellular adherence, proliferation, and ECM production in comparison to unmodified scaffolds. This has been done, for example, using proteins, such as collagen or fibronectin [85, 123], polysaccharides, such as hyaluronan [124, 125], and peptide sequences, such as the integrin-binding glycine-arginine-glycine-aspartic acid-serine (GRGDS) sequence [126]. Simply coating the fibres with adhesive proteins or peptide ligands can improve cell attachment; however, as the polymer material that makes up the fibres degrades, these signals are lost. Additionally, competitive binding of proteins and other molecules in the serum can displace these signal molecules from the surface. A better approach would be to incorporate adhesive binding sites directly onto the backbone of the polymer used to create the fibres; in this way, fresh peptide ligands will be exposed as the polymer degrades. Moreover, the mechanical forces generated by stretching the polymer fibres will be directly transmitted to the cells.

Of the peptide ligands derived from the ECM, those recognized by integrins have been the most studied with respect to their mechanotransductive action [127]. However, there is a significant lack of understanding of the role of integrins during ligament healing and in the mechanisms of integrin mechanotransduction. What is known is that the α5β1 integrin is up-regulated in ligament fibroblasts in response to cell injury [118, 128] and the expression of this integrin increases significantly when these cells are exposed to dynamic loading in culture [114], suggesting that the receptor for this integrin is important for ligament repair. Furthermore, ACLFs isolated from the midsubstance region of the ACL exhibited stretch-activated collagen gene expression levels that were dependent on integrin αvβ3-mediated cellular
adhesions [129]. This suggests that αvβ3-mediated stretch signal transduction might be effective in stimulating collagen gene expression in human ACLFs. Both the α5β1 and αvβ3 integrins bind to the RGD sequence [130]. MSCs also express these integrins [75], thus, it is reasonable to examine RGD containing peptides as ligands for promoting effective ligament tissue production.

There have been few studies wherein the influence of a peptide binding ligand has been employed to enhance ACLF interactions with a polymer substrate for ligament tissue engineering purposes. Of the studies done to date, all have used an RGD motif. Chen et al. grafted the GRGDS peptide onto aligned silk fibres and examined the influence of the GRGDS on human ACLFs and BMSCs under static culture conditions [75]. The presence of GRGDS improved initial ACLF and BMSC seeding efficiency but did not increase proliferation rates of either cell type. Furthermore, there was not a significant difference in collagen type I production per cell for either cell type after 14 days in the presence or absence of the peptide. In contrast, in a recent study by Wang et al., porcine ACLFs seeded onto GRGDS grafted poly (siloxane) films possessing nanometer-sized aligned grooves produced significantly more collagen type I per cell after 14 days in static culture [131]. Thus, the influence of RGD on MSC differentiation is presently unclear. The difference in the two results may be due to differences in the diameter of the silk fibres (~10–20 μm) versus the grooves (800 nm), the co-culture with MSCs in Chen’s study, or may have been a result of differences in the degree of RGD conjugation. Chen et al. report a GRGDS surface density of 7.2 pmol/cm [2], while Wang et al. did not quantify the surface density of GRGDS. The difference in results could also be influenced by the specific peptide sequence and method of conjugation. It has been shown that the spatial arrangement of RGD ligands is important in the formation of clusters and their effect on spreading and focal adhesions [132–134]; if RGD clustering is not occurring on the scaffolds, it could be responsible for the lack of improvement in cell attachment, proliferation, and ECM production. Additionally, both studies utilized static conditions and as noted above, cyclic loading during culture results in upregulated integrin expression, and so effective cellular responses would only be expected to be seen with dynamic culture conditions.

Although it is established that cyclic loading during culture improves tissue formation (in terms of tissue composition and cell morphology and orientation), there have been few long-term studies examining the influence of the loading conditions on the tissue formed. Noth et al. reported that 1 Hz, 8 h/day stretched to a strain amplitude of 12% and cultured for 14 days, increased collagen I and III gene expression of human MSCs seeded onto collagen scaffolds compared to nonstretched controls [120], while Kreja et al. reported that 1 Hz, 1 h/day, stretched to a strain amplitude of 5% and cultured for 15 days upregulated collagen I and III as well as tenascin C gene expression in human ACLFs seeded on poly(lactide) microfibres [135]. A frequency of 1 Hz was chosen by these researchers to reflect the physiological loading of the ACL [102]. We have shown that bovine fibroblasts seeded onto crimped poly(lactide) fibres and cultured at a strain rate of 10% with a frequency of 1 Hz for 3 h/day over 3 days generated tissue with a collagen to glycosaminoglycan ratio similar to that of native ACL [102]. Moreover, optimal
culture conditions with respect to tissue composition and mechanical properties were obtained upon straining the scaffolds beyond the toe region, and into the linear stress-strain region. The fibroblasts formed collagen bundles that resembled fascicles, a characteristic hierarchical feature of ligaments, and the biochemical composition of the tissue was similar to that of native ACL with respect to the collagen types present (I and III) and the ratio of collagen to sulfated proteoglycans [136]. These findings have recently been confirmed with ligament fibroblasts by another group who have used our crimping technique [136].

### 3.4.1 Decellularized Allografts

An alternative to the more common tissue engineered ligaments to date involves decellularizing native ACL tissue allografts, as has been done with porcine tissue in several studies. This approach aims to maintain the important architecture of the native tissue, in particular the crimped collagen structures, while reducing the possibility of an immunological response from the allograft/xenograft. Additionally, the use of an ACL allograft would better mimic the targeted physical and mechanical properties when compared to the current standard replacement tissue, i.e., tendon auto-/allografts. Portions of the femur and tibia may also be excised with the graft, to aid in fixation of the tissue, similar to current tendon grafts. Ideally, these tissues would be repopulated with fibroblasts from the patient’s own ACL before replacement. Decellularized tissues have successfully been used in many other applications [137–140] and could be translatable to ACL replacements.

Several researchers have examined the feasibility of decellularized allografts for ACL replacements using various decellularization protocols. Woods and Gratzer [141] compared sodium dodecyl sulfate (SDS), Triton X-100, and tributyl phosphate (TnBP) with respect to their effect on the biochemical composition and mechanical properties of a porcine bone-ACL-bone graft. SDS was the most effective at removing cell nuclei and intracellular protein from the graft. However, it also decreased the amount of collagen and GAG in the graft, and increased the stiffness of the ligament. TnBP matched the reduction in cell nuclei but was inconsistent in removing intracellular protein. It had a slight effect on the amount of collagen but did not significantly alter the mechanical properties. Triton was the least effective at cellular extraction but had no significant effect on the mechanical or biochemical properties of the graft. Based on the results of these studies, it was determined that all three protocols warranted further investigation. In another paper, Harrison and Gratzer [142] studied the re-popularization of the grafts using porcine ACL fibroblasts. Again, they compared the three different decellularization protocols and also looked at seeding density, the addition of epidermal growth factor, and culture duration. The Triton- and TnBP-treated scaffolds were more receptive to cellular ingrowth than SDS-treated samples; therefore, the Triton and TnBP decellularized grafts were recommended for further analysis.

Similarly, Vavken et al. [143] studied porcine ACL tissue decellularized using Triton-X, SDS, and trypsin. From their results, there was no significant difference in DNA content remaining after decellularization between protocols, though all were considered highly effective. Triton-X was chosen for further analysis, as it had the
lowest decrease in GAG compared to the intact controls. Grafts decellularized using the Triton-X protocol were then reseeded with human ACL fibroblasts; over 2 weeks, the DNA content remained constant while the amount of procollagen increased significantly. A study by Cartmell et al. [144] involved the decellularization of rabbit patellar tendons using either SDS or TnBP. Almost 70–90% of cells were removed in both protocols, and the mechanical properties of the tendon were conserved. Upon seeding with human dermal fibroblasts, cellular proliferation was reduced in the SDS-treated samples; in comparison the TnBP scaffolds were able to support cellular proliferation for 2 weeks in vitro. While these studies demonstrated the potential of decellularized ACL grafts, further analysis must be completed to determine whether they can remain cell-supportive and mechanically resilient long-term. In vivo studies must also be performed to examine the immunological response to these tissues.

3.4.2 Fixation Methods/Osteointegration

Another important consideration in the design of engineered ACL tissue is ensuring fixation of the graft. With contemporary tendon grafts, the attachment between the tendon and the bone is weakest post-transplantation [145, 146]. This is likely to also be an area of weakness in engineered ligaments, as they often aim to use the same fixation methods; tissue engineered ACL replacements would also benefit from improvements to the osteointegration during healing. Therefore, an emerging research topic is how to improve the osteointegration of the graft after fixation. The incorporation of osteoconductive materials, as well as the addition of different growth factors or stem cells, has been investigated in this regard.

Macarini et al. [147] investigated bioresorbable composite screws, made of PLL and hydroxyapatite, to improve osteointegration at fixation sites for replacements using human tendon grafts. When compared to PLL bone screws, those made with the inclusion of hydroxyapatite demonstrated greater primary stability and superior osteoconductivity. Further, the basic pH of the hydroxyapatite helped to mitigate potential inflammatory issues related to the acidic degradation products of PLL. Cho et al. [71] studied the improvement in osteointegration of a polyethylene terephthalate graft when coated with cationized gelatin and hyaluronic acid. Based on CT scans, the coated grafts caused a reduction in bone tunnel size at the distal femoral and tibial sites. Histologically, the coated grafts were shown to induce new bone formation between the graft and bone at the distal sites after 3 months.

Pan et al. [146] compared injectable biomaterials combined with bone morphogenic protein to study whether they enhanced the osteointegration of grafted tendon in rabbit ACL replacement surgeries. Injected calcium phosphate cement (ICPC) and injected fibrin sealant (IFS), both respectively combined with the protein, were studied using histology, micro-CT imaging, and biomechanical analysis. The IFS had a burst effect on enhancing the healing of the bone-tendon interface, while the ICPC had a more prolonged osteogenic effect, including greater ultimate load at failure. The ICPC composite group was selected for further work, particularly to improve its degradation properties and ideally increase new
bone formation. Sasaki et al. [145] used granulocyte colony-stimulating factor (G-CSF) to enhance the osteointegration of tendon and bone in canine ACL replacement surgeries. A cationic gelatin hydrogel was incorporated at the tendon-bone interface, either with or without the inclusion of the G-CSF. Histological analysis showed that microvessels were enhanced in the G-CSF conditions, while CT imaging showed smaller tibial bone tunnels at 4 weeks with the treatment, and RT-PCR showed heightened levels of vascular endothelial growth factor and osteocalcin. Further, the ultimate failure load at 4 weeks showed a significant increase compared to controls. Together, these results demonstrate that the granulocyte colony-stimulating factor can enhance osteogenesis and angiogenesis, resulting in a strengthened bone-tendon interface. Lim et al. [148] coated tendon grafts with MSCs in a fibrin glue carrier, and compared them to controls using histological and biomechanical analysis. While the control reconstructions showed mature scar tissue at 8 weeks, the treatment group displayed a zone of mature cartilage, transitioning from the tendon to the bone, similar to native tissue. Further, at 8 weeks, the MSC-treated grafts had higher stiffness and ultimate load at failure.

The osteointegration techniques discussed above were predominantly demonstrated using tendon grafts, the current standard in ACL reconstruction surgeries. Ultimately, as more successful scaffolds are developed for the midsubstance region of the ligament, these techniques can be employed to improve scaffold integration at the bone-ligament interface. Improved integration should result in faster healing with better load transfer and improved long-term success of the implant.

4 Conclusions

In a recent survey of British orthopedic surgeons, 86% of the respondents indicate that they would consider using a tissue engineering ACL, if it was biologically and mechanically successful, significantly improved patient outcomes or shortened surgical time [149]. Moreover, 76% of the respondents indicated that the tissue engineering ACL would be a better choice than the presently used autograft tissues. Thus, there is clearly a strong clinical incentive to create a suitable tissue engineered replacement ACL.

As the ACL is a complex tissue, a major difficulty in designing an appropriate polymer scaffold for tissue regeneration is balancing the mechanical and biological properties. While many studies primarily focus on one aspect or the other, it can be appreciated that all the criteria must be considered to develop a functional and clinically relevant engineered replacement. Despite the work done to date, no engineered ACL tissue has the appropriate extracellular architecture or mechanical properties leading to success following in vivo transplantation. This failure is primarily due to two factors: (1) the lack of scaffolds with biomimetic architecture and mechanical properties capable of supporting ligament tissue development in vitro and remodeling in vivo, and (2) the use of ineffective cell sources; neither MSCs nor ACLFs alone have generated appropriate ACL tissue, in vitro or in vivo,
regardless of the scaffold design. The different criteria and techniques discussed need to be further considered and employed to develop a tissue engineered polymer scaffold that can successfully supersede the current ACL replacement techniques.

References

Cell Encapsulation

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Abstract

The task of developing novel techniques for curing human illnesses is really a complex and tough challenge. This chapter gives a comprehensive discussion of various materials and techniques used in cell encapsulation. Cell encapsulation is a technique whereby living cells are entrapped into a selectively permeable polymeric material (membranes/beads) making them a potential tool for the treatment of various human illnesses such as Parkinson’s disease, hemophilia, lysosomal storage disease (LSD), cancer and diabetes. The encapsulated cells become immune, i.e., the immune system of the host could not recognize them; therefore, it does not develop any potential immune response against encapsulated cells. Overall, this chapter reviews a wide range of techniques that could potentially use in cell encapsulation and discuss how the capsule properties are related to the performance of the cell to treat various diseases. Furthermore, the use of different materials and their impact on the properties and performance in cell encapsulation are also discussed in detail.

Keywords

Cell encapsulation · Polymer therapeutics · Immune-isolation · Cytotoxicity · Permeability · Cell technology

1 Prospects of Cell Encapsulation

Entrapping the biological cells in physical polymeric membrane is the heart of medical sciences for treating the ailments that have been a dream of the medical professionals for years. This technique has been introduced as a basic research tool in 1950s to trap the living cells in a nonliving membrane where the cell carries out its normal metabolic activities. In 1964, Chang introduced the term “artificial cell” for encapsulated cells [1]. Cell encapsulation is a technique where the transplanted cells are protected from the host’s immune system or tissue rejection by enveloping the cell in an artificial, partially permeable polymeric membrane which potentially allows the grafting of the cells without using immunosuppressant drugs [2]. This technique allows scientists selectively enter the medicine in the cells through semipermeable membrane. Cell encapsulation has the ability to cure the diseases including lysosomal storage disease (LSD) [3], neurological disorders, e.g., Parkinson’s disease [4].
and dwarfism [5], and genetic disorder, e.g., hemophilia [6], diabetes [7], and cancer based on immune-isolation gene therapy [8, 9].

The encapsulated cell selectively absorb the essential nutrients that are required for its survival based on the differential permeability of the membrane, but the other molecules especially the large sized molecules cannot diffuse through the membrane [10]. Despite the nonliving nature of the membrane, it allows various cellular activities such as cell differentiation, metabolism, proliferation, and cellular morphogenesis while protecting the cell from the host immune system, this concept is called immunoisolation, depicted in Fig. 1. The main incentive in cell encapsulation is to incorporate the cells in the recipient’s body without using the immunosuppressant drugs [11]. These drugs are generally administered to suppress the immune response to the transplanted cells as these cells are crossing the recipient’s immunological barrier. The hindrance in using immunosuppressant drugs is their systemic administration, which would suppress homeostatic immunological physiology of the immune system leading to undesirable consequences including opportunistic infections, and it could adversely affect the incorporated encapsulated cells. Ethically it is not advisable to administer immunosuppressant drugs even to those patients for whom the organ transplant is needed. Therefore, it is the demand of the day to develop such methods which could be applied to the patients and treat disease without medicating the immunosuppressant [12].

Cell encapsulation is a technique in which living cells are enveloped by a nonliving semipermeable membrane in such a way that the cells can grow and express their genes even after encapsulation by the nonliving membrane (Fig. 1) [13]. Since the cells are encapsulated by the biocompatible polymer based nonliving semipermeable membrane, the patient’s immune system is unable to detect the presence of encapsulated cells. Therefore, the key role of cell encapsulation is to

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**Fig. 1** Immunoisolation of a cell encapsulated by hydrogel matrix [13]. (With kind permission of SciTech)
transplant the cells into the host body without any disturbance in the normal homeostasis of the body.

Whenever the donor naked cells are directly enter into the body of the recipient, the immune system of the recipient is triggered, the host immune system activated and releases huge number of defensive cells, e.g., phagocytic cells and lymphocytes in response to the foreign cells. The cells of the host immune system start producing cytotoxic molecules and cytokines that destroy the transplanted cells. The situation intensified especially in those cases where a less number of donors are available for cell donation or the required donor cells are difficult to grow on artificial culture medium. Cell encapsulation provides an adequate solution to these problems faced by organ transplant experts and also helps relieving the patients suffering from serious complications [14]. Various means of cell encapsulation has been used for curing the human illnesses like direct transplantation of the cells. This process implants bovine adrenocortical cells (BACs) encapsulated by alginate and used as a replacement therapy for adrenocortical insufficiency. It is always desirable to implant the immune-isolated foreign cells directly into the patients. However, this technique is limited in its use in clinical applications because of the unavoidable risk of direct contact of the foreign cells to the host immune system. In addition, the degradation of the matrix could lead to the escape of matrix components and develop sensitization to the newly exposed cells [15]. This limitation is now overcome by using the hydrogel that not only protect the implanted cells but also take care of the nutrients entering the cells for their survival. In order to understand the concept of cell encapsulation, a schematic representation of cell-selective encapsulation is given in Fig. 1a. The experimental illustration of hydrogel formation around the cells shown in Fig. 1a is further elaborated in Fig. 1b where the cells are encapsulated in two steps: (i) antibody-antigen interaction for identification of cell and (ii) formation of hydrogel around the cell by a reaction catalyzed by horseradish peroxidase (HRP).

Until now, hydrogels have been prepared from aqueous solution containing HRP of a variety of polymers including natural polysaccharides [16, 17], derivatives of polysaccharides [18, 19], proteins [20], and various synthetic polymers [21, 22].

Numerous polymeric materials are being explored for the encapsulation of therapeutic cells. Synthetic poly(ethylene glycol) (PEG)-based hydrogels are used in which gelation is done by photopolymerization using monomers, photoinitiators, and cross-linkers. Using microwave irradiation, PEG is chemically modified with methacrylic anhydride to get polyethylene glycol dimethacrylate (PEGDM), which was then exposed to 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone photoinitiator in presence of ultraviolet light [23]. Cross linked alginate was used for the first time to encapsulate islets protecting them from immune system when transplanted in the body of the patient [24]. Initially liquid-core encapsulation method and syringe pump extrusion techniques were employed for encapsulating islets, but later on a more matured and modified approach was developed [25]. In this approach, the islets containing alginate droplets are poured into a solution containing calcium chloride for gelation. This technique is found to be applicable to a large number of cells, e.g., dopamine-secreting cells. The cells encapsulated in alginate are found to be stable in in vitro cultures for 4 months and survive in vivo when
transplanted intraperitoneally in T1D human recipients [26, 27]. In spite of strenuous work by the scientists, they encountered some problems with the alginate beads that include nonuniformity, broad size distribution, and permeability of alginate beads. Wolters et al. [27] developed an air jet droplet generator system to address the existing problems of alginate beads. However, it was found that the beads produced by air jet droplet generator develop “tails” that causes immune responses. Moreover, the pressure applied during air jet operation causes damage to the cells. Sometimes air bubbles also get entrapped in alginate beads and hinder the diffusion of vital nutrients that is required for the survival of the cells [28].

Different materials follow different gelation mechanisms for the encapsulation of cells. In case of thermally sensitive gels, gelation mechanism may be controlled by temperature, e.g., gelation of gelatin and agarose. The gelation of photosensitive PEG is controlled by UV light [29]. However, the exposure of light and temperature could damage the cells during encapsulation. Therefore, the ion-based method is preferred in most of the cases for gelation purposes [30].

There are two types of polymers that used in cell encapsulation, namely (i) natural polymers such as alginate, agarose, collagen, and chitosan, and (ii) synthetic polymers such as PEG, poly(lactic-glycolic acid) (PLGA), poly(lactic acid) (PLA), and poly(glycolic acid (PGA). Among all of these polymers used in cell encapsulation, alginate is found to be the best material for applications in biological systems due to its easy crosslinking, biodegradability, and biocompatibility. Yet, alginate has a number of shortcomings that prevent them from using in in vivo experiments. One of the main drawbacks of alginate is cellular overgrowth that might be caused by immune response due to the release of foreign cellular material leaking through alginate membrane or to the exposure of the cells to the membrane due to the breakage of the cellular membrane. The other problems associated with the utilization of alginate capsules are that the alginate capsules are relatively weak, have increased pore size, and dissolve/destroy upon long term exposure to the host immune system. In order to increase the stability and maintain the porosity of the capsule, a polycation layer is usually added to the capsule, but most cases this polycation layer also cause inflammatory reaction to the host.

Another significance aspect of cell encapsulation is the drug delivery from the encapsulated cells. By adopting this system, it is possible to deliver/release the exact amount of drugs in a controlled manner that minimized side effects without repeating its use, which ultimately improve the quality of life [31]. Various types of polymers including both natural and synthetic polymers are being used for slow release of therapeutic agents, proteins, growth factors, and drugs. The drug delivery system was classically based on alginate-poly-l-lysine-alginate (APA) microcapsules. The main disadvantage associated with the APA microcapsules is their biodegradability. In order to address this challenging issue, the thickness of the membrane material must be optimized so that the capsule remains intact for the designated time period of use in the body of the patient. Ma et al. [32] have shown in his work that it is possible to optimize the thickness of the membrane which in turn varies the biodegradability of the APA capsules. For instance, an increase of 0.02% (w/v) to 0.08% (w/v) in the concentration of PLL solution causes an increase of 0.4 μm in membrane thickness of APA capsule.
Conversely, an increase in the pH of PLL solution from 5.8 to 9.2 results in the decrease of the thickness of the membrane from 9.8 to 8.6 μm. Therefore, it is possible to attain required thickness and biocompatibility of APA membrane by changing the specific reaction conditions under which the membrane is synthesized [32].

Research scientists are moving a step forward developing microcapsules with low durability and remarkable biodegradability that includes poly(lactic acid) and its co-polymers with glycolic acid, lactides and glycolides and their copolymers, polyalkyl cyano acrylates, polyanhydrides, and coropol. These polymers have been known to be biodegradable for many years. These newly designed 3D scaffolds without additional coating have the potential for cell delivery to the patient instead of just releasing the therapeutic agents [33]. Therefore, the release of drug is much easier and faster as compared to classical APA microcapsules. The rate of degradation of the microcapsule depends on the number of hydrolyzable functional groups present in the polymer composing the microcapsule wall. The increased number of hydrolyzable functional groups in the polymer backbone, the more will be the rate of degradation. Additionally, in certain circumstances the extremely high rate of degradation is required to release the cells, in these cases completely oxidized alginate-fibrin is used to accelerate the degradative process, e.g., injectable scaffolds in which calcium phosphate cement is used to release the cells quickly [34]. A comparison of degradative process and durability of the microcapsules is depicted in Fig. 2. However, drug delivery as well as cell delivery systems are becoming more and more attractive these days for curing serious illnesses, such as carcinomas especially brain tumors where the release of the cells or therapeutic agents are required directly into the specific cells [35]. In addition, the other encapsulated cells like encapsulated islets have been used as a therapy for curing diabetes saving the lives of a large number of patients across the world [27, 36]. Cell encapsulation could mimic the role played by gene therapy where the encapsulated cells containing gene of interest could be implanted directly into the body of the patient to release the therapeutic agents. Gene therapy is the technique to make up for the faulty genes to cure the illnesses.

Gene therapy can be classified in two ways, i.e., in vivo and ex vivo gene therapy. In vivo gene therapy consists of all the methods of gene therapy whereby the gene of interest is directly transferred into the body of the patient [37]. In vivo technique is preferred when there is no option of isolating and growing the individual cells from the affected part of the body as in case of neural disorders [38]. On the other hand, in case of ex vivo gene therapy, the gene is transferred into the patient-derived cells grown in the artificial culture medium. These cells are then expanded by culturing in vitro and ultimately transferred into the patient where the genes express themselves and make the desired product of interest. This technique is used when the removal of the cells from the body is easier where the cells are genetically modified and engrafted back into the body of the patient [39].

The first use of agarose came on the scene when Nilsson et al. used agarose for encapsulating the islets of Langerhans [40, 41]. They showed that a large number of cells were encapsulated in agarose, and the microcapsules were found to be stable. Unfortunately, this work was not advanced further, but at a later stage it was found that this technique appeared to be potential for the preparation of artificial organs.
such as biohybrid artificial pancreas (BAP) that consists of living cells and biomaterials [42]. Considering the stability, nontoxicity, and mechanical strength, the agarose is found to be suitable for encapsulating the mammalian cells. However, in vivo studies have shown that the effect of the agarose encapsulation decreases on the islets of Langerhans with the passage of time. In an experiment where the agarose encapsulated cells were implanted in the rat for normoglycemic activity, it was found that the activity of these cells has dropped to 50% in 15 days [43]. Although the agarose showed many promising and intriguing properties, there are many issues yet to be resolved concerning the encapsulated agarose islets of Langerhans before considering them for real-time application [44]. As a whole, the success of the encapsulation technique depends on the properties of the encapsulated cells, their performance in the living organism, and the materials used in the matrices.

Fig. 2 A schematic diagram showing a comparison between degradative process and durability of the microcapsules, i.e., both classical APA design and new scaffolds for drug delivery and cell delivery [35]. (With kind permission of Science Direct)
2 Materials for Cell Encapsulation

The materials used in cell encapsulation must be biocompatible and have reasonable mechanical strength to survive handling, implantation, and the mechanical, biochemical, and biological stresses imposed by the host. The capsules membrane must allow the exchange of oxygen, nutrients and metabolites, while obscuring the encapsulated cells from the host’s immune system. In the search for a better encapsulation design, many types of natural and synthetic polymers are being explored. Some of the most popular methods and materials, which have been applied to cell encapsulation, are shown in Table 1.

2.1 PEG-Based Hydrogels

Hydrogels are defined as 3-dimensionally cross-linked network of any profoundly water-soluble polymer. Additionally, hydrogels could be developed in different forms, e.g., slabs, micro- and nanoparticles, in the form a coating or a film, and are used for different clinical applications [68]. Polyethylene glycol (PEG) is a condensation polymer of ethylene oxide and water. PEG-based hydrogels are encapsulation systems that provide biocompatibility, long term stability, and permeability making PEG-based hydrogels ideal for protein release from the cells [45]. As the PEG has limited affinity for the cells and the biological molecules, its affinity can be increased by adding various cell binding peptides. The bioactivity of the PEG-based hydrogels can be increased by the addition of the cell binding peptides and cell modulators [69]. Though PEG is not considerably biodegradable, the different biodegradable components such as polyesters, fumarates, acetals, disulfides, and

<table>
<thead>
<tr>
<th>Methods</th>
<th>Materials</th>
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<tr>
<td>Bulk hydrogel</td>
<td>PEG-based hydrogel</td>
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<td></td>
<td>Photopolymerizable styrenated gelatin</td>
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<td></td>
<td>Silk fibroin</td>
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<td></td>
<td>Photopolymerized elastin-like polypeptide</td>
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<td>Chitosan chondrocytes</td>
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<td></td>
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<td></td>
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<td></td>
<td>6-mannitol crystals with photocrosslinkable MAC</td>
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<td></td>
<td>Alginate di aldehyde (ADA), gelatin, borax</td>
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<tr>
<td>Hollow fibers</td>
<td>Poly(acrylonitrile-vinyl chloride) [PAN-PVC]</td>
<td>[56]</td>
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<td>Chitosan, alginate in PAN-PVC</td>
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<td>Beads</td>
<td>Alginate, gelatin</td>
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<td>Alginate, poly-L-lysine (PLL)</td>
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<td>Agarose</td>
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enzyme sensitive peptides are incorporated into PEG hydrogels enhancing the biodegradability of PEG-based hydrogels [70]. For example, dimethacrylated PEG (PEGDM) and PEG urethane dimethacrylates (PEGUDM) are synthesized by chemical reaction using hydroxyl groups containing PEG chains with methacrylic anhydride and 2-isocyanatoethyl methacrylate, respectively, in presence of tetraethyl amine (TEA). Previously, PEG hydrogels have been synthesized by interfacial photo-polymerization in which PEG was coated around a single cell by a photo-initiator that absorbed onto the surface of the cell and activated by argon ion laser [71]. It is worth to mention that the photo initiated polymerization of hydrogels was proven to be a compatible technique for encapsulation of many cell types [72].

These hydrogels are known to have tunable properties. It is possible to change the structural properties of the hydrogels (e.g., crosslinking density, degradability) without changing the properties of the polymer [73]. PEG is known to be a biocompatible material and helps improving the mechanical strength of other encapsulating materials. Many of the structural properties mainly transport properties of the hydrogels can be easily controlled by changing the concentration and controlling the molecular weight of PEG chains in the initial reaction mixture. In general, the control of the transport properties of the gel is highly critical to maintain the balance of the nutrients and other metabolites for the survival of the cells while protecting the cell from the immune system of the patient [74].

Oligo poly(ethylene glycol) fumarate (OPF) is a polyester that is synthesized by the condensation reaction between PEG and fumaryl chloride in the presence of TEA. OPF was found to be a support for the formation of tissue in bones, cartilages, osteochondral cells, tendons, cardiovascular, ocular, and neural tissue [75]. OPF hydrogels could be used to encapsulate numerous types of cells such as chondrocytes, embryonic and mesenchymal stem cells, and connective tissues like primary tendon fibroblasts. In addition, the OPF hydrogels can also be used to deliver growth factors, DNA plasmids, small molecule, nanoparticles, and chemotherapy agents [76]. The crosslinked OPF prepared by thermal initiator is used as an injectable, biodegradable capsule for transplanting and regenerating the bone tissues. In an experiment, the rat marrow stromal cells (MSCs) were linked with the hydrogel precursors and encapsulated by using OPF solution at a concentration of 14 million cells/mL. These cells were then cultured in vitro in a medium containing osteogenic supplements (dexamethasone). The growth of the cells was monitored continuously and the results obtained at day 7, 21, and 28. It was found that the mineralized matrix is not formed at day 7, but at day 21 the mineralized matrix become visible, and at day 28 the cells have generated mineralized matrix throughout the samples, which clearly indicate that thermally cross-linked OPF hydrogels are highly useful as an injectable cell carrier for bone regeneration [77].

Despite the above advantageous properties, the OPF hydrogels do have certain disadvantages. Firstly, low tensile strength of hydrogel makes their use limited to low load bearing applications, and the softness of the hydrogels can result in dissolution and removal of the hydrogel from the site of implantation. Secondly and most significantly, the problem is the release of drugs from hydrogels particularly in case of nonpolar and hydrophobic drugs, the loading of exact quantity of
drugs while maintaining their homogeneity in hydrogels becomes difficult. Thirdly, rapid release of the drugs from the hydrogels due to high water content and large pore size [78].

2.2 Photopolymerizable Styrenated Gelatin

As we have seen in case of hydrogels, they suffer from the lack of considerable mechanical strength. Therefore, it becomes difficult to keep the cells fixed at a particular target site in the host [79]. Several biomaterials including natural and synthetic polymers have investigated for the improvement in the properties of these materials making them desirable biomaterials for cell encapsulation. An ideal cell encapsulating material should have four desirable properties: (a) it should have fix transplanted cells at the donor site, (b) should be biocompatible, (c) must have moderate biodegradability in order to avoid complications during post-transplantation period allowing gradual replacement of the cells from the body by the newly synthesized tissues, and (d) the biomechanical properties of this material should be similar to those of normal tissues of the host [47, 80]. Unfortunately, such material has not been developed until now, but continuous efforts are being made in this regard to synthesize a new material having these above mentioned properties.

The photo-polymerized hydrogels could be easily crosslinked by means of light-induced polymerization of a monomer or a macromer and achieve desired properties [81]. One of the salient features of photopolymerizable hydrogel is the formation of crosslinked hydrogel around the transplanted cells which could be carried out in situ from injectable solution and makes these hydrogels attractive for use in medicine. A huge number of research studies have been conducted that support the use of photopolymerizable hydrogel as scaffolds for culturing chondrocytes [47, 82, 83]. Accordingly, photopolymerizable styrenated gelatin have been developed that can undergo gelation simply by the photopolymerization induced by the visible light. A schematic representation for the synthesis of photopolymerizable styrenated gelatin is shown in Fig. 3.

The styrenated gelatin is synthesized by the reaction between the hydroxyl group of 4-vinylbenzoic acid and amino group of lysine residue of gelatin. These styrenated gelatin (95 kDa) chains are then used for gelation in two steps. In first step, the camphorquinone (CQ), a photoinitiator, undergoes hydrogens abstraction as it is exposed to irradiation by visible light and produces free radicals, and in the second step of initiation, it combines with styrene group and polymerization takes place [84].

Photopolymerizable styrenated gelatin has many advantages. It can tolerate huge load, show good cell viability, and appear as a viscoelastic biomaterial with elastic modulus of 13.41 kPa. These gelatin hydrogel has a great potential to be used as a sustainable biomaterial for biomedical applications. The significant advantage of the styrenated gelatin is its ability to undergo in situ gelation from injectable solution making it a desirable biomaterial for several clinical applications such as cartilage tissue engineering [85], adipose tissue culturing [86], and anticytokine antibody therapy in surgically restricted area [87]. In spite of the abovementioned advantages,
there is still roam to modify the photopolymerizable styrenated gelatin to enhance the cell viability and deserve optimization of the construction of the matrix while transplanted into the host [36].

2.3 Photopolymerized Elastin-Like Polypeptides (ELPs)

Elastin is a protein that is found in the extracellular matrix especially in connective tissues, i.e., blood vessels, skin, and ligaments [88]. Elastin is synthesized from its soluble precursor protein called tropoelastin, a protein consisting of hydrophobic and hydrophilic crosslinking domains. When tropoelastin is secreted into the extracellular space, it starts crosslinking through lysine residues resulting in the formation of insoluble elastin protein. Keeping the role of elastin in extracellular matrix (ECM),
it is becoming an interesting area in applications of drug delivery and tissue engineering [89]. Elastin-like polypeptides (ELPs) consists of pentapeptide repeats derived from the hydrophobic domains of tropoelastin. The most commonly used hydrophobic repeat in ELPs is \((VPGXG)_n\), where \(X\) represents “guest residue,” i.e., any amino acid other than proline, and \(n\) represents the number of pentapeptide repeats in ELP. ELPs consisting of \((VPGXG)_n\) sequence show a very unique property called inverse temperature phase transition. The ELPs are soluble below or above their transition temperature. Upon demixing of ELPs, they become insoluble in aqueous medium and aggregation of ELPs occurs into its structures called “coacervate” phase [52, 90]. The reversibility of transition temperature could be easily tuned by changing the composition of the guest residues and the length of the ELP chains. In addition to inverse temperature phase transition, ELPs also show response to stimuli and thereby belong to a stimulus responsive class of polymers called “smart polymers” [91]. These polymers show response to the changes in the environment of their solution. ELPs are not only responsive to the changes in temperature but also show response to change in pH, redox trigger, and light. Because of these tunable properties, ELPs are useful biological polymers that show response not only to the temperature but also other environmental factors such as pH and ionic strength. In addition, as ELPs are composed of amino acid sequences, it is possible to control the size of the ELP by controlling the size of the encoding gene [51, 92, 93].

A very precise control over size, stimulus response, and sequence of amino acids in ELPs make them an interesting platform to design drug carries for both local and systemic applications [94]. Tissue engineering of the ELPs allow the incorporation of the amino acids of interest into ELPs which make them targeting protein like a ligand to the receptor on the cell surface and promoting the uptake of the pharmaceutical compound into the cell. Because of the control on number and sequence of amino acids, it is possible to design the drug binding sites in ELPs for conjugation of the drugs or any locating probe [51, 95]. Because their biocompatibility, genetically encoded synthesis, and responsiveness to the stimulus are highly tunable properties, ELPs are becoming extremely attractive as macromolecular carriers of drugs for curing cancer [51]. By precisely exploiting the aforementioned properties, ELPs have been synthesized incorporating different proportion of valine, glycine, and alanine with pentapeptide repeats and transition temperature ranging in between 37 °C and 42 °C which could be obtained by focused ultrasound or microwave on the specific parts of the body. The ELP was then conjugated with rhodamine, injected intravenously, and monitored its location and accumulation providing local and slight hyperthermia to the tumor [51, 96].

ELPs are attractive biomaterials for encapsulating the cells. They are derived from extra cellular matrix (ECM), and they could provide ECM-like environment to the cells and tissues by providing the factors similar to their natural environment [93, 97]. Availability of highly tunable properties such as control of polymer chain length, composition, sequence of amino acids, ease of making different polymer architecture and type, number and location of crosslinking sites makes ELPs highly compatible for cell encapsulation. ELPs form triblock polymer by physical crosslinking in which the central block is of hydrophobic component and two hydrophilic blocks
on two sides of the central block. The hydrophobic portion forms the gel above the transition temperature by hydrophobic interactions, and the strength of these gels depends upon the length of the hydrophobic chain and solvent. Greater hydrophobic content allows higher elastic modulus. However, in some cases much greater strength is required, e.g., load bearing applications in case of cartilage repair, which could be sorted out by chemical crosslinking. Chemical crosslinking of ELPs could be accomplished easily in aqueous medium using hydroxymethylphosphines (HMPs). ELP hydrogel was formed through crosslinking between ELP chains and lysine residues of [tris(hyroxymethyl) phosphine] propionic acid (THPP). The hydroxyl group of THPP reacts with amino group of lysine residue in ELP chain resulting into the formation of hydrogel as a function of pH. The mechanical strength of the hydrogel could be controlled by controlling the number of lysine residues in ELPs chain [98].

2.4 Photopolymerized HA, Collagen, and Laminin

Hyaluronic acid (HA) is a biopolymer discovered by Karl Meyer and capitalized by group of researchers in the vitreous of bovine eye in 1934 [51, 99]. HA is a naturally occurring biopolymer found more abundantly in the connective tissues especially in the joint synovial fluid, vitreous fluid in eye, and umbilical cord [100]. It is naturally synthesized in the cells by special membrane proteins called hyaluronan synthases and is degraded by a group of enzymes called hyaluronidases. HA is composed of a disaccharide repeating unit consisting of D-glucuronic acid and D-N-acetylglucosamine linked together through β-(1-4) linkages [101]. Both sugar units resemble glucose, which in β position allows its bulky groups and anomeric carbon to be at the less sterically hindered equatorial position and smaller atoms like hydrogen to be at less favorable axial position giving HA a conformationally stable structure.

HA is a useful biological molecule. It plays an important role in many biological processes such as cell proliferation, wound healing, morphogenesis, inflammation, and migration [102]. In addition, hyaluronidase is an enzyme that could easily degrade HA to mark HA a biodegradable polymer. Based on these properties, HA could be considered as an excellent fabricating materials that could serve as an extracellular matrix for encapsulating the cells. In recent past, it was found that the photo-crosslinked HA hydrogels are excellent scaffolds for heart valve and cartilage tissues engineering [103].

In a recent work reported by Bae et al., HA hydrogel beads have been utilized for encapsulating bovine articular chondrocyte using a novel technique [104]. HA hydrogel beads were prepared by photopolymerization as shown in Fig. 4. In this process, presynthesized methacrylate-HA was added to the solution containing calcium chloride, triethylamine, alginate, and N-vinylpyrrolidone. 4-Benzoylbenzyltrimethylammonium chloride was used as a photoinitiator. In order to carry out the photopolymerization of methacrylated HA and N-vinylpyrrolidone, long wavelength UV light was used for 60 min in nitrogen atmosphere. The resulting beads were incubated in EDTA solution for a day to extract alginate and then washed extensively with deionized water.
The HA hydrogel beads (Fig. 4) were then used for encapsulation of bovine articular chondrocytes by microinjection technique in which the cells were directly injected into the beads by using a very fine needle with a tip diameter of 248 μm. An injection needle defect was created in the beads which was sealed by adding poly-L-lysine solution to the encapsulated beads to avoid the slipping out of the cells from the beads. The bovine articular chondrocyte encapsulated in this way was able to proliferate and survive in the beads (Fig. 5).

Collagen is another important natural protein of ECM and has been extensively used for encapsulation of cells as extracellular matrix. Collagen can be used in many forms including soluble collagen hydrogel, collagen sponges, fibrillary collagen cross-linked with glutaraldehyde. Collagen hollow fibers and microcapsules have been successfully used to encapsulate the artificial liver to encapsulate hepatocytes [105].
Though the naturally derived collagen has been used in many applications including bone repair, tissue engineering, and drug delivery systems, but it has some disadvantages like difficulty in modifying the sequences of the naturally derived collagen and possibility of immunogenic response [106]. In order to overcome these issues, collagen peptides have been chemically synthesized as an alternative biomaterial with improved properties. Since it is chemically synthesized, it is possible to control the amino acid sequence according to the requirement for providing the controllable materials for matrix engineering [107]. Recently, the covalently crosslinked collagen hydrogel has been synthesized by an 8-arm poly(ethylene glycol) star polymer with collagen-based peptide, which results a viscoelastic thermoresponsive hydrogel in nature. These crosslinked hydrogel is appeared to be with desirable properties such as stiffness and highly cross-linked network of pores where cells could easily reside while turning these hydrogels into promising scaffolds for cell encapsulation [60, 108].

Laminin is also an integral component of the neural tissues ECM that composed of three chains, and found in excess on the inner face of endoneurium where it is found in close contact with the Schwan cells and neurons [109]. The role of laminin in cell encapsulation is found to be of considerable impact with collagen and HA hydrogels. Recently, a study carried out by Suri et al. has shown that 3D interpenetrating network (IPN) hydrogels prepared from collagen and HA were used for encapsulating the Schwan cells [110]. Two types of hydrogels were prepared: one containing both collagen and HA in photocrosslinked form called IPNs and other containing only collagen in photocrosslinked form, and HA fibers are just entangled in the collagen network called semi-INPs. A schematic representation of synthesis of IPNs and SIPNs is given in Fig. 6. The synthesis of IPNs and SIPNs begins by suspending glycidyl methacrylate modified hyaluronic acid (GMHA) and collagen solution in a silicon mold where collagen is allowed to undergo fibrillogenesis at 37°C labeled as (1) in Fig. 6, and this allows the formation of SIPN which upon exposure to UV light results in photocrosslinking of GMHA leading to the formation of full IPN (2). The step (3) illustrates the effect of photo masking which allows photo patterning of the hydrogel leading to IPN and SIPN in the same bulk hydrogel. Both IPN and SIPN were applied as novel devices for neural regeneration therapies. It was found that the Schwan cells surrounded by 3D hydrogels containing laminin were not only able to survive for 2 weeks but also proliferated and carried out their secretary role to enhance the growth of the neurons. Overall, all these three components, namely, collagen, HA, and laminin, could mimic the role of extracellular matrix and could be used to encapsulate the Schwan cells that may serve as a nerve regeneration therapy. It may further enable us to understand the basis of Schwan cell interactions with neurons and different ECM components [111].

2.5 Silk Fibroin

The silk is considered as one of the best biomaterials due to its highly significant properties such as biocompatibility, easy to be chemically modified, gradual rate of in vivo degradation, and its potential to be changed into different forms of
biomaterial either in aqueous solution or organic solvent [112]. The silk is collected from silkworms especially *Bombyx mori*. The raw silk is obtained from cocoons, which have two components, namely, sericin and the core protein called fibroin. The sericin component is removed from the raw material and core fibroin is obtained, which is used for different biological applications. The sericin is composed of soluble glycoproteins. They are sticky in nature. The sericin completely surrounds the core fibroin protein of cocoon filament. Once the sticky sericin is removed, the remaining component is called silk fibroin [41, 113]. This silk fibroin showed great potentials to be used in the synthesis of different biomaterials shown in Fig. 7.

The silk fibroin consists of two chains: high molecular weight (MW) ($M_w \sim 390$ kDa) and low MW ($M_w \sim 26$ kDa). They linked together through disulfide bridges. The silk fibroin is a block copolymer and consists of hydrophobic $\beta$-sheet forming blocks which are linked together through hydrophilic cross linkers [41, 114]. The crystalline portions of silk fibroin consist of glycine-$X$ repeats, where $X$ represents serine, alanine, threonine, or valine amino acids, and in between these repeats they linked with other domains that consist of glycine, alanine, serine, and tyrosine residues. This arrangement of amino acids gives rise to a resiliently strong protein. The strength and toughness of this protein is further enhanced by the presence of $\beta$-sheets [115]. The toughness of silk fibroin is found to be much better than the best synthetic biomaterials, Kevlar. The strength of silk fibroin is even better than
commonly used degradable polymeric biomaterials especially collagen and poly(l-lactic acid) (PLA). A comparison of ultimate tensile strength of collagen (0.9–7.4 MPa) and PLA (28–50 MPa) with that of silk fibroin (740 MPa) shows that silk fibroin is far better in strength than the other two materials used for various applications [41, 116].

The silk fibroin is used for various applications at cell level. It has been found in an in vitro experiment that an aqueous solution of silk fibroin self-assembles and forms β-sheets leading to the formation of hydrogel. This sol-gel transition of silk fibroin is influenced by variation in temperature, pH, and ionic strength [117]. It is also found that the strength of the silk fibroin increases by increasing the concentration of silk fibroin in the aqueous solution. For cell level applications, it is always desirable that gelation may occur at a faster rate, i.e., in hours. However, in case of silk fibroin, the gelation takes place at longer time unless some modifications are executed in the natural silk fibroin. For example, it is seen that in 0.6–15% (w/v) solution of silk fibroin, sol-gel transition occurs in days or even weeks at 37 °C. Addition of salts has shown no influence on reducing the time of gelation, but lowering of pH decreased the time of gelation to few hours. These modifications have some effect on decreasing the time required for gelation, but they could compromise the normal physiological activities of the cell including their viability [45, 118].

In order to resolve this issue, a new method called ultra-sonication has been developed to accelerate the sol-gel transition of silk fibroin in a controlled manner. The gelation could be controlled by changing the power output and sonication time. This technique is based on the gelation by the formation of β-sheets due to change in

Fig. 7 Different formats of biomaterials derived from silk fibroin [112]. (With kind permission of Springer Nature)
the hydration of the hydrophobic component. In order to encapsulate cells or drugs in the silk fibroin hydrogels, the sonication conditions are optimized first without the cells, and the cells are then added before gelation and maintain a homogeneous and favorable medium that is required for cell encapsulation. For example, the cells have been encapsulated by autoclaving 4% (w/v) solution of silk fibroin. In another case, the human mesenchyme cells have been encapsulated by adding 50 μL of cell suspension to the sonicated silk at 37 °C. It is found that low concentration of K⁺ ions and lower pH accelerates the rate of gelation, but the presence of Ca²⁺ ions and higher concentrations of K⁺ ions reduces the rate of gelation. Nevertheless, the cells encapsulated by sonication of silk fibroin retained their physiological activity and proliferation over weeks [45, 119].

2.6 Chitosan

Chitosan is a derivative of chitin. When the deacetylation of chitin reaches to 50%, it becomes soluble in aqueous acidic medium. The reason for solubilization is the protonation of –NH₂ group at C2 of the d-glucosamine repeating unit in chitosan and appeared as a polyelectrolyte in aqueous acidic medium [120]. The degree of deacetylation can be determined by NMR spectroscopy, and the functionality of chitosan could be changed by changing the degree of deacetylation [121]. Chitosan is extensively used in controlled and slow release drugs due to its highly desirable properties that include nontoxicity, ease of biodegradability, and gel forming ability at low pH. Additionally, chitosan usually does not pose any stomach irritation as it has antacid and antiulcer activities; the formulations made from chitosan float and gradually increase in volume in acidic medium [122].

Chitosan has been used as a polymer matrix for cell encapsulation for curing Parkinson’s disease, hepatocytes, human bone marrow cells, and cardiomyocytes [123]. It is commonly used for cells that grow favorably in cationic environment or for biodegradable applications. However, chitosan has not been explored as much as agarose or alginate due to the fact that the chitosan is not easily dissolved in aqueous medium at pH greater than 6 except low molecular weight samples [124]. Nonetheless, chitosan is soluble at very low pH, that is, nonphysiological, and could turn out to be cytotoxic [125]. However, alginate-chitosan matrices have been successfully used for encapsulation and implantation of pancreatic islets in streptozotacin (STZ) induced diabetic mice [126]. But it is still not clear how the toxicity was avoided in this application. Actually, linking alginate with chitosan might improve both its biological and mechanical properties. Alginate and chitosan solution form hydrogel successfully at 37 °C when glycerol salt is added into the solution and has been applied for microencapsulation of insulinoma cells [127]. In certain instances, researchers have used N-acetylated chitosan instead of chitosan owing to its high solubility in the aqueous medium, but solubility decreases with increasing MW of the polymer [128]. The addition of alginate with N-acetylated chitosan resulted into a stable matrix for encapsulating the HPG2 cells that survived for 1 week [129]. Due to low compatibility of the chitosan and the encapsulating cells, it might not be
accepted as a strong candidate for cell encapsulation, but it could be considered as a potential coating material [130].

### 2.7 d-Mannitol Crystals with Photocrosslinkable MAC

Another aspect of considerable importance prioritized while encapsulating the cells especially the neural cells is the porosity of the 3D scaffolds. The size and dimensions of the pore are factors of prime importance because they regulate the transport of oxygen, nutrients, and other cell surviving signals. The materials that produce pores on heating or dissolution are termed as porogen, and these materials are added into the scaffolds to develop porosity [131]. The dissolution could be carried out both in vivo and in vitro to check whether the porogen is biocompatible, does not alter the cell microenvironment, e.g., pH, osmolality, and most importantly it is eliminated from the scaffolds after developing pores. d-Mannitol is an interesting biomaterial in this class as it is biocompatible, easy to dissolve, and nontoxic to the cells, it has been used in bone cement and bone scaffolds [64, 132].

Recently, the methacrylamide chitosan (MAC) hydrogel derived from chitosan is used for Neural Stem/Progenitor cells (NSPCs) as a highly tunable growth matrix that offers differentiation, proliferation, and cell migration. d-Mannitol is used in this study to control the porosity of the hydrogel according to the requirements of the cells [133]. In this technique, MAC hydrogel is chemically synthesized by photopolymerization MAC in the presence of UV light by using IRG-184 (1-Hydroxy-cyclohexyl-phenyl-ketone) photoinitiator for encapsulating NSPCs and then d-mannitol crystals were added at different concentrations to the polymerizing solution. 20 wt% d-mannitol was found to be the best for optimized pores in the 3D scaffolds for NSPCs. Overall, it is found that d-mannitol along with photopolymerized MAC hydrogel is an excellent biomaterial for controlling the porosity of the scaffolds used in neural cell encapsulation [134].

### 2.8 Mixture-Induced Two Component Hydrogels (MITCHs)

A new type of hydrogel called physical hydrogel has been developed by exploiting the physical aspects of the polymer whose viscoelastic properties are tunable by molecular level engineering [135]. Such physical hydrogels usually formed without any external environmental triggers are injectable and highly compatible for use in cell encapsulation and drug delivery systems [136].

The previously known physical hydrogels were synthesized by changing the external environmental stimuli such as pH, temperature, and concentration of cations [137]. The main disadvantage of this physical hydrogel was the mixing of the cells with the precursors in the solution phase in which the cells were momentarily exposed to nonphysiological conditions which was not only detrimental for the cells but also for the proteins inside them. In order to get rid of these issues, a new strategy was developed to exploit the specific molecular interaction between two naturally
occurring peptide domains to form two-component system, which has the ability to hetero-assemble into a hydrogel upon mixing under the constant physiological conditions, called the Mixture-Induced Two Component Hydrogels (MITCHs) [138]. By altering the sequence of amino acids in the primary structure, the binding affinity and the association domains can be tuned, and the physical hydrogels with highly controllable properties can be synthesized [139]. Figure 8 shows that there are two WW domains (CC43 and Nedd4.3) which have the ability to bind the proline-rich peptide (PPxY) mixed with hydrophilic spacers three polymer families are formed, i.e., \( C(x + 2) \), \( N(y + 2) \) and \( P(z + 2) \) [140]. (With kind permission of PMC)

**Fig. 8** There are two WW domains (CC43 and Nedd4.3) which have the ability to bind the proline-rich peptide (PPxY) and after mixing with hydrophilic spacers three polymer families are formed, i.e., \( C(x + 2) \), \( N(y + 2) \) and \( P(z + 2) \) [140]. (With kind permission of PMC)

2.9 ADA, Gelatin, and Borax

It is always desirable that the injectable polymer scaffolds used for cell encapsulation and drug delivery should be biocompatible and biodegradable [143]. The simplest approach is to directly inject the encapsulated cell or drug into the patient. The injectable polymer scaffolds provide ease of application, confined drug delivery for site specific action, better relief, and comfort for the patients [144]. Various injectable scaffolds have been prepared that includes pH sensitive, water soluble, temperature sensitive, and photopolymerizable hydrogels [145]. Hydrogels prepared from naturally derived polymers mimic the role of natural extracellular environment of the cells, and they cause the cell growth, differentiation and cell migration as well as stabilization of the encapsulated cells [146].
Many of these hydrogels are biodegradable and biocompatible, but they have certain disadvantages. These hydrogels are synthesized in situ using different crosslinking agents like enzymatic cross linker, chemical cross linker (such as glutaraldehyde, carbodiimide, adipic dihydrazide), and metal ions [147]. The photopolymerization requires a photosensitizer and longer duration of irradiation which is considered to be a limiting factor. The crosslinking by means of metal ions is reversible in the body and exerts cytotoxic effects [148]. The crosslinking agents that are incorporated into the hydrogel such as glutaraldehyde, polyepoxide, and isocyanate are highly toxic as they are gradually released while hydrogel degrade [149]. There are some crosslinking agents such as acyl azide and carbodiimide, which activates the crosslinking reaction without incorporation into the hydrogel, and are less toxic, but they slow down the crosslinking process [150].

Alginate is an anionic linear polysaccharide derived from brown sea weeds that constituents of 1,4-linked $\beta$-D-mannuronic (M) acid and 1,4-linked $\alpha$-L-guluronic (G) acid residues [55, 151]. A very important feature of alginate is its gelation in the presence of divalent cations, e.g., calcium ions. Alginate has found wide use in cell encapsulation. However, alginate hydrogels degrade in an uncontrolled manner releasing calcium ions, and different sized alginate fragments exposed to the host [152]. High calcium level has also shown to inhibit the growth of cells in culture medium. It has also been found that high molecular weight alginate is not biodegradable, but its di-aldehyde derivative is easily biodegradable [153].

Alginate di-aldehyde (ADA) and gelatin has been used to synthesize injectable, in situ forming, and biodegradable scaffolds in presence of borax. In this hydrogel, both gelatin and borax are found to be biocompatible and safe to use in biomedical applications. Gelatin has a long history of use as a wound dressing material, and borax has long been used as a lethal dose in human beings [154]. The gelation reaction between alginate di-aldehyde and gelation in the presence of borax would take place without any extraneous force or factor like change in pH or temperature. It is further convenient to carry out under constant physiological conditions, and hence, the cells would not be exposed to non-physiological conditions at any stage of cell encapsulation. Furthermore, there is no threat of releasing divalent ions like calcium ions, and therefore, it would not affect the viability of the cells.

The ADA-gelatin system is not only suitable for cell encapsulation but also for controlled release of drugs for curing diseases [155]. Primaquine has been used to conjugate with ADA- gelatin hydrogel and its release was monitored. It was found that the extent of drug release depends upon the degree of gelation. At lower gelation, the release would be faster, but it becomes slower at higher concentration of the gel. Overall, there is much potential in the hydrogels to be used in various biomedical applications including cell encapsulation and controlled release of the drugs according to one’s desire for effectively treating the diseases. Now researchers are trying to make highly efficient systems for cell encapsulation that are biodegradable, nontoxic, injectable, and undergoing gelation under the normal physiological environment of the body to facilitate and provide great relief to the patients.
2.10 PAN-PVC

Poly(acrylonitrile-vinylchloride) (PAN-PVC) is a thermoplastic polymeric material, which is extensively used to fabricate the cell encapsulating hollow fiber membrane (HFM) [56, 156]. The HFM is one of the many important biomaterials originally fabricated for ultrafiltration applications [157]. PAN-PVC-based HFMs are well known for their transport properties allowing sufficient exchange of soluble factors for the viability of cells for extended period of time [158]. Previously an enormous amount of works focused on transport of the biological molecules based on molecular weight cutoff (MWCO) value and the molecular size at which 90% of the molecular species are rejected under hydraulic pressure [159], but it is established that HFMs transport properties influence biomass, proliferation, and controlled release of therapeutic agents from encapsulated cells [160]. HFM prepared from PAN-PVC using various solvents including dimethylformamide (DMF), dimethylacetamide (DMAC), dimethylsulfoxide (DMSO), and different additives and was formed to be different wall architecture. The HFMs formed from DMF, DMAC, and DMSO have finger like macrovoid structure as shown in Fig. 9a–c, whereas the HFMs produced from additives are more symmetric with spongy architecture shown in Fig. 9d, e [161]. When the permeability of these HFMs was compared, it became evident that the HFMs prepared from DMF were more restrictive to the molecular diffusivity, while the HFMs produced from PEC additive were more permeable to the molecules for the growth of the cells. It was found that the permeability of PAN-PVC membrane increases irreversibly by washing with ethanol as compared to water [58].

The PAN-PVC HFM system was also examined for its ability to release the drugs in a controlled manner. This was achieved by encapsulating the PC 12 cells that have the neurosecretory function and have the ability to release dopamine, a neurotransmitter [162]. The cells were cultured for 4 weeks and the effect of different membrane diffusivities was tested. It became very clear from the experiments that the cells were not only viable but also consistently released dopamine under different conditions for 4 weeks. However, it is still unknown that whether this treatment would be stable over a long period of time or not. In addition, the dopamine release also continuously increases as the permeability of the cells increases. The cells encapsulated by PAN-PVC membrane are highly permeable to the solutes from external environment while protecting themselves from immune system [57]. Therefore, by controlling the diffusivity of the PAN-PVC HFM, it is quite possible to make the release of the dopamine much controlled and regulated which indicating the potential of HFM for controlled release drug delivery.

2.11 Polysulfone

Polysulfone is prepared by condensation polymerization of bisphenol A and dichlorodiphenyl sulfone [59, 163]. The material is an amorphous high performance thermoplastic with a glass transition temperature of approximately 190 °C [164].
Polysulfones provide the advantage of preparing semipermeable membranes with developed inner and outer walls [60]. Usually the membranes including polysulfone membranes are prepared by phase inversion process in which a homogeneous polymeric solution is cast onto the support as a thin film and then immersed into a bath solution containing a nonsolvent [165]. The polymeric film solidifies to form a membrane with symmetric or asymmetric structure as a result of exchange between the solvent inside and the nonsolvent outside the cast film. The asymmetric membrane has an outer dense layer and a porous sublayer. These membranes have been widely used in liquid and gas phase separation as the thin top layer acts as a selective barrier and the sublayer which is composed of macrovoids and micropores provides good mechanical support.

The enormous amounts of research have been conducted in the past to understand the principle involved in the formation of membrane by phase inversion process. Chakrabarty et al. have proposed two types of demixing processes that take place during phase inversion, i.e., instantaneous demixing and delayed demixing.
demixing [166]. According to this model, the membranes formed by instantaneous
demixing generally show a highly porous structure with macrovoids and a finely
porous thin skin layer. The membranes formed by delayed demixing show a
macrovoids free and thick, dense layer [167, 168]. In order to get the membrane
with desired porosity and structure, various additives are added during the fabri-
cation of the membranes. Actually, the additives change the properties of the
membrane by changing the phase kinetics or changing the solvent capacity and
the thermodynamic properties of the membrane [169]. Various additive systems
have been identified to play role in membranes like glycerol in a system of
polysulfone, dimethylacetamide (DMAC)/water, maleic acid in a system of cellu-
lose, dioxane/water/polyvinylpyrrolidone in a system of polysulfone [170]. Some
additives have the tendency to form macrovoids, while the others suppress the
tendency to form the macrovoids, thereby increasing the interconnectivities to
increase the porosity of the top and sublayer.

A study conducted by Boom et al. showed that the addition of PVP additive in the
formation of poly(ether sulfone) (PES) using NMP as a solvent decreases the
possibility of formation of macrovoids by reducing the possibility of delayed
demixing [171]. In another study, Yeo et al. reported that the addition of PVP to
the polysulfone membrane using DMF as a solvent increases the possibility of
macrovoid formation instead of suppression [172]. Jung et al. reported that the
macrovoid formation depends upon the MW of PVP [173]. The addition of different
MW of poly(ethylene glycol) such as PEG 400, PEG 6000, and PEG 20,000 Da
showed a significant effect on the performance and morphology of polysulfone
membrane prepared from the solvent NMP and DMAc separately [174]. Recently,
polysulfone membranes have been constructed with the developed inner and outer
surfaces. Such polysulfone membranes are obtained by means of special spinneret
with appropriate conditions of hollow fiber spinning [175]. The membranes devel-
oped in this manner have found applications in biomedical sciences especially in the
cultivation of the cells, e.g., hepatocytes [176].

### 2.12 Agarose

It is a polysaccharide which is used in cell encapsulation for many decades. It could
form insoluble membrane when polyvalent cations are added to its aqueous
solution [177]. Agarose is a gelling material which could be changed into ther-
ma reversibly gel by heating and cooling the aqueous solution of agarose
[67]. Agarose is different from other polysaccharides. It could be changed into
gel (~40 °C) below its gel-melting temperature (~90 °C). Agarose is stable at room
temperature, and its gelling temperature lower than 37 °C is considered to be
favorable [44, 178]. The bacterial strains could digest agarose, but mammals
cannot. In an experiment, it was found that the cells encapsulated by agarose
microcapsule can survive for 200 days when implanted in the peritoneal cavity
of the rat. Therefore, the cells surrounded by agarose microcapsules are quite stable
to be implanted in the human body [179].
Cell encapsulation techniques have been broadly classified as macroencapsulation and microencapsulation. In macroencapsulation, a large group of cells are entrapped inside the hollow devices usually tube shaped or disc like, whereas in microencapsulation smaller groups of cells are entrapped in a capsule. The preferability of the technique depends upon the properties of the encapsulated cells. The problems that arose in macroencapsulation are resolved by adopting microencapsulation, which provide better control of physical and chemical properties of the encapsulated cells. The capsules with the size ranging from 0.3 to 1.5 mm are usually called microcapsules [180, 181]. Their small size as compared to macrocapsules increases surface area to volume ratio for transport of solutes across the membrane. Microcapsules are more durable and mechanically stronger than macrocapsules. Microencapsulation is carried out very carefully as the technique works on microscale and cells encapsulated in hydrogels undergo gelation and ultimately change into solidified matrix.

3.1 Macroencapsulation

As discussed earlier, macroencapsulation is a process whereby cells are encapsulated in the form of large groups as compared to microencapsulation. The cells are entrapped in the hollow devices having semipermeable membranes. The homeostatic working of macroencapsulating devices depends upon the homeostatic environment of the host. In general, the transport of the materials in or out of the cells depends upon the concentration gradient. The membranes of the macrocapsules are made up of thermoplastic materials having unique properties such as structural, functional, and mechanical properties [182]. The macroencapsulation technique was basically developed in the laboratory for understanding the concepts associated with transplantation of tissues and the immunology associated with transplanted tissues. However, macroencapsulation became a tool in combating ailments associated with endocrine system, nervous system, and metabolic disorders [183]. Now many companies worldwide are putting their full expertise to meet the challenges in macroencapsulation technology by generating the following modern devices/properties for producing products at a substantial scale.

3.1.1 Diffusion Chambers

This device is developed to understand the transport properties such as endocytosis, exocytosis, electrical conductivity, and resistance. The chamber uses the cultured cells for understanding the cell permeability of the cell membrane. The electrical conductivity of the membrane is measured by using the electrodes in diffusion chambers. The components of the diffusion chambers consist of cell culture device, diffusion chamber, and micoreference electrodes. Cell culture device is 12 mm in diameter and 0.4 μm pore-sized polycarbonates and polyester membranes. The membranes are placed in the cell culture device using detachable rings. The cell culture device allows the placement of cells on the selectively permeable membrane.
Thus, the cells are formed into a monolayer placed in contact with selectively permeable membrane. The next step is to introduce the cells in the diffusion chamber that may be vertical or horizontal. The monolayer obtained above is placed in diffusion chamber, and the transport properties of cells are studied [184]. By mid-1970s the diffusion chamber became one of the most advanced and unique device for studying cell differentiation and cell growth in the host. Many of the cell properties such as morphogenesis, cell division, differentiation, and release of chemical compounds from the implanted cells remain unchanged while using the diffusion chamber. The earlier membranes used in diffusion chamber were common macroporous membranes with pore-size of 0.10, 0.22, or 0.45 μm. Later nylon reinforced mixed cellulose esters were used in the membranes to maintain very fine and uniform pore size [185]. Such membranes are also used for various sterilization techniques to remove the germs from the desired products.

### 3.1.2 Ultrafiltration Membranes

The newer membranes were developed for the better performance of the encapsulated cells in the human body. For the first time, the ultrafiltration membranes were prepared using phase inversion process in 1960s. These membranes are commercially available in the form of flat sheets or hollow fibers. The most commonly used material for synthesizing ultrafiltration membrane is poly(vinyl chloride-acrylonitrile) (PAN-PVC) copolymer, and this membrane series is called XM series. This new membrane had smaller pore size than the previously used membranes in cell encapsulation and provides more protection to encapsulated cells as compared to the previously used macroporous membranes. Therefore, these newly developed membranes allowed the xenotransplantation without using immune-protecting medicines.

In earlier studies, XM-50 membranes were used in a bioreactor for culturing the cells. This application leads to the studies of using XM-50 hollow fibers as intravascular encapsulation devices where these hollow fibers are loaded with cells namely islets of Langerhans and placed into the abdominal cavity of rats [186], while in some other instances, XM-50 was also used as extracellular devices where the cells were placed into the lumen of the hollow fibers and then both ends of the fiber were sealed by adhesives [187]. In earlier studies, the main focus was the rats and pigs. However, in an application, XM-50 hollow fibers were used for the first time as extravascular device in which cell suspension from the brain tissues from various animals including human was infused into the fiber, and then it was implanted into the brain of the rats suffering from limited growth due to the deficiency of growth hormone. It was found that the level of the growth hormone started to increase leading to the restoration of growth for 3 months [60]. These were the earlier developments on which the present era of macroencapsulation is based.

### 3.1.3 Cell Loading Chamber

The easiest way of loading a cell suspension in the encapsulation device is injecting the cell suspension directly into the device. However, sometimes this simple injecting technique results into the formation of cell clumps or aggregates as in
In the case of aqueous suspension, the driving force making the cells settle down by gravity, which limits the uniformity in packing of large numbers of devices. Various efforts have been made to resolve this problem and maintain the uniform cell suspension inside the devices. The removal of air becomes significantly important when the devices have only one opening. In this case, the air could be removed by attaching a vacuum pump to the device or introducing a venting mechanism in the device. In this process, the removal of the hydrophobic gas from the hydrophobic membrane is facilitated by wetting the membrane with a solvent, methanol. The wetting solvent is then removed by means of aqueous buffer maintained at the physiological pH. Following all these steps, the membrane becomes translucent and loading of cells could be viewed by means of stereomicroscope as the color of the membrane changes with loading of cells.

Another factor responsible for the loading of cells is the volume of encapsulation chamber. In some cases, a large volume chamber is required because the amount of compounds released from the cells is usually very low; therefore, a large number of cells are required for chronic liver failure. But in some other cases, the cells are required in smaller numbers because the cells can release potent chemical agents for site-specific action in the body [188]. Generally, the planar devices can hold the volume up to several millimeters, but in hollow devices for the encapsulation chamber can hold approximately 0.5–50 μL. Till to date, there is no method known that could exactly measure the encapsulation volume in the chamber making it difficult to predict the amount of biomass the hollow devices could retain.

3.1.4 Immobilization Matrices

One of the major problems of encapsulated cells is the necrosis of the cells (cell death due to tissue damage). The cells are dead with the passage of time and the amount of the pharmacological component starts to dwindle with time. To avoid this problem, the cells are loaded in the form of dilute suspension, but due to gravitational force acting on the cells the aggregates are formed slowly and exceed the transport limit of the membrane. This problem is resolved by immobilizing matrices that result into uniform dispersion of the cells in encapsulation chamber. For example, collagen containing gels along with glass beads is used in uniform spreading of the cells that require some support in the form of anchorage. On the other hand, hydrogels producing from alginate, chitosan, and collagen in which cells are suspended in the gels without an anchoring support. Both cationic and anionic matrices are used for these purposes. The neutral matrices such as highly cross-linked poly(ethylene oxide) and poly(vinyl alcohol) can also be used in cell encapsulation. These matrices help in uniform dispersion of cell suspension and prevent the formation of aggregates in the devices [189]. It is noteworthy to say that the viscosity of the matrix must be sufficient enough to allow the cells to disperse and eliminate the effect of the gravity avoiding the formation of the aggregates. Moreover, the matrix should also be mixed with such components that could provide food and nourishment to the cells.
3.1.5 Cell Engineering for Maximal Effectiveness

The cells used in transplantation could have different purposes in the host. For example, the cells may be used to release some hormone, a neurotransmitter for electrical signals, cytokines, growth factors, and growth inhibitors, etc. In most of the instances quickly proliferating cells, stem cells are used. Now a days, it is also possible to genetically engineer an encapsulated cell for the production of a specific compound required in the body of the patient. It is more feasible to genetically engineer the rapidly proliferating cells as compared to fully matured cells [190]. Another extremely important aspect of the encapsulated cells is to maintain the constant level of living cells after encapsulation because the encapsulated cells undergo proliferation and ultimate cell differentiation. Different views have been given to handle the issue of the cells, e.g., the number of cells could be maintained at constant level by using extracellular matrix to cause the cells to differentiate and prevent the proliferation of the cells. Latest approach has suggested the use of cell cycle regulators to control the proliferation of the cells so that they may not exceed the limit of the encapsulation membrane [191].

3.1.6 Long-Term Utility of Encapsulated Device

Demand of the medical sciences is the life of the encapsulated devices and the cells inside them. In many cases, the transplanted cells are required to stay in the body of the host for long time even for years [192]. In this case, there may be an instance where the device is required to remove from the host, and/or it may have to be replaced by another one. In order to fulfill this requirement, different approaches have been developed. The membrane should be mechanically strong enough to tolerate the force applied during its removal from the body. In one process, a tether is attached to the device and then it is transplanted, later on when there is need of removing the device from the body it could be pulled out. In order to make this device mechanically strong, a strain relief element is axially oriented in the fiber to avoid its breakage during eradication [193].

Another way is to replace the cells in the device instead of removing the entire device is to make an opening in the existing device replacing and/or refreshing the cells inside the device. In this case a suspension of cells is used. There is another approach to address the issue of retrieving the device. In this approach, the cells are mixed with polymeric solution that undergoes gelation just by changing the environment of the device. The polymeric solution undergoes gelation when the cells are transplanted into the host and hence the cells also become immobilized by the matrices formed by the polymeric materials [194]. Now it is possible to place, replace, or remove the cells from the interior of the devices without removing the device from the host.

3.2 Microencapsulation

Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules of many useful properties. Different polymeric
materials could be used for coating, e.g., polymeric matrices [195]. It could be seen that the cells are encapsulated by semipermeable membrane and matrix (Fig. 10). These cells are immobilized and supported by matrix which is also semipermeable and allows the exchange of essential delivery of the products for a longer period of time as cells release the products continuously (Fig. 1). Finally, the immobilized cells are surrounded by hollow device made up of semipermeable membrane that could be used for numerous applications. The encapsulated cells, called artificial cells, remain bound in the encapsulation membrane, which is permeable to vital components for the cells nutrients such as oxygen, nutrients, and electrolytes. While immunoactive components (antibodies, immunoglobulin, etc.) larger in size ranging from 160 to 900 kDa cannot enter into the capsule, the cells remain protected from the host immune system.

The eukaryotic cells have been implanted for more than 50 years since the first erythrocytes were encapsulated in 1966 by nylon microspheres [196]. The first application of microencapsulated cells was reported in 1980s when the encapsulated islets of Langerhans were implanted into the rat to treat diabetic. After a while, the first application in the human being was found in 1994 when the islets of Langerhans were implanted in a diabetic patient to release insulin. It was found that the glucose level was maintained in the patient for 9 months [197].

Cell microencapsulation is a technology with enormous clinical potential for the treatment of a wide range of diseases. In cell encapsulation system, cells are usually surrounded by liquid. Microcapsule can be prepared in different ways, which could potentially be used in cell encapsulation.

3.2.1 Microencapsulation by Polyelectrolyte Complexation

In this technique, the cationic and anionic polymers are used to form membranes for encapsulating the cells. If the polymeric compounds are soluble in the aqueous medium, then it becomes very convenient to carry out the encapsulation of the living cells. Both natural and synthetic polymers are used for this purpose. Natural polymers are considered to be the best polymer for encapsulation as they are more biocompatible to the cells. Among the natural polymers, alginate is the best studied and highly suitable material for encapsulating the cells. However, alginate has a
severe limitation, i.e., the isolation of the alginate from the living systems at a massive scale gives highly heterogeneous composition. Both of these factors contribute to the viscosity of solution affecting the production of microcapsules [198]. Nevertheless, these days a number of techniques have been developed for harvesting the homogeneous samples from the natural sources. Synthetic polymers on other hand could be produced on a large scale with uniform composition, but it has a problem of biocompatibility for the encapsulation of the cells.

One of the most widely studied microencapsulation system using polyelectrolyte complexation involves alginate-poly-L-lysine-alginate (APA) microcapsules derived from the protocol of Lim and Sun [199]. The alginate-PLL system is developed by complexation reaction between polyanionic alginate and polycationic PLL. Actually alginate-PLL system has been used extensively for encapsulating the islets of Langerhans, which has been used for treating the patients of Insulin Dependent Diabetes Mellitus (IDDM), Type-1 diabetes. Alginate is a linear binary copolymer composed of $\beta$-D-mannuronic acid (M) and $\alpha$-L-guluronic acid (G) residues. In this technique, the cell containing alginate polyanions was extruded through a needle with concentric air flow dropping into the gelation bath which contains a cross-linking agent solution of calcium chloride. Calcium ionically cross link with guluronic acid residue of alginate and form Ca alginate beads (Fig. 11). The Ca alginate bead is then suspended in PLL solution resulting in the formation of selectively permeable Alg-PLL membrane. The thickness and porosity of the membrane depends mainly upon concentration and molecular weight of PLL and the time of contact between the PLL and alginate. Finally, the Alg-PLL microcapsules are further dipped in a solution of negatively charged species most likely alginate solution so that any remaining positive sites on PLL could be neutralized and this in turn not only makes the microcapsules durable but also biocompatible [200]. The islets encapsulated by alginate-PLL system are reported to have an important role in normoglycemia in rodents and humans. The duration of the effectiveness of encapsulated islets varies from days to months and even for years in rodent [201]. However, these microcapsules show insufficient strength when implanted into larger animals such as dogs and presumably humans [202]. This may be due to their high water content or the loss of the polyelectrolyte overcoats.

3.2.2 Encapsulation Based on Interfacial Phase Inversion
A new technique has been developed to encapsulate the cells using a thermoplastic polymer with desirable properties when transplanted into the host. The main concern with these thermoplastic polymers using in cell encapsulation is the insolubility in water. In such case, the spraying method can be used for producing matrix type cell encapsulated microcapsules [203]. During free falling of cells-polymer suspension droplet that generated by blowing the air, a rapid interfacial reaction can occur by spraying an aerosol cross-linking solution towards it, which gives rise to liquid shell (Fig. 12). Droplets containing cells are generated by blowing the air, but this may exert shear force on the droplet causing the variation in size and damage to the individual droplets. The viscosity and density of the liquid in the phases is adjusted to maintain the desired uniformity of the droplets, approximately 400 μm [204].
**Fig. 11** Schematic representation of entrapment of cells in gel beads [13]. (With kind permission of SciTech)

**Fig. 12** Schematic representation of encapsulation of cells using the spraying method [13]. (With kind permission of SciTech)
The advancement in the interfacial cell encapsulation has led to the application of a new polymer namely hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA) in a ratio of 75:25. This polymer has led to the development of desirable characteristics such as permeability and mechanical strength of the microcapsules. This polymer has also shown promising results in dopamine released from the encapsulated cells that cure cerebral diseases [205].

### 3.2.3 Encapsulation by In Situ Polymerization

This technique was developed by Dupuy for encapsulating the cells in a membrane [206]. In this technique, a cell is surrounded by a healing polymer, which turn into a microcapsule with moderate strength. When these cells are transplanted, the microcapsule can be damaged and eventually ruptured which then triggers the healing polymer to undergo further polymerization to keep the cell protected. This technique provides the cells with much longer shelf life than ordinarily encapsulated cells. In recent past, a system has been developed in which microcapsules are made up of urea-formaldehyde (UF) to surround the healing agent, dicyclopentadiene (DCPD) [207].

### 3.2.4 Conformal Coating Techniques

This technique is quite advantageous as compared to aforementioned cell encapsulation approaches. It has been observed in encapsulated islets that show limited release of insulin in response to glucose when implanted in the body. The main reason behind this insufficient release of insulin is the limited diffusibility of molecules across thick and large sized (600–1000 μm in diameter) capsules. Additionally, the exact implantation of the islets into the target area of the body is also affected by the large sized molecules. In order to address these issues, a new technique called conformal coating has been developed to minimize the size of the capsule as well as that of grafting tissues to transplant them into the desired location.

The coatings are considered conformal as they fit to the size and shape of each cell. This is especially needed in case of islets because these cells vary in their shapes and sizes. In this technique, PEG hydrogel precursor is directly applied to islets to adjust according to their geometrical aspects. After PEG has surrounded the islets, the precursor is cross-linked into an elastic hydrogel. The cells are first surrounded by small amount of the coating precursor which is then cross-linked to form quite strong gel, also called shrink wrapping. In this way the cells get encapsulated in a thin layer of capsule that would give stability and uniformity to the islets making them more applicable [208].

### 3.3 Other Cell Encapsulation Techniques

#### 3.3.1 Silica Sol-Gel Entrapment

Sol-gel chemistry is used to develop a new type of membrane based on ceramics. These ceramic-based membranes are completely novel in the cell encapsulation
technology. The significance of these membranes lies in the ease of synthesis of these membranes from a solution of raw materials at room temperature and normal pH of the living cell. Secondly, their pore size could also be controlled easily even when the membrane is applied to the living cell. Additionally, the cells could maintain their normal physiological activities even under varied ionic concentrations, and cells could survive for 6 months in the presence of trypsin in an in vitro analysis [209]. The membranes spheres are synthesized by drop-tower sphere generation and emulsion polymerization. The pore size of the membrane was controlled in such a way so that only insulin and other biological molecules like cytokines can pass through while antibodies are not allowed to pass through these membranes [210]. Furthermore, the cells encapsulated in a ceramic membrane have good sustainability when transplanted in vivo and their recovery from the host has also shown that they were active even after 1 month. Although the cells encapsulated by sol-gel entrapment technique have not shown complete normoglycemia in some cases, they are potential alternative for other methods for different applications such as drug delivery and treatment of diabetes. They could also provide mechanical strength and durability to the cells in the host [211].

3.3.2 Thermo-reversible Gelation

This technique is based on the gels prepared by changing the temperature of the polymer solution. The aqueous solution of thermo responsive polymeric gel converted into a solution when the temperature of the polymer solution is lowered. However, when temperature is raised, these polymers form a coil in the solution which in turn changes into a three dimensional network and appears as a gel. However, there are some exceptions to this common behavior of naturally occurring thermo responsive polymers, i.e., they are liquid at room temperature but turns into a gel on heating [212].

This sol-gel transformation is utilized for developing gels that easily encapsulate the living cells. These gels especially made from methylcellulose could have shown considerable effect on the repair of the brain cells [213]. In another application, the cells encapsulated by this technique have shown sustained drug release for 60 min. But sometimes surfactants were used to stabilize the microcapsules, which could result into toxicity, and limit their applicability in an in vivo application [214].

3.3.3 Chondrocyte Membrane

This technique is based on a completely novel concept, i.e., instead of encapsulating the cells in a physical barrier, biological mechanism is used to protect the cells from the immune system. In general, the immune privileged cells, which are not attacked by the immune system of the body, for example, chondrocytes, are used to prepare the membrane [215]. In this process, the islets are entrapped within the capsule made up of chondrocytes. In a procedure developed by Pollok et al., islets were seeded on biodegradable polyglycolic acid polymer [216]. Afterwards the cells were encapsulated with monolayer of chondrocytes. The encapsulated cells along with controls were kept in culture for 5 weeks in which one group of cells were fed with glucose every 5 days. The amount of insulin in culture medium was monitored. It was found
that islets were functional. This indicates that chondrocyte encapsulation membrane permits diffusion of glucose and insulin with intact structure of the islets [217].

As a whole, the debate between macro- versus microencapsulation is an ongoing dispute, and neither technique has demonstrated clear superiority over the other. Above all, the success of any encapsulation technique ultimately relies on a systematic evaluation of capsule properties and encapsulated cell performance.

4 Assessment of Capsule Properties

The capsular properties are one of the many important factors that were accessed by the interaction properties of the capsule and the performance of encapsulated cells. Understanding the relationship between the performance of the encapsulated cells and the encapsulating capsules is vital in the development of a successful technology for cell encapsulation. In spite of the achievement of the desired aim of cell encapsulation, in depth understanding of the capsules could result into much improved properties such as permeability, mechanical properties, immune protection, and biocompatibility of encapsulated capsules.

4.1 Permeability

There are two factors of prime importance that determine the survival of the living cells. The cells permeability determine which compounds will enter and exclude by the cell, and the molecular weight cut-off (MWCO) will define the upper limit of the molecules getting into or out of the cells. The major hindrance is the inability to measure the actual pore size of the capsule. The indirect methods have been used to determine the pore size of the capsules. The permeation rate of different solutes into the cells can give estimation about the pore size of the capsule [218]. Some successes have been achieved in measuring the size of the macrocapsules; however, much efforts are being needed to determine the pore size of the microcapsules accurately.

4.1.1 Microcapsules Permeability

The pore size of the capsule could be determined by simply equilibrating the capsule with the known volume of desired solute and then the time taken by the solute to get into the cell and to reach equilibrium is measured [219]. This gives mass transfer of the solute which is used to determine the capsule permeability in the following manner:

The relationship between the rate of mass transfer, \( U \), and the membrane permeability, \( P_m \), is given by [220]:

\[
P_m = U \times d
\]

where, \( P_m \) = permeability of the capsule; \( U \) = rate of mass transfer as function of time; \( d \) = thickness of the capsule membrane.
The abovementioned technique gives the permeability of the capsule and provides some qualitative information about the pore size of the capsules. Another technique has recently been developed called inverse size exclusion chromatography to quantify the pore size of the microcapsules [221]. In this technique, the column is packed with microcapsules and solute is loaded into the column to pass over the capsules with the loaded mobile phase, and partition coefficient, $K_{sec}$, is measured which indicates the pore size distribution in the capsule membrane.

In another procedure for determining MWCO, 1 mL of the microcapsules is mixed with 1 mL of 0.05% (w/v) commercial dextran-FITC samples with nominal MWs of 10, 70, 150, 250 and 500 kDa. The microcapsules are then incubated for 24 h at 37 °C and observed with a confocal laser scanning microscope (CLSM). The intensity profile obtained by CLSM showed increasing in-diffusion with decreasing MW (Fig. 13), and the MWCO of the microcapsules were found to be 100–200 kDa [222].

4.1.2 Macrocapsules Permeability

The macrocapsule membranes are different from the microcapsule. The macrocapsule membranes are thicker and have the ability to withstand high pressure. The properties of these membranes depend upon the porosity and the ability of the membrane to retain a molecule of a particular size in the membrane is expressed in the form of nominal MWCO (nMWCO). The MWCO is the MW of the largest molecule that is permitted to pass through the membrane, whereas the nMWCO indicate that the 90% of the molecules of particular size are not allowed to pass through the membrane under higher pressures [223].

**Fig. 13** Top: CLSM middle sections of cell containing capsules exposed for 24 h at room temperature to 0.05% dextran-FITC with nominal MWs of (a) 10 k, (b) 70 k, (c) 150 k, (d) 250 k, and (e) 500 k. Bottom: Line profile from images as above [222]. (With kind permission of ACS)
In general, the transport properties of the macrocapsule membranes are determined by hydraulic permeability assessment, a technique based on consumption of ultrapure water. In this technique, the testing device consists of a flow rate controllable pump, membrane holder, pressure transducers, and balance that measure flow rate. Generally a lower pressure is maintained to reduce the chances of membrane deformation. The hydraulic permeability measured in this process is reported as unit of flow per unit surface area per pressure unit. This would indicate the pore size of the macrocapsule membrane [224].

4.2 Mechanical Properties

The determination of the mechanical properties of the capsules is important for the production. These properties usually give an indication about the strength as well as the integrity of the capsule. The durability of microcapsules is usually tested by subjecting the capsule to shear flow. The number of “failed” capsules is simply a measure of mechanical durability of the capsule membrane. The mechanical force faced by the capsule depends on the shear rate and viscosity of the fluid in this system. No doubt the method is very simple yet it is used as a screening tool to improve the process of capsule manufacture and produces capsules with better mechanical properties such as coating time, surface modification, and choice of polymeric additives to capsule membrane [225].

The mechanical properties of the capsules are generally determined by compression test [226]. In dry test, capsules are drawn into a pipette causing the release of a single capsule on the surface of the plate. An image of the capsule is taken before the compression test for determination of the initial diameter of the capsule, then the capsule is introduced to the apparatus for the compression test. In the compression machine, the capsule and punch is kept separated until the stepper achieves a steady state velocity. The compression program is started after positioning the punch above the capsule, and it gets terminated after failure of the capsule is observed. The immersion test is also performed modifying the dry test [227]. In case of immersion test, the capsules are dispersed in a bath of dicyclopentadiene (DCPD) and allowed to equilibrate for 24 h. As the capsule is dipped for long time in the bath, some diffusive mobility across the capsule membrane is observed. Afterwards a single capsule is taken by pipet and placed into the compression cell and small quantity of the fluid is added to ensure that capsule is completely immersed. The rest of the procedure is exactly the same as done for dry test to obtain the mechanical strength of the microcapsules [228].

4.3 Immune Protection

The immune protection of the transplanted cells depends on the diffusion of antibodies into the cells or onto the surface of the cells [229]. The diffusive permeability of the encapsulating membrane is dependent on the donor-recipient mismatch,
i.e., allogeneic versus xenogeneic tissues. In case of allogeneic graft tissue, reactive immunoglobulins are absent and hence the host’s immune reactive cells directly reach the allogeneic graft tissue which need to be prevented by the encapsulating membrane. In xenogeneic tissue, there are antigens derived from their surface and hence they activate host’s immune system especially CD-4 cells which in turn produces antibodies that stick to the grafted cell’s surface and interferes with the diffusion properties of the membrane [230]. Therefore, the approach for determining the diffusivity in allogeneic graft cells is called the direct pathway, whereas in case of xenogeneic graft tissue, the approach is called indirect pathway and is of secondary importance for allogeneic graft cells.

In actual practice, it becomes almost impossible to avoid the activation of host’s immune system because of the failure of small portion of the grafted capsules [231]. In this way, the direct pathway can be activated which could attack the failed grafted cells and hence the number of graft specific T-cells increases in the grafted area. The increase in the number of graft specific T-cells could be measured as a parameter for determining the cell’s diffusivity, but it is not clear up to what level these T-cells will affect the intact capsules in the body [232]. Another concern in direct approach is the occurrence of cytotoxic agents in the grafted tissue area. The cytotoxicity of the grafted cells could be measured by in vitro cell culture techniques. However, if the direct pathway is activated as an inadequacy in the capsule properties, then conditions for determining the diffusive properties of the cells will become highly stringent.

The permeability of the antibodies is simply measured by enhancing the transfer of the antibodies into the cells which is achieved by making an antibody sink in the membrane, but extra care should be taken if the large sized proteins are present in the antibody mixture. However, this issue could be resolved by using high purity proteins in the analysis and encapsulating membranes that are very reactive towards antibodies. The amount of the antibodies that have reacted with the capsule gives an indication about the response of the antibodies to the cell. Another way to determine immune protection of the capsule membrane is to mix the capsules with lymphocytes [229, 233]. However, the success of the techniques lies in the analysis of the immune protection in in vivo experiments as the cells are to play their final role in the body of the host. Nevertheless, the correlation between the in vitro and in vivo experiments could be very useful in determining the possible protection of the encapsulated cells in the body of the organism.

4.4 Biocompatibility Assessment

Biocompatibility of any biomaterial is defined by its performance in the body of the host with a tolerable host response in a particular application, e.g., the transplantation of artificial organs such as artificial breasts and hips, etc. [234]. A biomaterial is considered fully biocompatible if the cellular system having encapsulating membranes produces no or not more than a minimal foreign body reaction [235]. The response of the host is a serious problem to the clinical application in an
encapsulation technology. One of the serious consequences of nonbiocompatibility of encapsulated cells is the use of fibrotic overgrowth on the encapsulated cell’s surface affecting the transport of the molecules in and out of the encapsulated tissue [236]. The other factors that play a role in making the encapsulated cells biocompatible are the methods of surgical implantation, size of the device, morphology of the cell surface, the material composing the macroencapsulation device, and the content shed from the encapsulation chamber [200, 237]. When the device is implanted, the host immune response is initiated as a result of disruption of host’s vasculature. Initially, the cell surface is covered with activated platelets, humoral serum components, clot constituents, cell debris, and extracellular matrix. All the debris from the cell surface is removed by the tissue macrophages and the process of wound healing is initiated. In the final step, the mesenchyme cells produce the matrix and neovascularization around the implant completes the process [238].

The production of cytotoxic agents from the host cells also plays a role in response to the grafted encapsulated cells. The material of the capsule membrane plays a significant role in determining the response of the host [239]. Several studies have been conducted on these toxic species, and it has been found that there are two types of toxins mainly one with short life spans such as oxygen free radicals and second one are more stable and can permeate through the membrane of the capsule, e.g., cytokines [240]. Recently, several studies have shown material dependent production of cytokines in encapsulated cells [241]. The assessment of cytokine production can be carried out by using macrophages from peritoneal cavity, the site that is mostly utilized for implantation of the encapsulated cells [242].

Another significant aspect in encapsulation implants is the neovascularization around the grafted tissue in the host. It has been found that the outer topography of the membrane plays major role in determining the neovascularization response [243]. Generally, the membranes that have larger surface pores result in neovascularization very close to the host-material interface. The formation of a huge number of blood vessels is not desirable in many clinical applications as they may hinder the release of the desired therapeutic agent from the encapsulated cells [244]. In some applications, there might be a desire to maintain a high concentration of therapeutic agents systemically. In other applications, it is quite possible to implant the cells in the body for a shorter period of time and subsequent retrieval after the correction/cure the problem. In this situation, presence of huge number of blood vessels will hinder in the retrieval of encapsulated devices from the body of the host [245].

5 Efficacy and Survival of Encapsulated Cells

It has been found that a slight variation in the procedure of encapsulation could result into an important challenge in the performance of encapsulated cells. There is one common problem associated with encapsulated islets is that the implanted islets have never survived permanently into the host body [183]. This decrease in the efficacy of the implanted tissue is attributed to overloading amount of glucose in the blood and
the less quantity of the implanted tissue used in the host. Even after using large number of encapsulated cell, De Vos et al., showed that there was decrease in the efficacy of the implanted tissue with the passage of time, which indicates that the efficacy of the transplant cells neither depends upon the load of sugar nor the insufficient number of islets, and there are some other factors also responsible for deficiency in the efficacy of the implant [246].

A formulated hypothesis states that the number of renewing islets is not equal to the number of necrotic islets. Later this hypothesis was experimentally tested, and it was found that the actual reason is the life span of the insulin producing β-cells. These β-cells were not able to replicate after 3 months, and there were decreased in the number of β-cells leading to decrease in the efficacy of the implanted islets. The cell death is found to be more in the center of the implant than at the periphery. The reason for this behavior is the inability of the nutrients to arrive at the center of the implant [247].

6 Challenges in Cell Encapsulation

In spite of the abovementioned successes, there are still many challenges and promises that cell encapsulation has to meet for touching the new horizons in the medical sciences. For the last few decades, the researchers have been working day and night on developing the systems that have the ability to release the drug in a highly controlled manner so that the effectiveness of the drug could be improved with minimal side effects leading to the improved quality of life. Among these techniques, cell immobilization is the best approach whereby the cells entrapped and immobilized in the matrices act as the factories of therapeutic agents curing various ailments. The promising potential of cell encapsulation lies in the fact that the cells are not only made into drug releasing factories but also eliminate the need of administering immune-protective drugs during transplant of cells.

The history of cell encapsulation is full of many successes and defeats. At one edge, the continuous release of therapeutic agents has been proven to be successful in curing numerous diseases in animal models such as the diseases due to hormonal deficiencies, hemophilia, diseases of CNS, and cancer. Additionally, the technique has also been reported in many clinical trials for applications in curing human diseases. However, the general feeling is that this technique is not meeting up to the expectations. No doubt scientists from across the world have focused much of their efforts on the field, but the reality is that there is no product in the market until now due to the nonreproducibility of results in animal models, lack of standardized technology, immediate necessity of reproducible, and biocompatible materials for making biocompatible and stable devices. Moreover, their systematic administration is associated with unwanted side effects due to nonspecific suppression of the immune system that leads to a variety of undesired complications (e.g., opportunistic infections, failure of tumor surveillance, as well as adverse effects on the encapsulated tissues). Furthermore, the controlled release of drugs by the encapsulated cells is also another important consideration if the promises of cell encapsulation are to be met.
These ditches happening in the field of cell encapsulation have changed the attitude of many scientists leading to stepwise understanding of encapsulation technology. This stepwise approach is important to answer the research questions leading to in depth understanding of the factors that are responsible for limiting the success and help renewing the excitement and hopes surrounding this cell-based technology.

7 Conclusions

Nowadays the encapsulation technique is touching new horizons where encapsulated cells are playing role in gene therapy and drug delivery system in the human body. There are a large number of technologies that have been developed over the past years to improve the techniques and their outcomes in an effective manner. Cell immobilization and encapsulation has a wide range of applications. It appears likely that by the end of the decade clinical trials of encapsulated cells to treat many of these diseases will become a reality. It is worthwhile to mention that the biocompatible materials with much improved properties could serve the desired purpose in cell encapsulation. A better and in depth understanding of the host immune response could also result in developing appreciable biomaterials for cell encapsulation. A much better comprehension of the properties of the biomaterials with much control on the properties of the biomaterials during fabrication could lead to clinical applications of cell encapsulation. However, the biotechnological development including the sourcing of raw materials, the design and building of manufacturing facilities, the scale-up and optimization process, storage and distribution of the product, and quality control thus far is time-consuming, which requires collaboration between scientists and engineers from many disciplines. A discussion about the future application of encapsulated cells, design systematic protocol, and important objectives for the scientists and industries is yet to be considered for the benefit of all.

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# Hydrogel Properties and Characterization Techniques

Michael J. Majcher and Todd Hoare

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## Abstract

The unique structure of hydrogels as materials, including their soft mechanical properties, their typically high water contents, their capacity to respond to changes in their solvent environment, and their tunable and often multi-scale porosity, offers both significant challenges and specific opportunities in terms of their characterization. Herein, we describe the key properties associated with hydrogels (from both qualitative and quantitative perspectives) and review the major analytical techniques used to probe those properties, highlighting the

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strengths and weaknesses of various available strategies. Chemical, physical, and biological properties are all reviewed, with an emphasis on the techniques developed specific to hydrogels to measure swelling, mechanics, gelation time, and porosity.

## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AAS</td>
<td>Atomic absorption spectroscopy</td>
</tr>
<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared spectroscopy</td>
</tr>
<tr>
<td>JKR</td>
<td>Johnson-Kendall-Roberts</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NTA</td>
<td>Nanoparticle tracking analysis</td>
</tr>
<tr>
<td>PGSE-NMR</td>
<td>Pulsed gradient spin-echo nuclear magnetic resonance</td>
</tr>
<tr>
<td>SANS</td>
<td>Small-angle neutron scattering</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TRPS</td>
<td>Tunable resistive pulse sensing</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible light (as in UV/vis spectroscopy)</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
</tbody>
</table>

## 1 Introduction

Hydrogels have several key properties that must be understood both experimentally and theoretically in order to effectively design functional hydrogel-based materials for applications. However, the unique nature of hydrogels as materials (e.g., their high water contents, soft mechanics, internal porosities, networked chemical structures, etc.) makes many of the techniques typically used to assess the properties of other polymeric materials difficult if not impossible to apply to hydrogels. For example, contact angle measurements that are widely used to assess the hydrophlicity of a material interface are ineffective with hydrogels due to the typically rapid absorption of the droplet into the gel phase. Methods used to measure the porosity of hard porous materials (e.g., gas adsorption, mercury porosimetry) are ineffective at assessing the porosity of hydrogels given that hydrogels compress upon pressurization and the water inside hydrogels cannot effectively be displaced without significantly deforming the network structure. Many common techniques used to assess morphology (e.g., electron microscopy) or surface chemistry (e.g., x-ray photoelectron spectroscopy) require operation in a vacuum, posing challenges in the context of analyzing a highly water-swollen material. Furthermore, standard chemical characterization techniques used for polymers (e.g., nuclear magnetic resonance) are challenging or impossible to apply quantitatively to hydrogels based on the crosslinked networked structure which significantly alters relaxation times or other
fundamental mechanisms used for chemical analysis. As such, hydrogel characterization is frequently a significant challenge, particularly from a chemical perspective. However, at the same time, the unique properties of hydrogels have led to the development of several techniques beyond those used for conventional “hard” materials that have enabled a significantly better understanding of both the fundamental physics as well as the application performance of hydrogel-based materials.

In this chapter, we outline the key properties of hydrogels from the chemical, physical, and (briefly) biological standpoints, describe the conventional methods/approaches for measuring such properties, and outline the key theoretical relationships used to understand the fundamental influencers to those properties (the latter of which are also essential to understand in order to rigorously apply the techniques described).

2 Chemical Properties

Chemically, there is interest in analyzing both the chemistry of the building blocks incorporated into a hydrogel (particularly when two or more different building blocks are used as the basis of hydrogel formation) as well as the extent of reaction among available crosslinking groups. However, the network structure of hydrogels makes the chemical analysis of hydrogels challenging, as many of the traditional solution-based chemical characterization methods often do not give accurate or consistent results with hydrogel networks. As such, while most of the methods described below are highly imperfect, they can collectively give insight into gel composition and crosslink density.

2.1 Nuclear Magnetic Resonance (NMR)

Proton NMR is the most common method used for the chemical characterization of polymers [1, 2]. However, in the context of hydrogels, NMR is challenging due to the significantly extended proton relaxation times observed within hydrated network structures, resulting in peak broadening that effectively reduces signal-noise [1–3]. In addition, functional groups at the periphery of the hydrogel and in the bulk of the hydrogel are often exposed to functionally different chemical environments (particularly with regard to the balance between free and bound water) and local crosslink densities, resulting in asymmetric signal generation throughout the hydrogel and thus selective over-weighting of functional groups in local areas with faster relaxation times. Reducing the dimension of the hydrogel from the bulk scale to the nanoscale somewhat improves the analysis [4], but quantitative analysis in the manner of small molecules or linear polymers remains challenging. Solid-state NMR using magic-angle spinning has been applied to address this problem, with some success in particular for tracking changes in crosslinking during the process of gelation [5]. However, signal-to-noise can still be a challenge with solid-state NMR, particularly in probing hydrogels with lower crosslink densities if the main
purpose of the analysis is to calculate crosslink density, and experiments are slow and expensive.

2.2 Infrared Spectroscopy (IR)

IR spectroscopy is a useful tool to identify bond types in hydrogels and other polymeric systems. While the conventional potassium bromide (KBr) pellet technique for transmission-based IR can be applied to hydrogels, the difficulty inherent in grinding hydrogels (which remain somewhat wet due to bound water even when “dry”) often makes it difficult to create uniform pellets with substantial transmission [6]. Alternately, attenuated total internal reflectance techniques (AT-FTIR) may be used to probe the surface (0.5–5 μm) of the hydrogel [7], with the assumption that the surface chemistry is not substantially different from the bulk chemistry. This assumption should however be made with care, as AT-FTIR results are often carelessly interpreted in the literature. Sensitivity and quantification are also challenges in using IR for characterization of hydrogels (or indeed polymers in general). Interpretation of small crosslinker signals in particular should conservatively be limited to the qualitative appearance/disappearance of peaks related to the crosslinking compound before and after reaction.

2.3 Fluorescent Probes

Fluorescent probes that can covalently react exclusively with one or more functional groups on the hydrogel building blocks can be used as an indirect measure of residual functional group content and thus crosslink density, shown in Fig. 1 [8]. This method assumes (1) the probe can effectively diffuse through the hydrogel to bind with all accessible residual functional groups and (2) the effective yield of the reaction between the probe and those residual functional groups is ~100%. On this basis, care must again be taken in interpreting such results as fully quantitative, with the method particularly useful for comparing relative numbers of unreacted functional groups between different hydrogels of similar compositions and/or confirming the uniformity of hydrogels prepared with two or more functional precursor polymers [8].

2.4 X-Ray Photoelectron Spectroscopy (XPS)

XPS can identify the elemental composition of hydrogels and, when used in high-resolution mode, can give insight into the types of bonds present in hydrogels based on the subtle differences in binding energy depending on the types of substituents adjacent to the bond being studied [9]. This technique is particularly useful in cases where it is sought to prove a particular type of crosslink has formed, but IR techniques do not give the required resolution. However, as with any XPS measurement, only the surface (~10–50 nm) of the sample is probed [10]; furthermore, due to
the need to operate under a vacuum, the hydrogel must be dried prior to analysis, potentially altering the crosslinking chemistry of particularly dynamically crosslinked hydrogel systems relative to their solution state. Atomic absorption spectroscopy (AAS) may be a better choice in some cases, as it can probe the elemental composition of the entire bulk gel and thus give a clearer picture of overall crosslinking density (particularly if the crosslinker has a distinct element versus the building blocks) and/or monomer incorporation if the comonomers have different C/O/N/H ratios [11]. However, AAS has no capacity to give any information about the bonding between these atoms and, as a result, cannot distinguish between single (graft) or multiple (crosslink) reacted crosslinking molecules.

### 3 Physical Properties

For the design of hydrogels for targeted applications, three key physical measurements are typically performed: (1) hydrogel swelling, associated with thermodynamics and/or changes in the crosslink density within the gel over time; (2) hydrogel mechanics, associated with the viscoelastic properties and/or the load-bearing potential of the networks; and (3) hydrogel morphology as it pertains to the porosity of the gel network (affecting the rate of molecular diffusion in or out of the hydrogel) and/or the capacity for domain formation inside hydrogels (affecting both diffusion and the capacity of the hydrogel to adsorb chemicals in its environment).
3.1 Swelling

3.1.1 Theory

Swelling in hydrogels is typically reported either as the change in the percentage of water inside the gel network from the initial to the final state or a ratio of gel mass or volume before and after a swelling event (from which the percent water can be calculated if the initial water content of the gel is known). The two most commonly cited swelling ratios (SRs) are relative to the dry state (Eq. 1, representing the mass or volume change related to water adsorption/absorption to the hydrogel when a dry sample is placed in water) or the initial equilibrium swelling state of a prepared hydrogel before its conditions are changed (Eq. 2, representing the change in mass/volume between the initial and new equilibrium condition).

\[ SR_{\text{dry}} = \frac{X_{\text{swollen}}}{X_{\text{dry}}} \]  

\[ SR_{\text{wet}} = \frac{X_{\text{final}}}{X_{\text{initial}}} \]  

Here, \( X \) can represent either mass or volume, depending on the type of swelling ratio desired. Alternately, a percent water in the hydrogel can be calculated based on the dry swelling ratio according to Eq. 3. While the equation is written to calculate the percent water, the water fraction can be similarly calculated by not converting the final ratio to a percentage.

\[ \%\text{water} = \frac{X_{\text{swollen}}}{X_{\text{dry}} + X_{\text{swollen}}} \times 100\% \]  

A \% swelling value can also be calculated to express the wet swelling ratio of a hydrogel from its original swollen state \((X_{\text{initial}})\) to its new swollen state \((X_{\text{final}})\), as shown in Eq. 4. Note that the \% swelling value may be positive (gel swells in new condition) or negative (gel shrinks in new condition) depending on the nature of the swelling response observed.

\[ \%\text{swelling} = \frac{X_{\text{final}} - X_{\text{initial}}}{X_{\text{initial}}} \times 100\% \]  

Given the diversity of ratios or percentage changes that can be used to represent swelling, it is critical to clearly express what type of swelling ratio is being reported in any case. All of these expressions have clear uses and relevance in different applications, and all are routinely reported in the literature. Note that any of these expressions may be applied equally to equilibrium swelling responses or non-equilibrium swelling responses.

Swelling in hydrogels can be reasonably predicted in most cases by the Flory-Huggins model, first proposed by Flory in 1953 [12]. This model describes the balance of osmotic contributions to gel swelling via the thermodynamic affinity of
the gel building blocks for water ($\Pi_m$, Eq. 5), the elasticity of the crosslinks in the network that resist a swelling or deswelling event ($\Pi_e$, Eq. 6), and (for gels containing charges only) the Donnan equilibrium describing the role of counterion partitioning (and, due to water associations with those counterions, the additional osmotic gradient developed as a result of those counterions) within the hydrogel in driving gel swelling ($\Pi_d$, Eq. 7).

$$\Pi_m = -\frac{kT}{a^3} (\phi + \ln(1 - \phi) + \chi \phi^2)$$

$$\Pi_e = -\frac{kT}{a^3} \frac{\phi_a}{N_x} \left( \frac{1}{2} \left( \frac{\phi}{\phi_o} \right) - \left( \frac{\phi}{\phi_o} \right)^{\frac{2}{3}} \right)$$

$$\Pi_d = -\frac{kT}{a^3} \frac{\phi_o f}{N_x} \left( \frac{\phi}{\phi_o} \right)$$

Here, $k$ is the Boltzmann constant, $T$ is the temperature (K), $a$ is the characteristic length of the gel (m), $\phi$ is the volume fraction of polymer in the gel at the tested condition, $\phi_o$ is the volume fraction of polymer in the gel at the zero-strain state, $\chi$ is the Flory-Huggins solvent interaction parameter, $N_x$ is the number of monomeric repeat units between crosslinking points, and $f$ is the number of charged sites between crosslinking points. The polymer volume fraction at the new equilibrium state is defined as the value that drives the sum of all osmotic pressures influencing gel swelling to zero (Eq. 8).

$$\Pi_{total} = 0 = \Pi_m + \Pi_e + \Pi_d$$

For typical swelling measurements, the zero strain state is assumed to be equivalent to the polymer volume fraction during gel preparation, given that crosslinks should form in a manner to minimize the free energy (and thus strain) in the system. However, in other cases in which swelling changes between two different equilibrium swelling states are sought, the zero strain state may be taken as the initial equilibrium polymer volume fraction. The $a$ term can be simply defined in spherical micro/nanogel systems as the particle radius but can also be estimated for any shape of hydrogel in terms of the characteristic length and the ratio between the total volume and surface area of a gel of any shape/dimension. The mixing term $\Pi_m$ incorporates both enthalpic (i.e., polar interactions between the polymer and water) and entropic (i.e., entropy gains by allowing lattice sites to be occupied by polymer or solvent) terms and may contribute to either swelling or deswelling depending on the value of the $\chi$ parameter, which is directly related to the polymer solubility parameters of the gel building blocks. Similarly, the elastic term $\Pi_e$ may promote swelling if the other terms promote gel contraction (reducing entropy versus the zero strain state via chain compression/coiling) or deswelling if the other terms promote gel expansion (reducing entropy versus the zero strain state in terms of chain stretching). The Donnan term $\Pi_d$ can only drive swelling due to the increased
water content required to maintain high entropy of counterions to fixed charges on the gel network (if any).

More complex models can incorporate other effects on gel swelling, including chain stiffness (suppressing swelling/deswelling) [13], co-ion effects and background electrolyte (reducing Donnan equilibrium-based swelling) [13], direct repulsion between polymer-bound charges (increasing charge-driven swelling) [14], and counterion condensation (decreasing charge-driven swelling, particularly at higher overall ionic strengths) [13]. These more complex models have been applied to both bulk gels [13] and particulate gels with well-defined charge/crosslinker distributions [15]. However, unless hydrogels are made with liquid crystal-like building blocks (chain stiffness effects), very high charge densities (direct repulsion), or measured at high salt concentrations (counterion condensation/background electrolyte effects), these additional contributions are typically not necessary to consider to generate reasonable swelling estimates.

3.1.2 Experimental Measurements

Swelling in bulk hydrogels can be measured in multiple ways. The most common, and simplest, method is to track the change in the dimension(s) of the hydrogel upon exposure to a new solvent, allowing for the calculation of the volume-based swelling ratio. Measurements are made simpler when the hydrogel is fabricated in a container (e.g., a glass capillary) [16] that restricts gel swelling in only one dimension, increasing the accuracy of the volume swelling measurement at the cost of slowing down the response time of the gel due to the lower interfacial exposure of the gel to the new solvent conditions. Note that this approach inherently assumes the hydrogel is isotropic (i.e., swelling occurs equally in all dimensions if not otherwise constricted). Alternately, gravimetry is also widely used to estimate mass-based swelling ratios by weighing the hydrogel before and after exposure to the new solvent conditions [17, 18]. The hydrogel is most commonly contained in a wire mesh [19], a dialysis bag [20], or a cell culture insert [21] to enable facile handling of the gel between weighing steps without the need for tweezers that may break or damage the hydrogel; the pre-weighed container mass is subtracted from the overall mass at each time point. Gentle wicking of surface (nonabsorbed) water is typically performed using Kimwipes prior to measurement to ensure all water mass measured is adsorbed or absorbed within the hydrogel. Less commonly, the swelling pressure can be measured by loading a hydrogel on a mechanical test frame and tracking the change in measured normal force as a function of swelling [20]. Quantitative measurements of swelling using this method require the confinement of the gel to direct all swelling in the normal direction, as gel swelling in the lateral direction is not directly assessed using z-direction force measurements. As such, swelling pressure measurements are not routine and are typically used only in cases where the forces exerted by a swelling hydrogel are relevant to the target application.

Microscale gel swelling can be measured using laser diffraction instruments that can probe particle sizes on the microscale [22]. Alternately, optical microscopy can be used to track size changes visually, with particle size changes assessed using image analysis algorithms or software [23]. In this latter case, care must be taken to
ensure that an adequate number of representative particles (typically \( n > 100 \)) is assessed to achieve statistically relevant particle size results.

Swelling responses in nanogels can be tracked by any nanoparticle size measurement strategy, most commonly dynamic light scattering (DLS) [24]. While DLS is a powerful technique that is highly accurate for monodisperse nanogel populations, the intensity-weighted nature of the default particle size distribution coupled with the significantly higher weightings of larger scattering entities in those distributions (according to Mie light scattering theory) can lead to inaccurate swelling ratio measurements for broad or polydisperse nanogel populations. Volume or number distributions can be calculated if the refractive index of the micro/nanogel particle is known; however, no current analytical technique is available for such measurements, although reasonable estimates can in some cases be achieved if the water content of the nanogel is known by calculating a weighted average of the polymer and water refractive indices. As an alternative, newer methods such as single nanoparticle tracking analysis (NTA) [25] or tunable resistive pulse sensing (TRPS) [26] can directly measure number distributions of particle sizes with single-particle accuracy, using light diffraction to track particle diffusion paths in the former and the Coulter principle to track the electrical resistance as nanogels flow through a stretched pore in the latter. It is highly recommended for reliable swelling measurements of nanogels with broader particle size distributions to apply at least two of the available methods (DLS, NTA, TRPS) to ensure that accurate conclusions are drawn about the nanogel population; in particular NTA or TRPS will provide significantly better resolution of multiple nanogel populations whose modes are close together and/or very broad particle size distributions in which the higher size population will be much more intensively weighted by DLS analysis, while similar results are likely to be achieved with any method for more monodisperse nanogel populations (Fig. 2).

3.2 Mechanics

3.2.1 Theory

The mechanical properties of hydrogels are determined primarily by the crosslink density of the hydrogel, although the inherent viscosity of the polymeric building blocks (related to their molecular weight and degree of hydration) and the rigidity of the bonding structure within each hydrogel building block also contribute to the overall mechanical properties. Assuming a Gaussian statistical distribution of crosslinker within the hydrogel, the crosslink density \( \nu_e \) (representing the molar concentration of elastically effective strands) can be related to the stress/strain response of a hydrogel according to rubber elasticity theory (Eq. 9).

\[
\frac{F}{A} = \nu_e RT \left( \lambda - \lambda^{-2} \right)
\]

Here, \( F \) is the force, \( A \) is the cross-sectional area of the unstrained hydrogel perpendicular to the direction of the applied force, \( R \) is the gas constant, \( T \) is the
temperature, and \( \lambda \) is the extension ratio (i.e., the strain experienced by the sample). This correlation is most commonly used in practice by measuring a modulus (the ratio between stress \( = F/A \) and the strain \( \lambda \)) and converting the molar crosslinker concentration \( \nu_e \) to a number concentration of crosslinks per unit volume using Avogadro’s number \( N_A \). Eq. 10 shows this correlation for the plateau shear elastic modulus in the linear viscoelastic regime \( G'_p \), although other moduli (tensile, compressive, etc.) could also be interpreted in a similar context [27].

\[
\nu_e = \frac{G'_p N_A}{RT} \tag{10}
\]

The number concentration of crosslinks \( \nu_e \) can be converted to the number of monomeric units between crosslinks \( N_x \) (used in Eq. 11) based on a knowledge of the mass concentration of polymer per unit volume of the hydrogel, the average

---

Fig. 2 Example of NTA vs. DLS data from drug delivery nanoparticles made of or consisting of \( N \)-trimethyl chitosan (TMC), poly(lactic-co-glycolic) acid or PLGA, and liposomes. The left panel is showing a snapshot of the moving nanoparticle video, the middle pane is a size distribution of particles gathered from NTA (red) and DLS (blue), while the right-most panel is showing the NTA size distribution in 3D represented as size vs. intensity vs. concentration. (Reproduced with permission from [25])
molecular weight of the monomeric repeat units \( M_o \) comprising the hydrogel, and the density of the hydrogel building block polymers \( \rho \), as per Eq. 11.

\[
N_x = \frac{\rho \sqrt[3]{v_1} RT}{G_p M_o}
\]  

(11)

The estimates provided by simple rubber elasticity theory are only estimates, as any nonhomogeneity in the network can significantly alter the results. More complex models have been derived for some of these inhomogeneous network cases, although still assuming non-Gaussian statistical distributions of crosslinks and accounting for entanglement effects [28].

3.2.2 Experimental Measurements

For bulk hydrogels, mechanical properties in response to tensile, compressive, shear, or torsional strains can all be measured using rheometers or mechanical test frames with appropriate sample geometries and experimental assemblies. The type of mechanical test to be performed depends primarily on the anticipated nature of the mechanical stresses in the targeted application of the hydrogel being studied, although some practical limitations are also inherent in choosing measurement approaches. Given the low kPa range of most hydrogel moduli, standard rheometers are most commonly used for analysis and can accurately measure both compressive (normal force measurements as a function of head displacement) and shear (oscillatory force measurements as a function of frequency) moduli. As with any oscillatory rheology experiment, a stress or strain sweep should first be conducted at an intermediate frequency to first identify the linear viscoelastic range of the hydrogel being tested; the following frequency sweep should be conducted at a stress/strain value within this range to ensure that the storage \( (G') \) and loss \( (G'') \) shear moduli are calculated based on the correct assumptions. The use of mechanical test frames for both compressive and tensile testing is also possible, albeit with two limitations: (1) the load cell must be sufficiently sensitive to detect the low stresses resulting in most cases from hydrogel strain and (2) for tensile testing, a method must be identified to assemble a hydrogel sample with the appropriate geometry that can be clamped to the test frame. In this latter case, stiffer hydrogels can easily be molded and clamped using conventional techniques; however, weaker hydrogels must be measured using alternative clamping or immobilization techniques such as wirelike “rakes” that puncture the hydrogel at multiple places (Fig. 3a) [29]. Other methods such as gravity-induced bending or pressure-based bulging perpendicular to a gel cylinder [30], magnetic spherical ball inclusion (measuring displacement as a function of magnetic force applied to the entrapped magnetic ball) [31], deflection of silicone cantilevers during gel compaction [32], and indentation (measuring the force required to insert a spherically tipped indenter in a defined distance into the hydrogel) [33] have also been reported as alternative methods for measuring hydrogel mechanics (Fig. 3b–c), albeit with limitations in some cases for weaker gels (indentation) and in other cases for stronger gels (magnetic inclusion, gravity/bulge displacement).
While all the above strategies effectively probe the average moduli of a bulk hydrogel, an interesting alternative approach called cavitation rheometry has also been developed to measure local moduli within bulk hydrogels [34]. In this approach, a capillary or needle is inserted locally within a hydrogel sample and air is injected at a controlled rate. The corresponding pressure as a function of the air volume is tracked until the critical pressure of mechanical instability is reached, a pressure that can subsequently be correlated to the modulus of the material. This technique uniquely allows for multipoint modulus measurements within the hydrogel and can be performed using inexpensive instrumentation.

For microscale hydrogels, cantilever-based systems coupled with a microscope are available for performing compressive testing of single gel microspheres, with a capacity to perform measurements on microparticle-based hydrogels as small as 20–30 μm in diameter [34]. Micropipette aspiration measurements have also been developed in which the gel microparticle is aspirated into a capillary tube and the modulus is calculated based on either the percentage of the material sucked into the tube at a particular applied negative pressure and/or the angle of deformation at the tip-sphere interface [35]. Multisphere indentations coupled with Johnson-Kendall-Roberts (JKR) soft adhesion theory [36] and ultrasonic pulse-echo methods [37] have been reported as alternative strategies for gel microparticle modulus measurements.

For nanoscale hydrogels, atomic force microscopy (AFM) is the only viable technique for measuring a modulus via an indentation/compression mechanism [38]. Since this technique practically requires the adsorption or at least tethering of the nanogels to a substrate, care must be taken to ensure minimization of the known “pancaking” of nanogels upon interaction with surfaces that would complicate the conversion of the measured forces to moduli values.
3.3 Gelation Time

3.3.1 Theory
The gelation point for step-growth hydrogels can be predicted analytically based on the Carothers equation, which relates the fractional monomer conversion $p$ to the gelation point $\pi$ according to the average number of reactive functional groups on the monomer units $f_{\text{avg}}$ (Eq. 12).

$$p_{\text{gel}} = \pi = \frac{2}{\sum_i N_i f_i / \sum_i N_i}$$

(Eq. 12)

Here, $p_{\text{gel}}$ is the fractional monomer conversion at the gel point $\pi$ and $N_i$ is the number of monomers with a functionality of $f_i$ (note that at least one monomer must have an $f_i$ greater than two in order to induce gelation). An analogous approach could be used to predict gelation conversions of pre-functionalized polymers that crosslink via a step-growth approach, with the average number of functional groups per precursor polymer chain used as the functionality of the “monomer” units in Eq. 12. In this context, the kinetics of the functional group reaction driving crosslink formation determine the rate of gelation. Note that an analogous gelation point calculation for chain-growth hydrogels is not physically meaningful since crosslinks are formed simultaneous to chain formation depending on the statistical incorporation of the crosslinking or backbone monomer(s) during the polymerization process, as determined by the copolymerization kinetics of the monomer(s) relative to the crosslinker(s).

3.3.2 Experimental Measurement
The gelation time can be experimentally determined using a vial inversion test, in which a vial containing the pre-gel components is rotated at pre-determined time intervals until no flow is observed during the defined measurement time [39]. Alternatively, shear rheology experiments can be conducted to track $G'$ and $G''$ as a function of time, with the time at which $G' = G''$ (or $G'/G'' = 1$, conventionally defined as the gel point) corresponding to the gelation time of the hydrogel [40, 41].

3.4 Porosity/Network Structure

3.4.1 Theory
The simplest approach for estimating the average pore size of a hydrogel is to use the $N_x$ (number of monomer residues between crosslinks) value estimated via the swelling measurements (Eqs. 5, 6, 7, and 8) together with the known lengths/angles/numbers of bonds along the polymer backbone within each monomeric unit (calculated based on standard bond lengths/angles) to calculate an average separation distance between crosslinking points; the result corresponds to the (square)
dimensions of a pore in a perfect mesh with a defined crosslink density \( \nu_c \) with fully extended polymer chains. A somewhat more accurate estimate can be achieved by including the Flory characteristic ratio \( C_n \), the ratio between the mean squared unperturbed end-to-end distance for a real chain \( <r^2>_o \), to the value expected for a freely jointed chain with the same number of bonds of the same mean square length \( (N_x l) \), where \( l \) represents the length of the individual monomer units along the polymer backbone) to compensate for the non-idealities of real polymer chains. Equation 13 can subsequently be used to estimate the mesh size \( \epsilon \), which corresponds to the average pore size within the hydrogel [42].

\[
\epsilon = \varphi^{-\frac{1}{3}} (2C_n N_x)^{\frac{1}{2}} l \quad (13)
\]

However, such an approach ignores the substantial heterogeneities that are present in most hydrogel samples and are essential to understand, demanding the development of experimental techniques to gain an improved understanding over internal network structure.

3.4.2 Experimental Measurements

Electron Microscopy

Scanning electron microscopy (SEM) is the most common method used in the literature to assess hydrogel morphology, although transmission electron microscopy (TEM) is also frequently used to probe nanogel structures [43–45]. The obvious limitation of the technique in the context of hydrogel analysis is that it must be conducted under vacuum and thus requires removal of the water phase from the hydrogel prior to imaging. This is typically done either via a lyophilization process (i.e., quick-freezing the sample in liquid nitrogen or dry ice and then sublimating the water under reduced pressure) [46] or via a solvent exchange process followed by critical point drying (i.e., stepwise replacement of water with an organic solvent prior to drying) [47, 48]. Both these methods are inherently problematic. During lyophilization, free water inside hydrogels can form organized ice crystal structures upon quick-freezing that deform the gel and, following removal of the ice via lyophilization, leave behind macropores that are then imaged. Indeed, it is these micro/macropores resulting from ice crystal growth that are most commonly observed in SEM hydrogel images in the literature and are often highly misleading relative to the actual swollen state of an unperturbed gel, although it should be acknowledged that a correlation does exist between the size of the ice crystallites and the modulus of the hydrogel (i.e., stiffer gels result in smaller ice crystallites forming) [49]. The size of such ice crystals can be suppressed by improving control over the freezing process [50, 51]. Solvent exchange/critical point drying avoids these problems but introduces other challenges in terms of correlating the swelling state of the hydrogel in the exchanged organic solvent relative to that observed in water. Performing smaller gradient solvent steps helps to preserve the aqueous structure of the hydrogel [52] but is not a complete solution. Cryo-EM approaches can offer somewhat improved...
images by avoiding the need to remove solvent, as imaging is performed on a substrate maintained at low temperature typically using liquid ethane \[53\]. However, crystallite formation upon freezing can still deform the native microstructure to some degree, particularly in weaker hydrogels, and the resolution (absent the use of reconstruction algorithms) can still be limited. As such, while SEM/TEM has some degree of utility in terms of analyzing hydrogel morphology, results only from SEM/TEM must be interpreted judiciously in light of the sample preparation techniques required.

**Macromolecular Probes**

The uptake (or exclusion) of molecular or nanoparticle-based probes with well-defined hydrodynamic diameters into hydrogels can be used as an indirect strategy to characterize the pore size distribution. Analogous to the molecular weight cutoff of a membrane, macromolecules or nanoparticles with diameters larger than the maximum pore size of the hydrogel will be fully excluded from the gel phase, while probes of sizes smaller than the maximum pore size will be able to penetrate into the hydrogel to a degree proportional to the percentage of hydrogel pores equal to or larger than the size of the probe used. The most common probes used include fluorescein-labeled dextrans, which are commercially available in a range of molecular weights from \(~4\) kDa up to \(2\) MDa \[54\] (albeit with somewhat different polydispersities which must be considered in interpreting the data). Proteins are also highly useful as probes given their very well-defined geometries and molecular weights, eliminating any complications associated with the polydispersity of polymer probes. For example, using a set of fluorescently labeled proteins including bovine catalase (~240 kDa), glyceraldehyde 3-phosphate dehydrogenase (~146 kDa), avidin (68 kDa), trypsinogen (24 kDa), and myoglobin (17 kDa) allows for probing of pore sizes from the ~2 to 10 nm size range most typical of hydrogels, as successfully demonstrated on both bulk hydrogels and gel micro/nanoparticles \[55, 56\]. DNA can also be used as a probe given the precise control possible over the number of base units, often coupled with gel electrophoresis to accelerate the measurement \[57\]. Well-defined nanoparticles or microparticles are also useful as probes for analyzing larger pore sizes in hydrogels, as previously demonstrated for natural mucin \[58\]. It should be noted that this technique inherently assumes that the probe(s) can freely move through the gel micro/nanostructure without significant entrapment or adsorption. Given the noted capacity of proteins in particular to adsorb to interfaces, care should be taken to first understand any non-specific interactions that may occur to a substantial enough degree to interfere with the pore size measurements before quantitative pore size distributions are estimated from the data.

**NMR Relaxation**

As an alternative to tracking the physical size of the pores, NMR approaches can be used to instead investigate the dynamics and diffusion of water within hydrogel networks to gain insight into gel porosity based on the distribution of local confinements of water molecules by the network structure. The technique is based on the observation that the relaxation of bound water and free water within the gel is
significantly different; as such, as more water is bound (i.e., more of the water in the hydrogel is adjacent to the gel building blocks, correlated to smaller pores), the overall relaxation rate accelerates [59]. Two general approaches can be used. First, a spin-echo pulse sequence can be used to measure the longitudinal ($T_1$) and transverse ($T_2$) relaxation times of water within the hydrogel network. The resulting timescales of relaxation can be correlated with the self-diffusion coefficient of water based on the solvent viscosity and the size of the relaxing unit (i.e., water) [60]. Second, the attenuation of a spin-echo signal by the dephasing of nuclear spins due to both translational motion of the spinning nuclei and the application of well-defined gradient pulses (pulsed gradient spin-echo NMR, or PGSE-NMR) can be used to measure an apparent water diffusion coefficient that can again be correlated to the gel pore size. PGSE-NMR measurements are advantageous in that they allow for measurements of translational diffusion on longer timescales (i.e., ms-s compared to ps-ns for simple spin-echo experiments) and do not require making any assumptions about the mechanism of relaxation (Fig. 4). However, since water molecules interact with several nearest neighbors (i.e., both other water molecules and the gel) over the time frame of the measurement, it cannot be used to give explicit self-diffusion coefficients [61]. These strategies have both been widely used to estimate pore sizes in bulk [62, 63] and micro/nanoparticulate hydrogels [64, 65]. However, it should be emphasized that these methods explicitly allow only for the extraction of an average pore size, although some degree of insight into pore size distribution can still be attained. Furthermore, extracting an average pore size using either of these techniques requires extensive model fitting that is valid only if the key assumptions made to develop those models hold during the experiment.

**Small-Angle Neutron Scattering (SANS)**

SANS has been widely used for the study of the internal morphology of hydrogels on multiple length scales. Neutron scattering is uniquely capable of probing the hydrogel-relevant length scale (<100 nm, or even up to <500 nm with ultra-small-angle neutron scattering); is sensitive to C, H, and O commonly found in the building blocks of most hydrogels; can generate high scattering contrast by simply swelling gels in deuterium oxide (D$_2$O) instead of H$_2$O; and can examine bulk samples given the highly penetrative nature of neutrons [67]. Furthermore, unique to the other methods described above, SANS can simultaneously probe both the mesh size and the nature (i.e., average size and average spacing) of inhomogeneities within gel networks, which naturally occur in hydrogel systems due to the random inhomogeneity of the crosslinking process being locked into the resulting gel network. While these inhomogeneities are not typically detected in the zero-strain state during gel preparation, they become more significant when the gel swells (Fig. 5), creating static inhomogeneities within the network that significantly affect gel mechanics, transparency, and diffusion properties. These static inhomogeneities, coupled with the dynamic network fluctuations related primarily to temperature variations, provide information on gel structure on multiple length scales using a single SANS experiment.
SANS experiments generate radially symmetric scattering profiles that are converted to plots of scattering intensity $I(q)$ as a function of the scattering vector $q = 4\pi \sin(\theta)/\lambda$, where $\theta$ is the angle between the incident neutron beam and the detector and $\lambda$ is the wavelength of neutrons (typically between 6 and 10 Å). The resulting profile is then fit to appropriate scattering functions that allow for the extraction of microstructural information. Most scattering functions have the general form given in Eq. 14.

$$I(q) = P(q)S(q) = P(q)[G(q) + C(q)]$$  \hspace{1cm} (14)$$

Here, $P(x)$ is the form factor related to the scattering due to individual features and $S(q)$ is the structure factor related to the scattering from the inter-feature interactions.
or proximity between features (i.e., the sum of the dynamic mass fluctuations within the hydrogel network $G(q)$ and the static/frozen inhomogeneities $C(q)$ locked into the network via the inhomogeneous crosslinking process). Different hydrogels will require different structure factors to fit depending on their internal morphologies, with different information related to each morphology capable of being extracted. One of the more typical scattering expressions used to describe hydrogel morphologies is shown in Eq. 15 [68].

$$I(q) = \frac{I_{oz}(0)}{1 + \varepsilon^2 q^2} + \frac{I_{SL}(0)}{(1 + \Xi^2 q^2)^2}$$ (15)

In this model, the two key hydrogel parameters extracted are the correlation length $\varepsilon$ between the static inhomogeneities and the characteristic size of the inhomogeneities $\Xi$, parameters that give insight into both the size ($\Xi$) and spacing ($\varepsilon$) of inhomogeneous

Fig. 5 2D schematic representations of (a) a gel network during synthesis (at a polymer concentration well above the gelation threshold) and (b) the same gel swollen by water. Red dots represent the interchain crosslinks randomly distributed throughout the gel network. (c) Dynamic, static, and total concentration fluctuations with space coordinate $r$. (Reproduced with permission from [67])
features in hydrogels (note that both numerators are intensity-related weighting constants to facilitate fitting of the function to real scattering curves). Other functions including Porod, Lorentzian, and Guinier (some of which can be used to extract a correlation length that approximates the mesh size of the hydrogel as defined in Eq. 13) can also be used [67], with multiple static inhomogeneity terms added in some cases to account for the multiple types of inhomogeneities present in some gels [69].

Contrast matching experiments are also possible in which one of the hydrogel components is deuterated. By adjusting the ratio between D2O and H2O in the solvent used to swell the hydrogels, the scattering between the solvent and protonated component(s) and the deuterated component(s) can be manipulated to effectively suppress signal from one or the other component by matching the scattering length densities of the solvent with that of the polymer phase desired to be hidden [70]. This is analogous to observations with light, as zero net scattering is observed when a polymer phase and the solvent have equal refractive indices. The scattering length density is a direct function of the elemental composition of the hydrogel components, such that if the composition of the hydrogel is known, the H2O:D2O solvent ratio facilitating index matching can be reasonably calculated. Such analysis allows for investigation of the internal distribution of one gel component without interference from other components of the gel, another unique feature of SANS analysis of hydrogels. However, it should be noted that the information gained from SANS is highly model-dependent and requires both choosing the right physical model for the system and performing accurate fits to that model. In addition, highly specialized instrumentation is required to perform the experiments, requiring a neutron source with a sufficient flux and appropriate wavelength to probe the desired length scales of hydrogel features.

3.5 Optics

The transparency of hydrogels is critical for the use of hydrogels for optical applications. Transparency can be simply measured using UV/vis absorbance/transmittance measurements, with the threshold for transparency depending on the thickness of the hydrogel used and the needs of the specific application [71]. Refractive index measurements can also be performed, although the high water content of most hydrogels results in refractive index readings rarely far from those of water [72]; this is true even in hydrogels that are visually opaque due to the formation of internal scattering domains.

4 Biological Properties

While a comprehensive coverage of biological assays associated with the use of hydrogels as biomaterials is outside the scope of this chapter, the reader is referred to the excellent recent review on the use of hydrogels for cell culture applications from Burdick’s group for guidance on best practices for culturing cells on (2D) or inside (3D) hydrogels and assessing the capacity of those cells to remain viable, proliferate,
grow, migrate, or differentiate [73]. Protein adsorption to hydrogels is also of interest to measure, as the high hydrophilicity of hydrogels is often useful for suppressing non-specific protein adsorption and, subsequently, the inflammatory response following implantation in vivo [74–76]. Fluorescent [77] or radioactive probe [78] labeling of model proteins or of complex protein mixtures (e.g., whole blood or blood plasma) is typically used to enable detection of the fraction of added protein that binds to a hydrogel. Interpretation of the results of a protein adsorption experiment is however sometimes challenging with hydrogels given that proteins may both absorb (as with conventional hard biomaterials) and adsorb (due to the internal porosity) in hydrogels, depending on the size of the proteins relative to the hydrogel pore size; indeed, proteins are used as probes of gel porosity due to this capacity for non-specific absorption into the gel phase. As such, a hydrogel with smaller pores may be noted to exhibit less overall protein binding but actually interfacially absorb more protein, likely to ultimately result in a more severe inflammatory reaction. Confocal imaging of fluorescently labeled protein distributions across a hydrogel cross-section can assist in differentiating these contributions and improve the prediction of the potential compatibility of a hydrogel in a biological application [79].

5 Conclusions

The characterization of hydrogels is inherently more challenging than with many other materials given the dynamic nature of hydrogels as their environment (solvent type, humidity, etc.) changes. Indeed, many of the core techniques used to characterize in particular the chemistry of “hard” polymer materials (e.g., XPS, NMR) are at best challenging to apply to quantitative hydrogel characterization. There are significant needs (and thus opportunities) to develop improved techniques for (a) performing traditional characterization techniques accurately on hydrogels (e.g., developing new NMR pulse sequences) and/or (b) developing strategies of drying hydrogels (e.g., critical point drying) that minimally perturb the swollen structure of the hydrogel in water. However, significant recent advancements have been made in the context of accurately measuring the mechanics of hydrogels under various stress regimes using a variety of creative techniques. The continued development of such techniques is expected to yield further insight into the fundamental properties and physics of hydrogels, enabling improved engineering of hydrogel structures to achieve targeted hydrogel performance.

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Applications of Hydrogels

Michael J. Majcher and Todd Hoare

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Abstract

Hydrogels offer multiple unique properties in terms of their porosities, mechanics, interfacial dynamics, and biological responses that make them highly relevant to a broad range of potential applications. Herein, we review how hydrogels can address key challenges in biomedical, personal care, cosmetic, bioseparations, environmental (including natural resource extraction), catalytic, and agricultural applications, with an emphasis on how hydrogels can be rationally engineered in each case for optimal performance. Biomedical applications of hydrogels in drug delivery, tissue engineering, cell encapsulation, wound healing, and biological barrier materials are particularly highlighted in the context of how various
approaches to hydrogel synthesis and fabrication influence hydrogel performance in such applications.

**Abbreviations**

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<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>AAc</td>
<td>Acrylic acid</td>
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<td>AAm</td>
<td>Acrylamide</td>
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<td>AAS</td>
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<td>AgNPs</td>
<td>Silver nanoparticles</td>
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<td>AMPS</td>
<td>2-Acrylamido-2-methyl-1-propanesulfonic acid</td>
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<td>APTMACl</td>
<td>(3-Acrylamidopropyl)trimethylammonium chloride</td>
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<td>Cluster of differentiation 95</td>
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1 Introduction

The diversity of backbone polymer compositions, crosslinking strategies, and morphologies accessible with hydrogel-based materials opens a broad spectrum of potential applications for hydrogels. In general, applications of hydrogels tend to exploit one or more of three key properties that are unique to hydrogels relative to other types of materials. First, the capacity of hydrogels to imbibe large amounts of water (in many cases multiple times their dry weight) and bind that water inside the gel network by hydrogen bonding/water structuring is often leveraged for dewatering applications (e.g., in natural resource extraction), water retention applications (e.g., drought-resistant agricultural additives), or sorbency applications (e.g., wound dressings for absorbing exudate or personal care products such as sanitary pads or diapers). Second, the controllable porosity of hydrogels can be used to limit access to chemicals entrapped within the gel phase (e.g., controlled catalysis, selective biosensing, xenograft cell encapsulation), regulate the release of entrapped cargoes within the gel phase (e.g., drug delivery, agricultural release), and/or absorb targeted chemicals from the gel environment (e.g., environmental remediation, bioseparations); all such interactions are highly tunable (and, in the case of environmentally responsive hydrogels, dynamically tunable) based on both the size of the pores and the chemical affinity of the gel phase for the encapsulated species. Third, the soft mechanics of hydrogels strongly mimic those of biological tissues, leading to applications in tissue engineering and biological barrier materials in which a hydrogel can provide appropriate mechanical signals to surrounding cells.

In this chapter, we will outline the main applications of hydrogels across multiple fields, including biomedical engineering, personal care products and cosmetics, bioseparations, environmental applications, natural resource extraction, catalysis, and agriculture. Emphasis is placed on the mechanism by which hydrogels can address key application needs in each area and how hydrogel properties can be effectively engineered to optimize the performance of hydrogels in each application.

2 Biomedical

The use of hydrogels in biomedical applications was first demonstrated by Wichterle and Lim in 1960 via the crosslinking of hydrophilic poly(2-hydroxyethyl methacrylate) (PHEMA) polymers into a network to form a contact lens [1]. Contact lenses remain a key application of hydrogels, with the more recently developed long-wear
contact lenses based on interpenetrating polymer networks between silicone and PHEMA that provide significantly improved oxygen transport relative to PHEMA lenses only of particular note [2]. However, since this initial demonstration, hydrogels have been applied in a wide variety of biomedical applications, the key applications of which will be summarized in the following section.

2.1 Tissue Scaffolds

The number of papers pertaining to the use and/or design of hydrogels for use as scaffolds in tissue engineering applications has dramatically increased since the earlier work in this field in the 1990s, and the interested reader is referred to critical reviews on the development of tissue-engineered hydrogels by Zhu and Marchant [3] and Brandl et al. [4]. In general, hydrogels are attractive candidates as scaffolds for tissue engineering applications since they are naturally hydrated and porous networks that can be engineered to have unique architectures and mechanical properties that mimic native soft tissues to a greater extent than other polymeric biomaterials [3], providing cells with the mechanical and topographical cues required to develop into functional tissues.

Hydrogel tissue engineering scaffolds have primarily been designed by drawing inspiration from the organization of the native extracellular matrix (ECM) of cells [3]. Natural ECM offers mechanical support to cells in addition to containing many essential cell signaling molecules, fibrous proteins (primarily based on collagen, elastin, and laminin), and proteoglycans (glycosaminoglycans) [5]. Cells can respond to biochemical or mechanical cues from their ECM; alternately, cells can actively restructure their ECM by releasing enzymes to direct its dynamic degradation and remodeling; in this latter case, cells effectively signal each other through these dynamic ECM restructuring processes [6]. An effective tissue scaffold should mimic these key properties of native ECM, including a capacity to support cell adhesion/growth; provide biomimetic physical, mechanical, and chemical cues to direct cell behavior; and facilitate effective exchange of nutrients, gases (e.g., O2 and CO2), metabolic waste, and biological signals to and from the cells [7].

The use of hydrogels as ECM mimics for tissue regeneration requires a combination of control over hydrogel chemistry and structure. From a chemistry perspective, scaffolds should ideally mimic specific functions of the natural ECM such as cell adhesion [8, 9], proteolytic degradation [10–12], growth factor/cytokine binding [13, 14], and matrix protein binding [15, 16]. Most useful hydrogel chemistries are either injectable [17, 18] or can be formed via ultraviolet (UV) photopolymerization [19, 20] since the use of mild synthesis conditions is required in the presence of cells. From a structural perspective, the macroporosity and fibrous nanostructure of native ECM is mimicked by a variety of methods to make macroporous hydrogel scaffolds including salt or porogen templating, bicontinuous emulsion templating, gas foaming, cryogelation techniques, electrospinning, and additive manufacturing techniques like 3D printing, all aiming to provide space for cells to grow, proliferate, and signal other cells (Fig. 1) [21]. Hydrogel structure can also be manipulated spatially...
within the gel to promote desired cell responses by structured signaling. For example, gradient hydrogels with spatially defined mechanical gradients, typically prepared by locally altering the monomer and initiator concentrations of photopolymerizable precursors, have been demonstrated to direct cell spreading or expansion in a particular direction [22]. The addition of cell signaling chemical gradients (e.g., cell-adhesive peptides) into the scaffold can further control the spatial distribution of cells on or within a hydrogel [23].

Natural polymers are commonly used to fabricate tissue engineering scaffolds due to their inherent biodegradability and the potential for their degradation products
to modulate the native biological system. The four main types of natural polymers used include natural proteins (e.g., collagen, elastin, fibrin, gelatin, or silk [3], the first three of which are found in native ECM), genetically engineered proteins such as calmodulin [30, 31], polysaccharides (e.g., hyaluronic acid or dextran [32, 33]), and DNA [3]. Collagen has been particularly broadly used since it is the key native structural protein in many tissues and can be degraded by metalloproteinases (MMPs) such as collagenase, allowing for controlled matrix degradation. Additionally, gelatin is popularly used since it naturally contains arginylglycylaspartic acid (RGD) residues which promote cell adhesion, migration, differentiation, and proliferation while also showing capacity for in situ thermogelation. Some carbohydrate-based building blocks also have native biological properties that can be leveraged for functionality. For example, hyaluronic acid (HA) is a non-sulfated glycosaminoglycan (GAG) that plays a key role in regulating the mechanics and hydration of the extracellular matrix as well as promoting cell spreading [8, 34, 35]. The combination of both proteins and polysaccharides together, inspired by the structure of native ECM, can overcome any deficiencies associated with either building block alone, with collagen/HA [36], laminin/cellulose [37], and fibrin/alginate [38] interpenetrating networks among the many examples reported. With DNA, it is common to add arms and/or “sticky” (i.e., self-assembling) ends to DNA’s polynucleotide chains that can be specifically tailored for relevant applications [39, 40] while still taking advantage of the native self-assembly of complementary DNA under physiological conditions.

Naturally secreted ECMs can also be recovered from cells in vitro for use as hydrogel tissue scaffolds. The most common of these systems is Matrigel™, a protein mixture secreted from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. The collected protein mixture from these cells contains critical ECM proteins such as laminin, collagen type IV, elastin, and growth factors [41]; reconstitution of these proteins into a gel has thus shown beneficial properties in various cell and tissue studies [42]. Similar gelatinous mixtures of proteins can be constructed in the lab using chemical crosslinkers (e.g., glutaraldehyde or epichlorohydrin), although the possible cytotoxicity of unreacted crosslinker must be considered if this latter approach is pursued.

Synthetic polymers have also been applied to tissue engineering applications since they offer more tunable and reproducible physical and chemical properties. Nonbiodegradable synthetic polymers are most widely used, with hydrogel scaffolds based on 2-hydroxyethyl methacrylate (HEMA), 2-hydroxypropyl methacrylate (HPMA), acrylamide (AAm), acrylic acid (AAc), N-isopropylacrylamide (NIPAM), poly(ethylene glycol) (PEG), Pluronic or poly(ethylene oxide) and poly(p-phenylene oxide) PEO-PPO block copolymers, and poly(vinyl alcohol) or PVA most frequently used [43]. On the other hand, biodegradable synthetic polymers have displayed distinct advantages for tissue scaffolds since the rate of polymer degradation can be tuned to match the healing/regeneration rate of the damaged tissue [44]. However, these polymers are mostly not water-soluble and thus cannot be used directly to form hydrogels. Many hydrophobic biodegradable polymers (including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone)
(PCL), and their respective co-polymers [44, 45]) can though be grafted to water-soluble polymers to enable the formation of hydrophobically-associative physically crosslinked hydrogels that can degrade at a rate related to the degradation rate of the degradable hydrophobic graft. Hybrid hydrogels based on combinations of synthetic polymers with natural polymers can also be applied to exploit benefits of both types of polymers to precisely engineer scaffold properties. Functionalization of synthetic polymer building blocks before or after hydrogel formation with bioactive moieties such as cell-adhesive sequences [46, 47], enzyme-sensitive linkages [48–50], and/or growth factors [51] can also introduce the desired biological properties of the scaffold in an all-synthetic hydrogel system, with the work of Anseth’s group in creating dynamically biofunctional PEG hydrogels being particularly notable on this topic [52].

Significant effort has also been invested to try to reproduce the fibrous network structure of natural ECM (Fig. 1). Synthetic self-assembling peptides such as self-complementary peptides and peptide amphiphiles (PAs) have been extensively used to create fibrous hydrogel networks [53], exploiting the native fiber formation of such materials. Electrospinning of hydrogel nanofibers via either rapid UV photo-polymerization of extruded fibers [54] or rapid in situ gelation upon extrusion [55] can create nanofibrous structures directly. However, biofabrication strategies that apply additive manufacturing techniques to form complex three-dimensionally structured tissue constructs with high shape fidelity have attracted particular recent interest for the augmentation, replication, or replacement of native tissues of interest. By combining computer-aided manufacturing approaches with biomaterials and cellular components, scaffolds with applications in tissue engineering [56] as well as drug screening [57] and in vitro disease models [58] can be designed with prescribed structures and thus functions. Commonly used bioprinting techniques for hydrogels include inkjet, laser-assisted, extrusion, and stereolithographic bioprinting, as shown schematically in Fig. 2 [59].

The mechanisms available for hydrogel biofabrication inherently limit the types of hydrogels that can be used. The final matrix must facilitate cell migration, growth, and proliferation [60], but the materials must be printable in a manner appropriate for each mechanism and be capable of rapidly forming stiff structures following printing to maintain shape fidelity; these are often conflicting properties from a hydrogel design perspective. Mixtures of bioinks with different molecular weights (e.g., high and low molecular weight or MW alginate) can offer benefits in this regard, with the low molecular weight component reducing the viscosity of the ink (to enable printing) but the high molecular weight component enabling rapid gelation to maintain shape integrity [61]. In addition, while optimal shape fidelity is attained with stiff hydrogels with high crosslinking densities, this stiffness can also limit cell migration, growth, and differentiation [62, 63]. On this basis, medium crosslinked hydrogels are often considered optimal for the biofabrication of tissues or tissue mimics, gaining improved cell responses within the scaffold at a cost of losing a degree of geometrical accuracy. As a result of this suite of required properties, relatively few bioinks are now available on the market, with most hydrogel-based commercial processes using the rapidly gelling calcium-alginate chemistry [64].
Leveraging the properties of in situ covalently gelling hydrogels in this application is likely to lead to significantly more diverse (and thus biofunctional) tissue chemistries while still maintaining the key properties required for printability.

2.2 Cell Encapsulation

As a subset of tissue engineering, the encapsulation of a few or single cells inside hydrogel microparticulate gels has been extensively explored to create microscale matrices with the capacity to support cell viability (e.g., facilitating cell adhesion and supporting the diffusion of nutrients/oxygen into the encapsulated cells) while also excluding undesirable agents (e.g., inflammatory cells) [65]. Such an approach has been used to encapsulate cells of various lineages including islet cells (of particular interest as protected insulin-releasing materials), kidney cells, fibroblast, myoblasts, and various stem cell lineages [66]. Early work with cell encapsulation technologies aimed at the immunoisolation of cells producing therapeutic proteins for various diseases [65], requiring ideally nondegradable hydrogel matrices to enable the use of any cell from any lineage to produce the therapeutic protein. Now, while immunoisolation is still being explored, the principal interest in cell encapsulation lies in developing models for understanding cellular physiology and for cell delivery in vivo in regenerative medicine applications with musculoskeletal, neural, dermal,
hepatic, and cardiovascular applications. In this case, the degradation of the gel or gel microparticle at targeted times appropriate for the regeneration of the tissue of interest is essential for the proper function of the gel.

Hydrogels used for cell encapsulation must be cytocompatible, prepared using mild crosslinking conditions so the cells do not get killed or highly stressed during the encapsulation process, stable over the appropriate period of time, and contain sufficient porosity to ensure that the microstructure has enough space for cells to move/rearrange, communicate, respire/reproduce, and access nutrients [18]. Additional porosity can be introduced if required via the use of porogens or other methods described in Sect. 2.1 for fabricating macroporous bulk scaffolds.

Calcium-crosslinked alginate is by far the most frequently used biomaterial for cell microencapsulation based on its simple, rapid, and biologically friendly crosslinking mechanism and its safe degradation products [67]. However, the calcium-alginate system is prone to high levels of swelling and/or cation exchange to remove the calcium crosslinker in vivo, limiting the maximum long-term stability achievable [63]. Other divalent cations (e.g., barium) have been used to improve the stability of the gel microbeads [68]; the use of functionalized alginates such as perfluorinated alginate [69] can also improve the stability of the ionic complex formed. UV photopolymerizable systems have more recently been explored by using inverse emulsions, microfluidic processes (which can enable few or single cell encapsulation) [70], or other assembly techniques to template a microparticle form that is subsequently covalently crosslinked in place via photoinitiation with a cytocompatible initiator (e.g., Irgacure 2959). Cytocompatibility is maintained for most cell types provided the irradiation time remains relatively short. Cell adhesion to the matrix is also essential to ensure long-term cell viability. For hydrogels without inherent cell adhesivity (e.g., hydrophilic synthetic polymers like poly (ethylene glycol)), hydrogels are typically biofunctionalized with a peptide or ECM protein such as fibronectin or the RGD cell-adhesive peptide, with the peptide most commonly used since it is less expensive and easier to isolate and has less diverse functions [71]. Surface decoration of gels with antibodies including apoptosis antigen-1 (APO-1 or APT), cluster of differentiation 95 (CD95), and tumor necrosis factor receptor superfamily member 6 (TNFRSF6) [72] can also modulate the inflammatory response to the material, in particular to avoid the “walling-off” fibrotic response that would reduce transport in and out of the capsule.

2.3 Drug Delivery

Hydrogels have several properties that have made them widely exploited in drug delivery applications. The high internal porosity of hydrogels enables loading of drugs into the scaffold and the controlled diffusion of those drugs out of the scaffold at a rate dependent on the tortuosity (i.e., the combination of pore size and pore connectivity) of the hydrogel network, a parameter that can be tuned by the method of fabrication and the crosslink density [73]. The chemical flexibility of hydrogels can also be applied to introduce affinity sites for charged [74, 75], hydrophobic
or other specific types of small molecules to improve loading and/or prolong release kinetics. The high water content of hydrogels leads to generally low inflammatory responses due to low non-specific protein adsorption, at least compared to other types of biomaterials useful for drug delivery [78]. Biodegradability or dissolution may also be introduced via judicious selection of crosslinker and/or building block material.

The terms “controlled release,” “sustained release,” “extended release,” and “long-term delivery” are largely used interchangeably in the literature to describe drug release from hydrogels. However, in any case, the main goal of most hydrogel-based drug delivery systems is to maintain the local concentration of the therapeutic agent between the limits of toxicity (upper limit) and the subclinical baseline below which the agent has no functionality (lower limit), termed the “therapeutic window” (Fig. 3) [79]. For long-term delivery profiles, the engineering goal is to prolong the amount of time in which release occurs in this window and to reduce the slope of the release profile leading up to a leveling off or a plateau region.

Many properties of both the hydrogel phase and the hydrogel environment contribute to determining the release kinetics from a hydrogel, including both the rate and magnitude of water penetration into the gel (i.e., swelling) and the effects of

![Fig. 3](image)

An example of a drug release profile with the line indicated as “maximum desired level” being the toxic level and the line labeled as “minimum effective level” being the subclinical limit, while the grayed region in the middle encompasses the therapeutic window. The blue dotted line represents conventional release in which multiple administrations of a drug are required, while the red curve shows a standard zero-order kinetics curve for a controlled release vehicle in which the drug concentration is maintained long-term within the therapeutic window. (Reproduced with permission from [79])
swelling on gel porosity, the degradation rate of the hydrogel, any change in pH of
the gel matrix due to the degradation of polymers, the amount of drug present in the
matrix, the diffusibility and solubility of that drug in the matrix and the release
medium, the adsorption/desorption equilibria between the drug and the hydrogel
building blocks, and the partitioning equilibria between the gel phase and the release
media [80]. The complexity of these dynamic interactions is significant, and
depending on the number of different contributors and the relative magnitude of
those contributors to regulating drug release kinetics, different mathematical models
have been developed to predict drug release rates. The most widely used of these
models and equations for modeling drug release from gels include the following:
(1) zero-order kinetics, relevant to reservoir-type vehicles (including reservoirs
surrounded by hydrogels, which act as a membrane to mediate release) [81, 82];
(2) first-order kinetics, relevant for purely diffusion-controlled release of drug from
hydrogels [83]; (3) the Baker-Lonsdale model, relevant for diffusion-based drug
release from spherical matrices such as gel microparticles or micro-/nanogels [83];
(4) the Hixon-Cromwell model, relevant when drug needs to dissolve within the gel
matrix prior to diffusion-based release [83]; (5) the Korsmeyer-Peppas model,
relevant to account for drug-polymer affinity-retarded diffusion of drug through a
hydrogel [83, 84]; and (6) the Hopfenberg model, relevant to describe hydrogels that
undergo heterogeneous surface erosion during the release process [83]. Of these
models and empirical formulae, the most prominent within the hydrogel community
include zero-order kinetics for reservoir-type delivery systems (primarily for
hydrogels incorporated into transdermal devices), first-order kinetics for hydrogels
with no specificity to the drug (i.e., only diffusion-controlled release), and the
Korsmeyer-Peppas model for hydrogels with non-specific affinity for the drug (i.e.,
both partitioning and diffusion-controlled release). Use of these models requires
knowledge or measurement of the diffusion coefficient of the drug within a gel,
which can be assessed using a diffusion cell in which a known thickness of hydrogel
separates the source (the drug of interest dissolved in a suitable buffer) and receiver
(containing only the buffer); the appearance of the drug in the receiver over time can
be correlated to the diffusion coefficient of drug in the gel [85].

Hydrogel-based drug delivery vehicles have been successfully fabricated to
deliver drugs via a range of different administration routes. While this is by no
means an exhaustive list, it is meant to highlight the key areas in which hydrogels
have been applied commercially for drug delivery.

2.3.1 Oral
Hydrogel-coated capsules, commonly referred to as GelCaps, have been widely used
to deliver drugs orally. The gel capsule typically consists of a stiff gelatin-based
hydrogel which surrounds either dispersed solid drugs or a liquid core containing
drugs and some suitable dispersing agent (often non-crosslinked poly(ethylene
glycol)). The presence of the gel layer enables facile swallowing via its lubrication
effect as well as controlled release over time due to drug diffusion through the
capsule and the slow degradation of the capsule in the digestive tract [86]. A variety
of pH-responsive hydrogel coatings or delivery vehicles has also been designed in an
attempt to prevent or minimize release of a drug cargo in the stomach (pH 1–2) but promote release in the small intestine (pH 5–6), where most drugs need to be released and stable in order to be absorbed by the body in a bioactive form. Poly(acrylic acid) or PAA and related polymers have been widely used for this purpose based on their protonated/collapsed state at acidic pH but ionized/swollen state at near-neutral pH values characteristic of the intestine [87], with natural polymers such as pectin [88], chitosan [89], and carboxymethyl cellulose [90] that display the similar ionization equilibria also used. Some buccal hydrogel-based delivery vehicles (e.g., delivery vehicles immobilized in the mouth) have also been developed with mucoadhesive hydrogels based on chitosan or Pluronics [91], formulations that have the advantages of self-administration and more direct entry of the drug into the systemic circulation compared to conventional oral delivery methods.

### 2.3.2 Topical
Topically applied hydrogel formulations are most commonly used for wound healing applications, the fundamentals of which are covered in depth in Sect. 2.4. The combination of the beneficial properties of gels to absorb wound exudates and maintain local hydration [92] together with their capacity to control the release of drugs that can promote sterility at the wound site (e.g., antibiotics or antifungals) and/or active cues toward tissue regeneration (e.g., steroids and/or growth factors) [93] offers significant benefits in terms of facilitating more effective wound closure while avoiding infections.

### 2.3.3 Transdermal
While the hydrophobic stratum corneum lining the skin is a barrier to the use of hydrophilic hydrogels directly for transdermal delivery, hydrogels have still been used in two contexts for skin delivery. First, hydrogels can be used as carriers for nanoparticles that can penetrate into the skin via cellular transport mechanisms, prolonging retention of nanoparticles at the targeted site as well as extending the time period over which the drug is delivered [94]. Second, hydrogels can be used in the reservoir of a transdermal patch to prolong the release of drug through the porous membrane into the skin and/or improve the suspension of an insoluble drug inside the patch through the increased viscosity of the continuous phase [95]. The capacity of a hydrogel patch to continually hydrate the skin is also beneficial to avoid skin irritation, often observed with longer-term patches [96]. Hydrogel-based micro-needles have also been recently developed that can penetrate through the outer layer of the skin and quickly form a continuous and unblockable aqueous conduit between the contents of the microneedle and the sub-dermal tissue, avoiding some of the inflammatory challenges often observed with conventional microneedle delivery vehicles [97].

### 2.3.4 Vaginal
Hydrogels have long been used as intravaginal delivery vehicles given their capacity for easy application, their favorable mechanical properties relative to viscous solutions that prevent or minimize leakage of the delivery formulation following
administration, and their capacity for mucoadhesion [98] to promote both retention and intimate contact with the vaginal tissue to improve drug absorption. Thermoresponsive gels that can selectively gel at the temperature of vaginal mucosal tissue (e.g., Pluronics mixed with mucoadhesive carbohydrates such as hydroxypropylmethylcellulose (HPMC) [99] or chitosan [100]) have been particularly commonly applied. Liposome-containing hydrogels have also attracted attention due to their combination of improved drug delivery rates (particularly for hydrophobic drugs) and their provision of a physical barrier for infective agents to transfer through the vaginal wall [101]. Given the different pH values of vaginal fluid (pH 3.5–4.5 in healthy patients) and semen (slightly basic, pH 7–8), pH-responsive hydrogels have also been designed to either selectively gel to provide a dynamic biological barrier [102] or burst release an anti-infective drug only upon semen exposure [103], both of which have been shown to reduce transmission rates of sexually transmitted diseases.

2.3.5 Ophthalmic

Drug-eluting contact lenses that can facilitate longer-term and more effective delivery of drugs to the front of the eye relative to traditional eyedrops have long been a topic of research interest. While some benefits have been identified based on simply soaking commercial lenses in drug solutions and allowing them to release via simple diffusion on the eye [104], more advanced nanocomposite drug delivery strategies in which liposomes [105], nanoparticles (NPs) [106], or drug-loaded degradable films [107] have been integrated inside contact lenses have achieved significantly longer delivery times coupled with more desirable release kinetics (e.g., zero-order in the case of the degradable film, in which the hydrogel encasing the film effectively acts as a membrane) [107]. In situ gelling and/or highly shear-thinning hydrogel systems have also been used to increase residence time in the tear film, with Pluronic thermogelling hydrogels [108] or hydrophobic self-associative polymers (typically containing a highly hydrated backbone polymer coupled with hydrophobic grafts) [109] most commonly applied. Hydrogels have also been used for delivery to the back of the eye, following either implantation in the subconjunctival space or injection into the vitreous humor [110]. Particular recent efforts have focused on injectable hydrogels based primarily on hyaluronic acid (a major component of the vitreous humor) for the delivery of anti-vascular endothelial growth factor (VEGF) inhibitors, aiming to extend the efficacy of intravitreal injections for treating acute macular degeneration [111].

2.3.6 Intranasal

Intranasal delivery offers the advantages of capacity for self-administration, rapid absorption due to the high local vasculature, the avoidance of first-pass metabolism, and the capacity for direct drug delivery to the brain that circumvents the challenges of the blood-brain barrier [112]. However, practical hydrogels for use in this context must be in situ gelling and ideally mucoadhesive to ensure their facile delivery (via spray/aerosolization) and subsequent retention in the nose. Formulations including both mucoadhesive and thermogelling combinations such as chitosan/
hydroxypropyl methylcellulose [113] or gellan gum/Carbopol [114] (the latter a lightly crosslinked poly(acrylic acid)-based polymer with known mucoadhesion properties) have been among the more effective formulations according to these requirements. Self-assembled nanogels have also been successfully used for delivering both drugs and vaccines to the nose, exploiting the capacity of mucous to capture nanoparticles and the capacity of nanoparticles to penetrate through the mucous and into (or through) the nasal epithelium [115].

### 2.3.7 Rectal
While generally less used than some other routes, hydrogels have advantages in rectal drug delivery given the capacity of this route to increase patient compliance versus injections and improve bioavailability versus oral delivery. For example, mucoadhesive hydrogels such as chitosan-catechol have been successfully used for the treatment of colitis [116], while hydroxypropyl methylcellulose hydrogels have been used for delivery of anti-seizure drugs in children [117], a key target population for rectal delivery formulations due to the inability of children to swallow pills.

### 2.3.8 Injectables
Many of the in situ gelling hydrogels based on ionic interactions, hydrophobic interactions, supramolecular chemistry, or dynamic covalent chemistry [118] have been used for drug delivery, primarily following either subcutaneous or intramuscular injection if systemic delivery is the objective. However, in other cases, injection can be targeted to the desired site of action, including sites of local pain (local anesthetic delivery) [119], cancer tumors (chemotherapeutic delivery) [119], or arthritis (immunosuppressive drug delivery) [120].

### 2.3.9 Micro-/Nanogel Delivery Vehicles
Nanogels offer the advantages of nanoparticles in the context of drug delivery (i.e., long circulation times, capacity for targeting to specific tissues, capacity for cell uptake) with the advantages of hydrogels (low non-specific protein adsorption, capacity to load hydrophilic drugs, mechanical deformability to penetrate tight junctions). Of particular note, Akiyoshi’s group has intensively investigated cholesterol-modified pullulan nanogels for delivering small molecules, peptides, proteins, and vaccines [121], with the high degree of control exerted over nanogel size coupled with the capacity of these nanogels to deliver both hydrophilic and hydrophobic payloads making this material particularly attractive. Smart thermo-responsive micro-/nanogels can also be used as an in situ gelation strategy for localizing delivery at a specific site [122]. Other micro-/nanogels have been developed to deliver drugs to treat a range of diseases including cancer, autoimmune disorders, or neurodegenerative conditions, among others [123] (Fig. 4).

### 2.3.10 Hydrogel-Mediated Smart Drug Delivery Vehicles
Environmentally responsive hydrogels or micro-/nanogels can be exploited both for promoting thermal in situ gelling responses (as described previously) and for enabling “smart” dosing of drugs in a disease-responsive [124] or doctor-/patient-
responsive manner. Glucose-responsive hydrogels, gel microparticles, or nanogels for insulin delivery have been most commonly explored [125–128] although a range of other chemoresponsive hydrogels (e.g., antigen-responsive or molecularly imprinted hydrogels) have been similarly applied [129]. Thermoresponsive hydrogels have been applied to facilitate triggered burst release of drugs upon hydrogel heating based on the convective transport of water (and dissolved drug) from the hydrogel upon activation [130]. Alternately, hydrogels can be used as gates for the on-demand release of drugs from a reservoir in response to a particular chemical signal (e.g., insulin with glucose) [131] or remote signal (e.g., membranes featuring a combination of magnetically responsive super paramagnetic iron oxide nanoparticles or SPIONs and thermoresponsive nanogels that can be remotely heated via an oscillating magnetic field) [132].

2.4 Barrier Materials

Hydrogels have been frequently used as biological barrier materials by exploiting their high interfacial hydrophilicity and thus low bioadhesion to prevent undesired tissue adhesion following surgery due to fibrin deposition. The most common scenario in which hydrogels are used in this context is to prevent peritoneal adhesions following cardiovascular or abdominal surgery. Such adhesions between the peritoneal wall and underlying organs (most commonly the intestine) often become sites of postsurgical infection and account for 1% of all hospital admissions [133]. Relative to the viscous polymer solutions traditionally used for such applications [134], hydrogels have substantially longer residence times at the barrier site while also retaining more water per unit volume, facilitating longer healing times before the barrier degrades away; relative to lyophilized sheets or sponges of watersoluble polymers [135], hydrogels (and in particular injectable hydrogels) offer improved coverage of all possible adhesion sites due to the capacity of the precursor polymers to flow and fully wet the tissue interface prior to gelation. Kohane’s group has contributed significantly to developing injectable hydrogels for this purpose, including hydrazone-crosslinked carboxymethyl cellulose (CMC)/dextran hydrogels [136].
hydrazone-crosslinked hyaluronic acid (HA)-based hydrogels [137], and highly shear-thinning physical hydrogels based on rheologically synergistic physical blends of hyaluronic acid and hydroxypropylmethyl cellulose (Fig. 5) [138]. Loading such hydrogels with drugs such as glucocorticoids, anticoagulants, fibrinolytics, antibiotics, or steroids can exploit the capacity of such hydrogels for drug delivery to treat the underlying biological causes of adhesion formation in addition to providing a transient physical barrier [133, 139]. The mechanical properties of such barrier of hydrogels are typically less important aside from the requirement that the gels are stiff enough to withstand the typical shear forces at the peritoneal wall over the required healing time (typically 1–2 weeks).

Fig. 5 In vivo assessment of adhesion prevention by hyaluronic acid/hydroxypropyl methylcellulose rheological blend hydrogels in a rabbit double injury model: (a) application of the hydrogel by extrusion immediately following the second injury, (b) adhesion formation 7 days after repeated injury following hydrogel treatment, (c) adhesion formation 7 days after repeated injury following treatment with saline, showing substantially higher adhesion between the intestine and peritoneal wall. (Reproduced with permission from [138])
Several commercially available hydrogels have been marketed for postsurgical adhesion prevention, including Oxiplex® (an ionically crosslinked hydrogel comprised of CMC, polyethylene glycol (PEG), and calcium chloride) [140], Intergel® (an ionically crosslinked hydrogel comprised of HA crosslinked by ferric chloride [141], although clinical trial results have been mixed [142]), Carbylan-SX (formed by disulfide crosslinking of thiolated HA) [143], and Spraygel® (an in situ gelling PEG hydrogel) [144]. Photopolymerizable hydrogels, predominantly based on gelatin [145] or methacrylated PEG or PEG-block-poly(lactic-co-glycolic acid) or PEG-PLGA polymers [146, 147], and thermogelling hydrogels, primarily based on Pluronic/Poloxamer PEO-PPO-PEO block copolymers, have also been demonstrated to have efficacy for reducing postsurgical adhesions. Self-assembled particle-based hydrogels based on bacterial cellulose have further been explored, with the capacity of such hydrogels to retain large amounts of water suggested to be effective for their role as a physical adhesion barrier [148]. Other in situ gelling chemistries such as Schiff base interactions (e.g., N,O-carboxymethyl chitosan/aldehyde-functionalized HA) [149] or thiol-ene click reactions (e.g., multi-thiol and multi-ene PEG) [150] have also been explored with early promise, although further investigation is required to confirm their utility relative to the other more widely investigated materials.

2.5 Wound Healing

The skin is the largest organ on the human body and is prone to damage such as burns, wounds, and ulcers through exposure to the environment. The two most frequent challenges involve the treatment of burn or laceration victims [151] or the treatment of diabetic or pressure ulcers [152]. The level of treatment required for treating such damage depends on the depth of the wound into the epidermal and dermal layers, the location of the wound on the body, the amount of area covered by the wound, the longevity of the wound (i.e., chronic or acute), and the nature of the wound (i.e., open or closed). Common methods of addressing such injuries include auto-/allografts, tissue-engineered skin substitutes, simple wound closure (i.e., suturing), and wound dressings, the traditional woven versions of which are significantly limited by potential infection and adhesion to the wound site. In contrast, hydrogels offer an attractive option for wound healing/tissue regeneration [153]. A hydrogel can seal the wound to prevent exudate from leaking out (bioadhesive) and bacteria/fungi from accessing the wound (antibacterial/antifungal), keep the system hydrated, absorb exudate and inflammatory media (high swelling ratio), have sufficient mechanical strength to remain intact over the required period, resist the applied stresses faced (including physical handling), allow proper gas exchange, decrease the healing time compared to normal physiological process (gas permeable), and avoid provoking a large inflammatory reaction that would result in adhesion between the wound dressing and the underlying wound that could result in re-irritation of the site and/or patient discomfort (low protein adsorption) [154]. Compared to synthetic adhesives, hydrogels typically offer lower adhesive strength but also lower
inflammation and improved capacity to absorb exudate and/or deliver active agents to assist with the wound healing process [155–157].

Common types of hydrogels used for wound healing include synthetic polymer-based gels like poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and poly(urethanes) (PU) as well as bio-based, polymer-based gels such as dextran, silk, gelatin/collagen, alginate, cellulose, and chitosan [158–160]. To address the key challenge of anti-infective properties, nanocomposite hydrogels incorporating silver nanoparticles (AgNPs) have been successfully demonstrated to inhibit bacterial and fungal growth [161], although the incorporation of copper ions, zinc oxide [162], and/or charged polymers (e.g., zwitterionic or quaternary ammonia grafts) [163] also assists in maintaining sterility. Multifunctional hydrogels can also be designed that combine the favorable properties of wound healing hydrogels with the release of therapeutic agents in order to accelerate healing and/or deliver anti-infective drugs, further controlling the microorganisms’ growth at the site of the wound [164].

3 Personal Care Products and Cosmetics

A personal care product describes any substance used for the purpose of personal grooming and hygiene [165], while a cosmetic has been defined by the US Food and Drug Administration (FDA) as an “article an intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body...for cleansing, beautifying, promoting attractiveness of altering the appearance” [166]. Many commercial and household products can be classified as personal care products or cosmetics, including skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial makeup, hair colors and dyes, deodorants, mouthwashes, and toothpastes [167]. Newer markets are being addressed as novel chemistries are developed, such as the use of smart supramolecular hydrogels [168] and/or hyaluronic acid-based gels [169] for anti-aging/anti-wrinkling products.

While many traditional cosmetics are based on creams, aerosols, and other hydrophobic and often colloidal delivery forms, hydrogels can play a role in such materials as viscosity enhancers to improve the delivery (i.e., the emollient feel of a cosmetic) and/or introduce additional viscosity to reduce the rate of emulsion or suspension instability [165]. Nanocomposite hydrogels containing liposomes [170], block copolymer self-assembled nanoparticles [171], and other nanoscale/microscale emulsions or particles capable of loading hydrophobic drugs [172–174] offer the dual benefits of prolonged drug delivery of molecules likely to penetrate into the skin (small hydrophobes) coupled with the capacity to retain moisture. Most hydrogel-based additives for these and other cosmetic applications apply polysaccharide-based gels since they are abundant in hydroxyl groups (and are thus moisturizing), are good rheological modifiers, produce inert degradation products, and promote sustainable manufacturing practices [175–177]. “Green” cosmetics based on replacing oil-based materials with hydrogel-based materials have also attracted interest (e.g., tamarind seed-extracted polysaccharides coupled with
hyaluronate and xyloglucan as a cosmetic formulation for skin creams [175]) based on consumer trends.

Hydrogels have been heavily used in the diaper and sanitary pad industry for over 30 years, with superabsorbent polymers (SAPs) particularly broadly employed due to their favorable water retention properties and thus capacity to absorb bodily fluids, wick moisture away from the skin, and prevent rashes or skin discoloration [178]. While Harper [179] and Harmon [180] filed patents for superabsorbent hydrogels in 1966, one of the earliest SAP hydrogels used for personal care products was based on starch-g-polyacrylate for use in feminine napkin/pad hygiene products [181]. The first use of SAPs in the diaper industry was proposed by Umicharm in 1982 in Japan, allowing the production of diapers with less leaks and a more ergonomic design that assisted also in suppressing the colonization of germs and the risk of fecal contaminations [181]; current leading diapers continue to use similar SAPs in their design [182]. More recently, to address the challenges associated with the ecological fate of disposable diapers, degradable SAP hydrogels based on carboxymethyl cellulose, hydroxyethyl cellulose, or starch derivatives have been explored [183], although the total capacity for water retention remains lower than the best nondegradable polymers reported. The coupling of hydrogels with fibrous fillers has also enabled improvements in the retention of absorbed fluids into the gel even under external pressures and/or forces. For example, the combination of strong synthetic fibers (polypropylene, polyesters, copolymers of polyesters and polyamides) and a highly swellable acrylamide hydrogel can absorb 1 g of a 0.9 wt% NaCl solution in an hour even after being subjected to loads of 21,000 dynes/cm² [184].

Other compositions of hydrogels have been applied in other personal care applications. Hydrogels made from polymers like Carbopol (lightly crosslinked poly(acrylic acid), a SAP hydrogel), hydroxypropyl (methyl)cellulose, methylcellulose, Poloxamers, carrageenan, and alginate have been used to create high hydrated ointments and creams to maintain moisture [181, 185, 186]. Combinations of hydrogels with lipids, often also containing glycerin and/or short alkylglycols as compatibilizing solvents, have also been explored, with the lipid component of the formulations serving to enhance adhesion of the formulation to the hydrophobic skin. Lipids such as palmitic acid, oleic acid, and ceramides, all of which are natively found in the epithelium of human skin, have been found to be particularly effective additives.

4 Bioseparations

Hydrogels have been applied to bioseparations since the 1990s, leveraging the capacity of hydrogels to swell/deswell in aqueous solutions and exhibit relatively low non-specific adsorption [187]. The most common application of hydrogels in bioseparations is gel electrophoresis, the standard technique used in biochemistry to separate and semi-quantitatively assess the type and amount of different proteins and/or DNA molecules within a complex mixture. Hydrogels based on polyacrylamide (polyacrylamide gel electrophoresis, or PAGE) are most commonly used due
to their controllable pore sizes and ease of manufacturing, with the surfactant sodium dodecyl sulfate (SDS) typically added to the test solution (SDS-PAGE) to equalize the surface charge of all proteins/DNA being analyzed and thus promote protein separation via size alone (i.e., the size of the protein relative to the hydrogel mesh size). The electric field is applied to direct the diffusion of the biomolecule and accelerate the separation process. For very high molecular weight proteins or larger DNA segments with thousands to millions of base pairs, agarose gels are often used instead due to their larger average pore sizes and thus capacity to separate larger molecules [188].

Hydrogels can also be used for the purification of biomolecules, generally using one of two possible techniques. First, bioconjugation techniques can be used to graft antibodies for the desired antigen target to the hydrogel and provide affinity sites to promote antigen binding to the hydrogel. This approach is generally effective provided the 3D conformation of the antibody and the access to the active site are both maintained during the grafting process; however, subsequent recovery of the bound antigen can be challenging, particularly for stronger antigen-antibody binding pairs. Second, stimuli-responsive hydrogels triggered by temperature or pH (most common) have been exploited in order to dynamically tune the size selectivity of the hydrogel (via swelling/shrinking transitions) and/or the affinity of the hydrogel for the target molecules (via hydrophilic/hydrophobic transitions) [188]. For example, Feil et al. used a poly(N-isopropylacrylamide-co-butyl methacrylate) membrane to separate a mixture of uranine (MW = 376 g/mol), low molecular weight dextran (MW = 44,000 g/mol), and high molecular weight dextran (MW = 150,000 g/mol) by changing the temperature to alter the overall mesh size and thus the separation selectivity. Smart hydrogel pore-filled membranes can be used in a similar way to successfully separate proteins, enzymes, small solutes, polymer latex particles, viruses, and other biological contents [189, 190]. Alternately, the sol-gel transition observed in many thermo-responsive hydrogels can be leveraged to recover bound target molecules and/or resolved bands of specific biomolecules following a separation step. For example, thermoreponsive gel electrophoresis matrices have been reported that can solubilize upon cooling to enable effective “de-gelling” following the separation and thus facilitate the recovery of each separated protein [191, 192] in a spatially controlled manner [193] depending on where the gel is heated/cooled.

5 Environmental

The major application of hydrogels in environmental applications relates to their use as sorbents for heavy metals [194] or ionic dyes [195], both of which are frequent contaminants in industrial wastewater/effluents and must be removed prior to return of the effluent to the environment. The capacity of charged hydrogels for ion exchange can be exploited to replace lower affinity counterions (e.g., sodium) with heavy metal ions and/or ionic dyes that must be removed from water effluent [196]. The high swelling ratio of many ionic hydrogels also provides
high accessible surface areas for ion sorption, with superabsorbent polymers that typically contain both high charge and high swelling ratios (Sect. 3) being particularly attractive in this regard. Superporous hydrogels (SPHs) that combine high swelling capacity with macroporosity (typically templated based on similar strategies to those used to make macroporous tissue scaffolds, Sect. 2.1) are also highly attractive for similar types of sorbent applications given their higher accessible surface area compared to traditional SAPs. The earliest examples of SPHs were based on acrylamide, salts of acrylic acid, and sulfopropyl sulfate [187], while newer compositions are typically interpenetrating networks (e.g., divinyl crosslinked poly(acrylamide) interpenetrated with calcium-alginate) that maintain high swelling capacity but with significantly improved elastic properties [197]. It is this capacity for swelling that allows hydrogels to effectively compete with, or in some cases exceed the performance of, other types of micro-/macroporous sorbents (e.g., activated carbon) on a per mass basis [198] while avoiding the disadvantages of those alternative materials (e.g., processing costs, disposal costs, etc.) [194]. The capacity of hydrogels to swell and deswell as a function of ionization (i.e., pH) also offers the potential to regenerate hydrogels following their saturation with target heavy metals and/or dyes, with reuse over multiple (albeit not infinite) cycles observed in many cases [195]. The hydrogels must be highly stable for reuse to be effective, with nanocomposite-reinforced hydrogels often applied to improve gel toughness and thus the potential for repetitive sorption/desorption cycles [199]. Based on these collective properties, hydrogels can effectively address all steps of the required sorption cycle for continuous heavy metal/organic dye removal (Fig. 6).

The most common types of hydrogels used for heavy metal removal are based on biomass sources grafted with polyanionic polymers, with cellulosics, starch, and clay the most common biopolymers and poly(acrylic acid) or 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) the most common polyanions [200]. Hydrogels based on neutral but highly water binding polymers such as poly

![Fig. 6](image-url) Schematic of the use of hydrogels in the adsorption/regeneration process for binding heavy metal ions or organic dyes in wastewater. (Reproduced with permission from [195])
(vinyl alcohol) or polyacrylamide either grafted with polyanions [201] or derivatized to introduce anionic groups directly on the polymer backbone (e.g., sulfomethylation of polyacrylamide to form sulfonate groups) [201] have also been successfully used. Alternately, chelating grafts or even cationic grafts containing lone pair electrons can be used to bind cations through Lewis acid/base interactions, grafts that have been observed to offer the potential for higher selectivity for specific cations relative to simple ionic hydrogels [202].

For removing different types of organic dyes, the specific functional groups present on the hydrogel building blocks (e.g., –OH, NH₂, –SO₃H, –COOH, or –CONH₂) can be altered to control the affinity of the hydrogel for different targeted pollutants [200]. Cationic polymers such as poly(N,N-dialkylaminoethylmethacrylates), poly((3-acrylamidopropyl)trimethylammonium chloride) (PAPTMACl), or poly(diallyldimethylammonium chloride) (PDADMAC) are widely used for the sorption of anionic dyes [195], while hydrophobic comonomers will also often promote dye sorption since many of these pollutants are highly aromatic [203]. Other additives are often incorporated into particularly micro-/nano-sized hydrogels to enable their effective removal from the water to allow for regeneration and reuse [198], with magnetic nanoparticles (enabling physical and low-energy separation of the gels from the wastewater via the application of a magnetic field [204]) particularly useful in this regard. Other nanoparticles have also been added to create active remediation hydrogels that have the capacity to actively bind and degrade organic dyes, with titanium oxide nanoparticles that can degrade organic compounds when UV-activated being the most common [205]). The excellent review by Khan and Lo is recommended for a broad-picture look at the various polymers and additives used to prepare hydrogels for environmental sorbent applications [195]. It should be noted however that truly selective removal of specific pollutants is typically challenging based on competitive binding and thus steric interference to the binding of the target molecule(s) in real wastewater samples [195].

The efficiency of pollutant removal depends on the kinetics, overall capacity, and pH activity range of a given hydrogel [195]. In a typical sorbent hydrogel, the degree of swelling (related to the crosslink density plus the solvent affinity of the gel building blocks) primarily controls the equilibration kinetics while a combination of the degree of swelling and the chemical nature of the backbone polymer and/or crosslinker used to prepare the hydrogel regulates the sorbent capacity of the hydrogel. Note that the use of micro-/nanogels instead of bulk gels can significantly enhance the kinetics of the response given the higher surface area to volume ratio and lower average diffusional path length to accessing all hydrogel binding sites (albeit at the cost of requiring a subsequent separation step to isolate these particles from the effluent) [198]. The pH must be equilibrated in a range that maintains the desired ionization of any ionic functional groups on the hydrogel important in driving heavy metal/dye uptake, with functional groups exhibiting very broad or indefinite pH ranges of ionization (e.g., sulfonates instead of carboxylates or quaternary amines instead of primary amines) offering benefits for maintaining high activity over a broad range of potential wastewater properties [195].
6 Natural Resources

The main application of hydrogels in natural resources lies in enhancing the recovery of oil from mature oil wells. Once easily accessible oil is pumped out, the well is typically flood with water in an attempt to utilize the hydraulic pressure of the added water to drive out residual oil trapped in the smaller pores of the reservoir. If a gel or a viscous polymer is co-injected with the water to fill a significant fraction (typically up to one half) of the now-empty part of the reservoir, the high viscosity and/or elasticity of the gel phase results in any additional water pumped into the well being specifically diverted through the (lower viscosity) residual oil-containing pores [206], based on the lower resistance to fluid flow through these pores. Such a process typically enhances the total oil recovered from a well by 5–30% [207].

Materials useful in this context must be low cost (~0.5 kg polymer required per barrel of additional oil production), highly water binding even in the presence of substantial salt concentrations, resistant to mechanical degradation, highly stable over time at high temperatures, and insensitive to oilfield chemicals [208]. As such, inexpensive and highly hygroscopic linear polymers such as partially hydrolyzed polyacrylamide or xanthan gum are most commonly used. However, relative to viscous polymer solutions, hydrogels have been demonstrated to improve this process in two ways. First, the capacity of superabsorbent hydrogels for binding water and resisting pressure (via the elasticity of the network) facilitates similar viscosity buildups and resistances to flow at substantially lower total polymer concentrations. Conventional superabsorbent hydrogels are limited in this context given that most depend on ionic interactions to drive swelling, interactions that are strongly suppressed in the high-salt oil well environment; furthermore, since the polymeric additive is often added to the well over the course of several years as more and more oil is removed (and thus water-filled pores form), the stability of conventional SAPs at high temperatures over this timeframe is non-ideal. Instead, a variety of clay-based superabsorbent nanocomposite hydrogels have shown promise in this context, with the clay component substantially improving the thermal stability of the gel [209]. Double crosslinking strategies combining covalent and ionic interactions have also been applied to this problem, allowing for increases in the mechanical resistance and stability of the gels to both pressure and degradation while maintaining superabsorbent swelling properties [210].

Second, the use of particulate hydrogels and/or in situ gelling hydrogels can overcome the challenges associated with pumping highly viscous linear polymer solutions into narrow pores within oil wells, as both particulate gels and in situ gelation precursor polymers have substantially lower viscosities at the same overall mass concentration relative to bulk hydrogels [211]. In either case, the lower viscosity of the pre-gel materials can improve the plugging of alternate water flow pathways and thus promote more effective recovery of residual trapped oil while requiring less energy to administer. Hydrophobically functionalized polyacrylamides that can self-associate at rest but are shear-thinning on injection are of particular interest for this purpose, enabling facile injection but effective visco-sification once inside the well [212]. Alternately, ionic interactions have been used...
to crosslink partially hydrolyzed polyacrylamide/clay nanocomposites using a dynamic crosslinking mechanism that facilitates injection [213]. Microgels (again primarily based on polyacrylamides and derivatives) [214] have also been successfully used in this context, with high viscosities being achieved in the well either via latent crosslinking interactions or colloidal close packing at sufficient concentrations [215]. Microgel nanocomposites containing clay or other additives can provide similar benefits as observed on the bulk scale in terms of thermal resistance, swelling, and stability [216].

7 Catalysis

Hydrogels have been applied in both biological and chemical catalytic reactions due to their unique compositions and porosities. First, hydrogels can prevent the aggregation and thus maximize the accessible surface area of catalytic nanoparticles to the catalytic substrate [217], maximizing both the efficiency and the kinetics of the chemical reaction. Second, the porosity of the hydrogel (and thus the accessibility of the substrate for the catalytic surface) can be altered as the gel swells in a given solvent condition and/or responds to an external stimulus [218, 219], allowing for control of the rate of reaction by altering the diffusibility of both the reagents to and products from the active site. Third, specific functional groups integrated within the hydrogel matrix can serve as both chelating and capping agents for metal nanoparticles, promoting their stabilization and helping to protect them from potential oxidation/deactivation [220].

Hydrogel-embedded metal nanocatalysts (termed hydrogel-M, M for metal catalyst) are commonly implemented for improving the production of hydrogen gas via metal hydride-catalyzed hydrolysis (e.g., the hydrolytic dehydrogenation of ammonium borane) [221–224]. Nanoparticles may be prefabricated and entrapped within a gel or formed in situ following gel preparation, with several ex situ catalytic nanoparticle-hydrogel systems having been described in which nanoparticles are deposited and immobilized on hydrogel matrices [225]. As an example, NiNPs can be formed in situ within the hydrogels during the reduction of 2- and 4-nitrophenols and other nitro groups [222, 226, 227].

Nanoparticles can also be formed in situ inside hydrogels by pre-soaking the hydrogels in a solution of the appropriate metal salts and then adding a reducing agent (e.g., sodium borohydride (NaBH₄), hydrogen gas (H₂), citrate, or ethylene glycol, depending on the metal ions used) to precipitate the salts into metallic nanoparticles [220]. However, reduction of the metal into nanoparticles is not always required for catalysis. For example, 4-vinylpyridine hydrogels have been shown to form complexes with chromate ions that themselves can be used to catalytically oxidize benzyl alcohol to benzoaldehyde or alkyl alcohols into carboxylic acids, the latter of which is a very useful reaction in carbohydrate chemistry [228]. Extrapolation of these hydrogel-M systems for the degradation of both organic and watersoluble dyes/pigments, pesticides, and herbicides for environmental applications is also possible.
Other hydrogels can serve complementary roles in catalytic reactions. For example, hydrogels have been used to coat anodes and cathodes to control the movement of ions for various electrochemical processes, with the role of the hydrogel in promoting the dilution of ions at the electrical source having been demonstrated to increase the overall efficiency of electrochemical reactions [229]. Additionally, enzyme-loaded hydrogels can be used to facilitate hydrogel crosslinking and self-healing [230, 231] and/or catalyze biochemical reactions; as an example of the latter, aptamer-crosslinked polyacrylamide hydrogels with molecular recognition have been used to immobilize glucoamylase on a paper substrate and support cascaded enzymatic reactions for signal amplification and subsequent sensor readouts for point-of-care diagnostic devices [232]. Catalytic activity can also be applied in the context of hydrogel synthesis. For example, in the case of double network hydrogels, the first network can be used to catalyze the formation of the second using orthogonal chemistries, as shown in Fig. 7 [233].

8 Agriculture

Finally, hydrogels have been applied in various agriculture and horticulture applications. The most basic of these examples involves the loading of hydrogels with active agrochemical agents, either directly (e.g., simultaneous drug loading and gel synthesis) or indirectly via passive diffusion/solvent evaporation/centrifugation following gel formation. A variety of agriculturally active agents such as pesticides [234], fungicides [235], herbicides [236], and fertilizers [237] have been formulated...
in hydrogel delivery vehicles for field use. The most popular starting materials for preparing agricultural hydrogels include polysaccharides (facilitating high water retention and degradability) and superabsorbent polymers (SAPs) like poly(acrylamide) or poly(acrylic acid) [238, 239]. The use of SAPs is particularly of interest since the hydrogel can not only improve the release profile of the active agent but also promote a higher amount of water retention in the soil, beneficial to facilitate seedling emergence [240] and plant sustenance during drought conditions [241].

Controlled fertilizer release is the most common target of hydrogel formulations, enabling less frequent reapplication of fertilizer by farmers. Hydrogel coatings on fertilizer particulates are most widely used in slow release fertilizer (SRF)/controlled release fertilizer (CRF) formulations, with the coatings designed to protect the fertilizer from the soil microenvironment and release the contents over a time dependent on its degradation rate [242]. Alternately, to avoid any risk of loss of typically small molecule fertilizer compounds via leaching, oxidation, or evaporation, hydrogels can be designed that directly incorporate vital species for plants (i.e., N, P, K) into the hydrogel itself that can then be released gradually as the hydrogel degrades. As an example, Davidson and coworkers crosslinked a carboxymethyl cellulose (CMC) hydrogel with iron and calcium salts that degrades over time via ion exchange to allow for plant root-targeted delivery of various fertilizers [237].

Hydrogels can also be applied in other agricultural or horticultural applications. For example, Mori’s group has developed hydrogel formulations for promoting seed germination. Plant seeds are placed directly on top of agar hydrogels to maintain an environment with controllably high water content in order to maximize the probability of seed emergence, avoiding the inconsistent and/or extreme wetness issues that can induce root burn or seed damage in soil [243]. The biodegradability of the natural polymer-based agar (and in other applications or chitosan [240]) hydrogels makes them useful either as a plant starter (after which the plant is transplanted in normal soil) or for growing full-sized plants in some applications [243]. Similarly, somatic embryoids have been encapsulated in alginate hydrogels and used as artificial seeds, with the design and functionality of such “synseeds” representing an alternative pathway to forming plants with a higher resistance to desiccation [244].

9 Conclusions

Hydrogels have tremendous potential to solve technological challenges across a broad range of applications. The diversity of building blocks and crosslinking strategies available for forming hydrogels leads to a broad range of accessible properties that can be rationally engineered according to the needs of specific end uses. Current research aiming to directly address the existing drawbacks of hydrogels – including the limited capacity of hydrogels to bear loads, maintain high hydration long-term in dry environments, prolong out-diffusion of loaded molecules, and further reduce protein adsorption – is expected to even further
broaden the applications of hydrogel-based materials. Furthermore, a growing understanding of how to process hydrogels to enable them to be printed both in 2D and 3D, structured internally by user-friendly, rapid, and highly controllable protocols, and delivered non-invasively (particularly for biomedical applications) will both improve the functionality of hydrogels in current applications and open new application areas. Fundamental modeling work to better predict the structure and physicochemical properties of nanostructured hydrogels also has significant potential to improve our understanding of structure-property correlations and thus design materials that still meet the key criteria of hydrogels but exhibit diverse physical, mechanical, and/or biological properties.

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Stimuli-Responsive Membranes for Separations

Raja Ghosh

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Abstract
Synthetic membranes are increasingly being used for a varied range of applications, in industry, medicine, agriculture, environmental sciences, geoengineering, and medical diagnostics, to name just a few. In the chemical industry, membranes are more commonly used in separation processes. Such separations are usually carried out based on one of two basic working principles, i.e., relative permeability and relative sorption. Between the two, permeability-based separations are more common. The physical form and functionality of conventional membranes are not expected to change appreciably during a separation process. This is to ensure reliable performance during separation and thereby ensure consistent product quality. However, the ability to alter membrane permeability in a controllable and reproducible manner increases its scope and range of application. This includes membranes

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for sequential multiple component separation, controlled release membranes, antifouling membranes, self-cleaning membranes, and membranes for biomedical devices. Such unconventional membranes are generally referred to as “stimuli-responsive” or in some case “environment-responsive” or “smart” membranes. This chapter reviews different kinds of stimuli-responsive membranes, including both permeability-based and sorption-based membranes.

1 Introduction

Synthetic membranes are used for a wide range of applications in industry and medicine [1–4]. A membrane, broadly defined, is a thin barrier which separates two phases and allows selective passage of material through it. In some sense, a membrane could be thought of as a highly specialized filter. On one hand, a membrane could be used to purify extremely large volumes of material as in applications such as water treatment [5], while on the other, they could be used for regulating the release of miniscule amounts of drugs for the treatment of serious ailments such as cancer from delivery systems such as microcapsules [6]. Figure 1 shows the working principle of a reverse osmosis membrane used for desalinating seawater [7]. This figure summarizes the different forces responsible for transport of different species through a membrane. In a desalinating membrane, the principal driving force is a hydrostatic pressure drop which drives the flow of water from a high-pressure zone to a low-pressure zone through microscopic openings (or pores) present in the membrane. The salt electrolytes being bulkier cannot go through the membrane and tend to accumulate near the surface of the membrane. There is usually a fine balance between the amount of electrolyte accumulating near the membrane surface and that diffusing back into the bulk feed, this being dependent on the system hydrodynamics, i.e., how well the feed side is mixed. If significant amounts of electrolytes accumulate, their presence results in the so-called osmotic back pressure, which acts against the applied hydrostatic pressure. In a typical desalination process, the magnitude of osmotic back pressure could be very significant. Other factors and mechanisms typically at play in most membrane separation processes include Fickian diffusion, electrostatic attraction or repulsion, Donnan effect hindered diffusion and convection. Figure 2 shows a membrane-based osmotic pump [8] used for controlled release of drugs. Here, osmosis and liquid absorption are the main mechanism involved. For most applications, membranes are expected to demonstrate consistent permeability for a specific permeating species, i.e., for a given driving force, such as pressure or concentration difference, and operating condition such as temperature, the same amount of the species should go through the membrane per unit time. While the permeability of most membranes does change to some extent during operation due to factors such as concentration polarization [9] and membrane fouling [10], it is expected that this change is not very big. This is to enable the design and operation of reliable and reproducible processes with low run-to-run variability. However, there are situations where the ability to alter membrane permeability in a controllable manner significantly increases the scope and range of
Such membranes are referred to as “stimuli-responsive” or in some case “environment-responsive” or “smart” membranes [11–18]. The concept of a stimuli-responsive membrane is by no means a novel creation of the human mind. Almost all natural membranes such as the cell membranes that surround the innumerable cells in our body are stimuli responsive to varying extents. They precisely regulate what goes in or comes out of a cell based on very specific triggers and control mechanisms. For instance, they regulate the concentration of ionic species within our cells by controlled uptake and elimination through the utilization of different passive and active transport processes. Our cell membranes are dynamic in terms of their morphology and permeability and have, within them, very specific solute and solvent transport routes such as ion channels and aquaporins. Figure 3 summarizes some of the more important transport routes and mechanisms present in a typical cell. Controlled elimination and uptake of species are important in biological processes ranging from hemostasis and osmoregulation in individual cells to glomerular ultrafiltration and tubular reabsorption in our kidney.

Fig. 1  Desalination using reverse osmosis membrane

Fig. 2  Controlled release of drug from osmotic pump

application. Such membranes are referred to as “stimuli-responsive” or in some case “environment-responsive” or “smart” membranes [11–18].
Therefore, stimuli-responsive membranes currently used in medicine and industry are at best poor imitations of biological membranes, both in terms of complexity of form and function.

Stimuli-responsive membranes have been successfully used for many applications in the fields of separation science and technology [11–21] and drug delivery systems [5, 22–27]. The main motivation behind the development of stimuli-responsive membranes (see Fig. 4) is to be able to modulate the permeability of a species through the membrane by providing an appropriate stimulus such as a change in temperature [21, 28, 29], pH [30], ionic strength [28], light [31], voltage [32], current [33], or charge density [34] or by the addition or removal of specific chemical entities [19] to/from the immediate environment of the membrane. This chapter focuses on stimuli-responsive membranes used in separation processes. For the reader interested in stimuli-responsive membranes for drug delivery and similar biomedical applications, there are some excellent articles and reviews [6, 22–27]. The term “stimuli-responsive membranes” could also be broadly used to include pressure- or shear-sensitive material used in touchscreens and thin layers coated around sensors and actuators [35]. Unfortunately, any discussion on these “membranes” is beyond the scope of this chapter. Also not included are geomembranes [36] used in construction and geotechnical engineering and stimuli-responsive textile material [37] which are better discussed in other more relevant forums.

Stimuli-responsive membranes used in separation processes could be broadly categorized into two groups. The first group includes membranes that respond to a stimulus by altering the solute or solvent permeability, i.e., true stimuli-responsive membranes or stimuli-responsive permselective membrane [17, 19, 38–40]. The second group includes membranes, the selective passage of solutes through which is not governed by “permeability” but by other factors such as physical adsorption or chemical bond formation [13, 29, 41–44]. Membranes of this group could be called
apparent stimuli-responsive membranes or simply stimuli-responsive adsorptive membranes. These are basically adsorbents prepared in a membrane-like format, i.e., in the form of thin microporous sheets.

Stimuli-responsive membranes are generally prepared using two methods. The first method (see Fig. 5) involves combining a stimuli-responsive polymer with a standard membrane scaffold polymers to produce a copolymer blend [11, 45–50] which is then cast into a membrane using standard fabrication techniques such as deposition, coating, and phase inversion. With such membranes, the entire structure is stimuli responsive, i.e., the functional properties are directly built into the structure of the membrane. The second method involves the attachment of a stimuli-responsive polymer component to a pre-existing porous membrane scaffold [51–53]. Three different types of membranes can be prepared using the second method. The first type includes “grafted membranes” (see Fig. 6) which are made by attachment or growth of a stimuli-responsive polymer on/from the surface of the pre-existing scaffold membrane [47, 51, 53–60]. Members of the second type are referred to as “filled membranes” (see Fig. 7) which are prepared by filling the pre-existing porous membrane with a polymer solution followed by cross-linking to immobilize a stimuli-responsive polymer network domain within the pores [17, 61–66]. Members of the third type are frequently referred to as “coated
membrane,” and these are prepared by interpenetrating network formation (see Fig. 8) which effectively results in the formation of a thin stimuli-responsive layer over a pre-existing supporting polymer network [13, 29, 41–43, 67]. The immobilized polymer or polymer network described above could be tweaked using an appropriate stimulus to alter its swelling properties in the case of permselective membranes or its binding properties in the case of adsorptive membranes.

2 Stimuli-Responsive Permselective Membranes

Before discussing the specific methods for making stimuli-responsive permselective membranes, it is perhaps better to understand the different possible motivations behind making such membranes. In separation processes carried out using regular or non-stimuli-responsive membranes (see Fig. 9), two overall product fractions are obtained, i.e., the permeate consisting of all species that go through the membrane and the retentate consisting of species retained by the membrane. Therefore, such a process is not suitable for fractionating a multicomponent mixture. A stimuli-responsive membrane, the permeability of which could be sequentially changed using appropriate stimuli would be more suitable for such applications. A simplified scheme for multicomponent separation using stimuli-responsive membrane is shown
in Fig. 10. Using a membrane whose permeability could be changed in a sequential manner using multiple stimuli, multiple permeate fractions, each having a different composition, could be obtained using a single-membrane device. In order to carry out similar separations using fixed permeability (i.e., non-stimuli-responsive) membranes, several membrane devices arranged in the form of a cascade would be required [68].

The second type of application where stimuli-responsive membranes would be useful is the control or regulation of the concentration of chemical species within a compartment such as a chemical reactor [39, 40]. This application would require a stimuli-responsive membrane that shows a higher permeability when a substance has to be removed from the compartment and a lower permeability when it has to be retained, just as a cell membrane would regulate concentration of different species within a cell. The third type of application of a stimuli-responsive membrane is as an “antifouling” membrane [69–71]. Fouling refers to the normally irreversible binding of material present in the feed solution on the membrane surface or within the pores. Fouling is widely considered the Achilles heel of membrane separation as cleaning of fouled membranes is very difficult. In most current applications, fouled membranes are simply replaced. By contrast, a severely fouled stimuli-responsive membrane could quite easily be cleaned either by forward or back flushing of the foulant (see Fig. 11).

2.1 Copolymer Blend Membranes

The main advantages of a copolymer blend membrane include the ease and reproducibility of fabrication. Precise amounts of the different polymer components, i.e., the
scaffold and the stimuli-responsive polymer, could be blended using appropriate solvent systems and cast into membranes. Different casting methods such as coating followed by solvent evaporation, spray coating, coacervation-phase separation, and phase inversion could be used for preparing such membranes. Alternately, the different polymer components could be cross-linked together using appropriate linker molecules.

The main disadvantages with membranes made using the above approach include low permeability and limited stimuli-responsive properties. This is because the structural and functional properties of these membranes are intertwined. Due to this, the response of the stimuli-responsive polymer could be “quenched” to some degree by the scaffold polymer. Another significant disadvantage of using this approach is the limited range of stimuli that could be used with such membranes. Some of these disadvantages could be overcome by decoupling the fabrication of the scaffold membrane and the stimuli-responsive membrane using the different grafting, filling, and coating methods described below.

2.2 Grafting to Membranes

This is the easiest strategy for making a grafted stimuli-responsive membrane. An appropriate pre-existing porous scaffold membrane is selected, and preformed stimuli-
responsive polymers are chemically bonded or physically attached to it [47, 51, 54, 55]. However, physical attachment is less commonly utilized as the resultant membranes tend to be relatively more unstable than membranes prepared by chemical attachment, thereby limiting the operating stimuli windows such as temperature, pH, and ionic strength ranges. The surface of the scaffold membrane is first activated followed by the addition of a polymer possessing an active functional group. The location of the active functional group determines both the density of polymer attachment and the orientation of the responsive polymer. The main advantage of using this approach is the preciseness of the identity and behavior of the stimuli-responsive polymer. After synthesis, a polymer could be purified to a high degree of homogeneity before being attached to the surface of the scaffold. By doing so, the permeability and responsiveness of the resultant membrane could be very precisely controlled. The stimuli-responsive polymer could be selectively attached in a brushlike configuration close to the pore entrance to create a surface (or lid) valve or could be attached in a brushlike configuration within the pores to create an internal valve. If required, the grafted polymer could be further cross-linked to create mesh-like valves. The main advantages of the brushlike configuration include high membrane permeability and a large range for the stimuli-responsive permeability. The main disadvantage with the brushlike configuration is low stability, i.e., drastic change in permeability in response to small change in the stimulus. When the polymer brushes are cross-linked, the magnitude of response is low but better controlled.

The main disadvantage of the grafting to approach is the low grafting density. When attaching preformed polymer chains to surfaces, steric hindrance limits the number of chains attached per unit surface area, particularly when grafting on concave surfaces. This problem can be solved by using the grafting from approach described next.

### 2.3 Grafting from Membranes

The grafting from approach involves growing polymer chains on the surface of the scaffold membrane [53, 56–60]. The first step typically involves the attachment of an initiator molecule on the surface. The polymer chain is then grown by controlled monomer addition and reaction. Different chemistries such as atom transfer radical polymerization [53, 56–58] and reversible addition-fragmentation chain transfer [59, 60] are used for the grafting from approach. Depending on the method used, different approaches are available for obtaining specific monomer composition and chain length of the responsive polymer. In addition to the potential for increasing the graft density, the grafting from approach is generally cheaper than the grafting to approach as the polymer purification step is not necessary.

The main disadvantage of the grafting from approach is the relatively greater heterogeneity of the attached responsive polymer when compared with the grafting to approach. On account of this, the magnitude and responsiveness of permeability are less controllable. Both the grafting to and the grafting from approaches rely on the covalent attachment of the responsive polymer on the membrane. On account of
this, the range of material that could be used for preparing stimuli-responsive membranes gets limited. This problem can be overcome using the filled and coated membrane approaches described next where covalent attachment of the responsive polymer to the scaffold membrane is not required.

### 2.4 Filled Membranes

A filled membrane [17, 61–66] could be prepared by filling the pores of a macro-porous scaffold membrane with a reaction mixture consisting of appropriate monomer/s, initiator, and cross-linker followed by thermal or photochemical polymerization and cross-linking. The resultant cross-linked bulk polymer domains are physically retained within the pores of the scaffold membrane due to their bulk, shape, and continuity (see Fig. 12). Better retention of the polymer can be ensured by using a scaffold with high degree of pore connectivity. A simpler but perhaps a bit more expensive variant of the above approach is to use a reaction mixture consisting of preformed polymers and cross-linker. By using this approach, better control over the magnitude and responsiveness of the membrane permeability could be ensured.

The main disadvantage of using the pore-filling approach is the relatively low permeability of the resulting membrane. Also, the selectivity of such membranes tends to be low. The problem of low permeability can be solved to some extent using the coated membrane approach discussed next.

### 2.5 Coated Membranes

The method for making coated membranes [13, 29, 41–43, 67] is indistinguishable from that used for making filled membranes. The main differences are in the nature

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**Fig. 12** Micrograph of filled stimuli-responsive membrane
of the supporting material used and the quantity of responsive polymer in the resultant membrane. With a filled membrane, the scaffold material is usually macro-porous in nature, while that used for making a coated membrane is microporous. With the filled membranes, the responsive polymer remains in the form of bulk domains, while with the coated membranes, the responsive polymer intermeshes with the scaffold polymer, effectively forming a thin stimuli-responsive coating (see Fig. 13). Therefore the coated membrane is somewhat structurally similar to an interpenetrating polymer network. Figure 14 shows the change in appearance of the microporous scaffold polyvinylidene fluoride (PVDF) membrane due to the addition of a polyvinyl caprolactam coating layer. A comparison of Figs. 12 and 14 shows that the effective porosity and pore size of a coated membrane is greater than that of a
filled membrane. Therefore, the permeability of a coated membrane is expected to be greater. Also due to the greater room for volume change of the responsive polymer, the range of permeability is also expected to be greater.

3 Stimuli-Responsive Adsorptive Membranes

Membrane adsorption or membrane chromatography involves the use of a stack of porous membranes as chromatographic media [72]. The main advantage of membrane chromatography over column chromatography is the high speed of separation due to the predominance of convective mass transport. Due to convective transport of solutes, the time taken by solute molecules to travel to and away from their binding sites is significantly lower than in resin-based columns. Membrane chromatography is typically faster than column chromatography by more than one order of magnitude. A shorter process implies higher productivity and reduced product degradation. Despite the obvious advantages of membrane chromatography, it is not that widely used, primarily due to lower solute-binding capacity compared to resin-based media. The binding capacity of conventional membranes used for membrane chromatography is low as solute binding is limited to the pore walls and external surfaces only (see Fig. 15). The use of coated and filled membranes discussed in the previous section offers the opportunity for creating three-dimensional binding domains within the membrane pores [13, 29, 41–43, 65, 67]. As shown in Fig. 15, a filled membrane offers a substantially magnification of available binding sites. With conventional membranes, solute binding takes places on a predominantly concave surface. With large solutes such as proteins and nucleic acids, this may result in significant steric hindrance and consequently low solute binding. With a filled membrane, the binding surface is predominantly convex which implies lower steric restrictions and thereby greater binding [65].

![Fig. 15 Comparison of solute binding on conventional and stimuli-responsive adsorptive membranes](image-url)
The use of a stimuli-responsive membrane allows greater flexibility in fine-tuning the adsorption/desorption process and thereby leads to the potential for greater resolution in multiple solute separation. For instance, conventional hydrophobic interaction membrane chromatography relies on the binding of solutes on media prepared by grafting hydrophobic ligands such as phenyl, butyl, and octyl on support material. Such media is always hydrophobic, and therefore the selectivity in solute-solute separation is limited. As shown in Fig. 16, the selectivity of such separation could be significantly enhanced using a stimuli-responsive membrane with tunable hydrophobicity, prepared by grafting polymers such as polyethylene glycol and polyvinyl caprolactam on appropriate scaffold membranes [13, 29, 41–43, 67]. In the presence of lyotropic salts, such polymers exist in a collapsed state, and therefore the membrane surface is effectively hydrophobic and thereby suitable for binding hydrophobic solutes. In the absence of lyotropic salts, these polymers exist in an extended hydrophilic state. Therefore, hydrophobic solutes are released from the surface of the membrane. The recovery of solutes from such stimuli-responsive surfaces is far greater than that from conventional hydrophobic interaction media. Moreover, the change in the hydrophobic-hydrophilic property of the stimuli-responsive membranes is generally graded; thereby high-resolution separation can be carried out.

4 Conclusions

Conventional membranes are suitable for binary separation, i.e., where a feed material has to be fractionated into two components, the permeate and the retentate. On the other hand, stimuli-responsive membranes whose permeability could be changed in a sequential manner using multiple stimuli are suitable for carrying out multicomponent separations. This opens up endless possibilities about how a separation process could be designed and operated. It also increases process efficiency by
replacing a multi-device process with a single device one. Stimuli-responsive membranes could potentially be used for the regulation of concentration of a chemical species within chemical reactors just as the cell membrane regulates the concentration of different species within a cell. The same principle could be applied for regulating species concentration in bioreactors, such as those used for mammalian cell culture or tissue engineering. Membrane fouling is widely considered the Achilles heel of membrane filtration. In most conventional membrane processes, a membrane needs to be frequently cleaned, and this increases the cost of production. Stimuli-responsive membrane can function as good “self-cleaning” or “antifouling” membranes as they could quite easily be cleaned either by forward or back flushing of the foulant in conjunction with an appropriate stimulus which “opens up” the membrane. Using such membranes, separation processes could easily be carried out in a sustained or semicontinuous manner by implementing some simple in-process cleaning strategies. Stimuli-responsive membranes used in separation processes could be broadly categorized into two groups: (a) true stimuli-responsive membranes or stimuli-responsive permselective membranes that respond to a stimulus by altering the solute or solvent permeability, and (b) apparent stimuli-responsive membranes or stimuli-responsive adsorptive membranes, the selective passage of solutes through which is not governed by “permeability” but by other factors such as physical adsorption or chemical bond formation. Stimuli-responsive membranes could be prepared by “copolymer blending,” i.e., by mixing a stimuli-responsive polymer with a standard membrane scaffold polymers and then casting this into a membrane using standard fabrication techniques. However, these membranes tend to have poor permeability and smaller responses to stimuli. Grafted membranes can be prepared using a “grafting to” or a “grafting on approach.” However, grafting limits the range of material that could be used as scaffold or as the stimuli-responsive polymer. This problem could be overcome by preparing filled or coated membranes. Filled and coated membranes are prepared in a similar way, but they differ in terms of the morphology of the stimuli-responsive polymer. In a filled membrane, the responsive polymer forms the bulk domain within the pores, while with coated membranes, the responsive polymer just coats the surface of the scaffold membrane. Overall, the field of stimuli-responsive membranes is a promising new area for research, development, and application.

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Specialty Application of Functional Biopolymers

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Abstract

In today’s world, we have been facing global challenges like pollution explosion, resource depletion, changing climate, and demand for food and potable water forcing us to move forward toward sustainable development. Among these hurdles, especially shortage of fresh water across the globe has made us to look toward more efficient, lower-cost, robust technology to decontaminate and
disinfect water from the source to the point-of-use. For this, membrane processes play a key role in water treatment technologies, due to their low energy consumption and involve no phase change. Compared to synthetic polymeric membranes, biopolymer-based membranes have drawn the attention of researchers due to its biocompatible, nontoxic, biodegradable, easily available, and environmentally friendly nature. The development of cellulose- and chitosan-based membrane put forward the new benchmark for desalination, heavy metal ion removal, dye rejection, wastewater treatment, drug delivery, wound healing, and other applications. Efforts are in progress to invest more and more time and research to make abundant use of these naturally occurring hydrophilic materials. These materials serve to be a cleaner substitute to the synthetic polymers that are currently in use and are dominating the market. This chapter gives a detailed overview of cellulose, chitosan, and their derivatives for membrane applications. Further, key scientific encounters are adopted on the path to industrially applicable membranes comprising these biopolymeric-based materials.

**Keywords**
- Biopolymer
- Blend membrane
- Pervaporation
- Heavy metal ions

**List of Abbreviations**
- BSA: Bovine serum albumin
- CA: Cellulose acetate
- CAB: Cellulose acetate butyrate
- CAP: Cellulose acetate propionate
- ECH: Epichlorohydrin
- EGDMA: Ethyleneglycol dimethacrylate
- FRR: Flux recovery ratio
- HOBt: Hydroxybenzotriazole
- HPC: Hydroxypropyl cellulose
- MTBE: Methanol–methyl tert-butyl ether
- NIPS: Induced phase separation
- P(VP-co-AA): Poly(vinylpyrrolidone-co-(acrylic acid))
- P(VP-co-Vac): Poly(vinylpyrrolidone-co-(vinyl acetate))
- PAA: Poly(acrylic acid)
- PAN: Polyacrylonitrile
- PEG200DMA: Polyethyleneglycol 200 dimethacrylate
- PEG600DMA: Polyethyleneglycol 600 dimethacrylate
- PEI: Polyethyleneimine
- PPEES: Poly(1,4-phenylene ether ether sulfone)
- PVA: Poly(vinyl alcohol)
- PVP: Polyvinylpyrrolidione
- PWF: Pure water flux
- RAFT: Reversible addition-fragmentation chain transfer
- SMM: Surface modifying macromolecules
- TEEMA: Triethoxy ethyl methacrylate
The recent developments in current technologies are focused on bio-based materials, as they can provide sustainable, eco-efficient, and green product for the next generation [1, 2]. Biopolymers are obtained from naturally available materials such as mushrooms, wood, plant-derived materials, and crustacean shells with well-defined structures [3]. The functional biopolymers such as cellulose acetate, starch, chitosan, lignin, and its derivatives have been extensively investigated the greener way to produce the actively used products. Moreover, these functional biopolymers have several environmental benefits compared to synthetic polymers, as they raised detrimental human health and ecological issues. The progress in polymer science extends possible applications range from biomedical, agriculture, paper, water treatment, corrosion prevention, textile, membrane science, and other industrial applications. Since, bacterially or plant-extracted biopolymers are strictly biodegradable, biocompatible, and having the abundant number of polar function group for facile chemical modification, to further improve the material physico-chemical properties. A broad classification of biopolymers is presented in Fig. 1.

Novel functional bio-based materials promise to deliver high-performance, environmentally friendly biopolymers that can potentially replace some of the synthetic materials in various applications.

**Fig. 1** The classification of biopolymers (source: Diagnostyka 16(4):63–70, 2015)
Particularly, present research in membrane separation process is now aiming more on functional biopolymeric materials with well-defined structure and properties [4]. Novel biopolymer membranes facilitate separation based on driving forces like electrical gradient, potential gradient, temperature gradient, or pressure gradient. Therefore, surface physicochemical properties and appropriate functional groups offer effective interaction with wide spectrum of applications, which include removal of toxic metal ions, hazardous organic compounds, dechlorination, and pervaporation with tunable hydraulic permeation [5]. The various functional biopolymers obtained from natural resources such as cellulose, chitosan, starch, and alginate have been extensively employed for sensor devices, artificial muscles, controlled drug release, membrane separation, and other applications. Most importantly, these naturally occurring functional biopolymers are having good film-forming capacity, chemical resistance, and proper responses to changes in external physicochemical conditions [4, 6, 7]. This chapter gives an insight into fundamentals of functional biopolymers for the membrane applications. The presence of reactive polar functionality in biopolymers offers facile chemical modification and imparts hydrophilicity to the membrane; these characteristic properties aid in higher permeation flux and selectivity toward the large variety of contaminants and other applications. This chapter also describes a detailed overview of biopolymer-based membranes for desalination, heavy metal removal, dye rejection, antifouling, and pervaporation applications.

2 History of Membranes

Synthetic membranes are extensively employed today in various technically and commercially related separation processes including desalination, water treatment, and gas separations. They are also key components in preparation of artificial organs, drug delivery devices, energy conversion, and storage systems. The earlier investigation of membrane phenomena and the discovery of osmosis dates back to the middle of the eighteenth century [8]. Nollet was the first to describe the effect of osmotic pressure on semipermeable membranes. More methodical investigation on mass transport in semipermeable membrane was conducted by Graham. The first synthetic semipermeable membrane was prepared by using cupric ferrocyanide in thin layer of porous porcelain to understand the osmosis phenomenon. Later, Fick and coworker illustrated the theoretical behavior of osmosis, understanding of osmotic phenomena, and mass transport through the semipermeable membrane. However, most of the developments in the membrane science and its phenomenon were theoretically interpreted in the early nineteenth century [9]. Thereafter, membrane science and technology move into a new phase. Bechhold discovered an approach to prepare the first artificial membranes by immersing a filter paper with a solution of nitrocellulose in glacial acetic acid. Nitrocellulose membranes were also the first marketable membranes that became accessible in 1937 in a series with various pore sizes. This development changed membrane field drastically from 1950 onward, when the real use of membranes in technically significant approach became
Upgrading of synthetic asymmetric membrane at the University of California by Loeb and Sourirajan (1962) for the separation of salt from seawater has led to a substantial development in the membrane science and technology. Since then, the application areas have been extensively spread out in many areas such as beverage, food, pharmaceutical, electronics, paper, textile, etc. Membranes have been generally used in the wastewater treatment for the recovery of valuable materials like dyes, metals, and other dissolved components present in aqueous media. Drinking water treatment is another key area where membrane separation processes have recently been introduced [11]. Popularity of the membrane technology in environmental applications has emerged due to the potential scarcity of fresh water and the stringent environmental regulations. Meanwhile, the quick growth of the membrane material science over the recent years has also assisted the widespread use of membrane technology. Due to the availability of membranes from a wide range of materials with different structures and properties, including synthetic polymers, cellulose- and chitosan-based natural products, and others like inorganic, ceramic, and metals [12], the membranes are broadly classified into:

(i) Biological membranes
(ii) Synthetic membranes

Generally, most extensively used synthetic polymers for the fabrication of membranes are polyether sulfone, polysulfone, polyvinylidene fluoride, polyamide, polypropylene, polyimide, polyacrylonitrile, polyethylene, polytetrafluoroethylene, and polyvinylchloride. Most of these synthetic polymers are hydrophobic in nature, thereby showing less affinity toward water molecules and offering higher resistance to mass transfer across the membrane. The hydrophobic membranes are also prone to have higher fouling propensity and less membrane shelf-life during the separation process. Hydrophobicity, fouling, higher hydraulic resistance, and other surface properties decrease the performance, efficiency, and selectivity and increase the membrane cost. In addition to this, stringent environmental policy in the current scenario made us looking forward for hydrophilic, biodegradable, and efficient membrane matrix for the real field applications. In order to overcome the drawbacks of synthetic polymer membrane, naturally occurring functional biopolymers such as sodium alginate, cellulose acetate and its ester derivatives, and chitosan and its derivatives have been attracting the attention of researchers [10, 11]. These naturally occurring polymers with nontoxic and biodegradable nature plays a vital role in the membrane technology. Moreover, thin film-forming ability and restricted mass transport of biopolymers and derivatives offer excellent membrane materials and find its applications in the various fields. Chitosan, cellulose acetate, and its derivatives have attracted significant attention due to their biological activities such as antimicrobial, antitumor effects, immunity-enhancing, hypocholesterolemic, drug delivery, etc. [13]. Application of biopolymer into membrane-based separation technology started from the past three and half decades. During that time itself,
biopolymer proved to be a sustainable membrane material for microfiltration, ultrafiltration reverse osmosis, nanofiltration and pervaporation applications (Fig. 2).

3 Biopolymers as Membrane Material

Natural biopolymers have been attracted as one of the extensively used materials for membrane-based separation process [14]. The most important advantages offered by these materials comprise of the following:

(i) Hydrophilic nature of these polymers exhibited by the presence of polar functional groups such as –OH, –C=O, –NH in the glucose unit.
(ii) Presence of abundant numbers of reactive functional groups in the polymer backbone offers facile chemical modification, which is easy to alter the physicochemical properties.
(iii) Their large-scale availability and relatively low cost.
(iv) Flexible structure of the polymer chains and their chemical reactivity.
(v) Biocompatibility and biodegradability.

\[\text{Fig. 2} \quad \text{The schematic representation of (i) membrane process and (ii) pressure-driven membrane separation. (source: Prozesstechnik)}\]
In recent years, these environmentally friendly benign as new class of materials wastewater treatment, heavy metal ion removal, desalination, reactive dye removal, pervaporation and protein separation.

4 Cellulose

Cellulose is one of the most abundant natural biopolymer materials. It is a polysaccharide obtained in cell walls of hemp, wood, cotton, green plants, and other plant-based materials. The ubiquitous sources of cellulose in nature are presented in Fig. 3. Cellulose is described as a macromolecule, an unbranched chain of variable length of 1–4-linked β-d-anhydroglucopyranose units [15]. The intramolecular hydrogen bonding interaction between oxygen and hydroxyl groups of adjacent repeating units stabilizes the linkage, which resulted in linear configuration of the cellulose. The van der Waals, inter- and intramolecular hydrogen bonding between the basic building blocks endorse parallel stacking of multiple cellulose chains forming primary fibrils that further aggregate into larger microfibrils (5–50 nm in diameter and several microns in length). Inter- and intramolecular hydrogen bonding interaction network creates cellulose a comparatively more stable polymer and gives the cellulose fibrils high axial stiffness [16]. Schematic representation of hydrogen bonding interaction is presented in Fig. 4.

Cellulose and its derivatives are the most traditional and primitive membrane materials which still continue to relish the propensity of being one of the most extensively employed membrane material due to its reusability and abundance. Other parameters include its chirality, hydrophilicity, selective transport,
Fig. 4  The schematic representation of hydrogen bonding interaction between cellulose. (source: Cellulose - Fundamental Aspects)
biodegradability, and low cost. However, unlike most of its hydrophilic counterparts, cellulose is insoluble in most of the common organic solvents and water due to extensive intrastand hydrogen bonding and intramolecular hydrogen bonding interaction, which is ascribed to the presence of an abundant hydroxyl groups in its backbone [13]. This gives emphasis to the need for proper derivatization or chemical modification of cellulose for its wide spectrum and versatile use as a potent membrane material. Facile chemical modification ability of cellulose significantly alters properties w.r.t. hydrophilicity, adsorption capacity, ion exchange, elasticity, or water uptake capacity, microbial resistance, mechanical strength, and thermal stability. The presence of reactive primary and secondary hydroxyl functionality in the cellulose building blocks offer possible sites for modification of cellulose and its derivatives. The possible modifications include esterification, oxidation, etherification, halogenation, and grafting of different polymers [17]. Commercially accessible cellulose derivatives include cellulose triacetate, cellulose butyrate, cellulose acetate, methyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, and water-soluble hydroxyethyl cellulose (Fig. 5). Also, cationic derivatives of cellulose comprising nitrogen has been reported in recent years; however, their applications in membrane preparation are yet to be explored.

Fig. 5 Structure of (i) cellulose acetate, (ii) cellulose acetate butyrate, (iii) cellulose triacetate, (iv) carboxymethyl cellulose, and (v) cellulose acetate phthalate
5 Application of Cellulose-Based Membranes

5.1 Nanofiltration and Ultrafiltration

First critical breakthrough in the field of asymmetric membrane preparation for water treatment was attained preparing cellulose acetate (CA) membranes via a phase separation method. Development of CA membranes for reverse osmosis process has explored the new approach for fabrication of various membranes to several applications such as microfiltration, ultrafiltration, nanofiltration, and pervaporation [6, 13, 15]. Generally, CA is exploited as one of the major cellulose derivatives for membrane application due to its good toughness, potential flux, low cost, stability, chemical resistance, biocompatibility, and biodegradable. Moreover, CA is easily soluble in most of the common organic solvents, and its hydrophilic nature offers in mitigation of membrane fouling.

Extensive investigation has been carried out to explore the performance of cellulose derivative with respect to surface properties, morphology, mechanical strength, and permeation behavior by altering the concentration, solvent, coagulation bath condition, bath temperature, and blending with other polymers. In 1997, Sabde et al. illustrated the preparation of ultrafiltration membranes from cellulose acetate butyrate polymer. The variation in casting solution environment such as concentration, composition, solvent evaporation, and bath temperature resulted in significant changes in the morphology, hydrophilicity, porosity, and permeation properties of resultant membranes [17]. Transport property of symmetrical CA membranes was described by using sodium chloride (NaCl) solution under unsteady-state reverse osmosis method. During this study, the concentration and diffusion coefficient of NaCl were taken into consideration. Concentration dependency of “D” can be described by considering two types of membrane modules (imperfect or porous and perfect or dense). The phenomenon was explained by the below equation

\[ D_{p,c} = D_d \exp(\gamma \times W_w) \]

where “\( D_{p,c} \)” is the diffusion coefficients of NaCl in porous part, “\( D_d \)” diffusion coefficients of NaCl in dense part, “\( \gamma \)” water content coefficient, and “\( W_w \)” water content in the membrane.

From unsteady-state analysis of diffusivity of the salt solution, the osmotic pressure on the membrane has significant influence on the diffusion coefficient and permeation properties. However, diffusion coefficient through dense part of the membrane “\( D_{d,c} \)” does not depend on water content in the membrane. Their whole averaged value \( D_{d,c} = 3.2 \times 10^{-4} \text{ m}^2/\text{s} \), which is close to Yasuda’s reported value of \( 6.5 \times 10^{-4} \text{ m}^2/\text{s} \) for CA film cast from an acetone solution [18]. Furthermore, Takagi et al. also reported the effect of streaming potential and charge density of CA membrane under aqueous NaCl solution environment. The significance of Donnan potential was about two orders higher in the charged membrane that of potential in the absolute value for NaCl solution of 0.1 mol m\(^{-3}\). It is well known that streaming potential is related to charge density on the membrane surface, while the Donnan
potential is related to charge density expressed in the volume density. The effect of membrane charge density and pore radius on transport of sodium chloride solution is presented in Fig. 6. Since the effective membrane charge density expressed in volume density increases with decrease in the pore radius and magnitude of Donnan potential depends on the pore radius [19].

Applicability of CA-based membranes for ultrafiltration and nanofiltration applications was extensively studied by many researchers, although they exhibit relatively low mechanical strength, chemical stability, and thermal resistance compared to synthetic polymer-based membranes. Most interestingly, pristine CA membranes are highly hydrophilic, impart negative charge density and higher water uptake capacity which are benefit of being highly resistant to fouling phenomena and economically viable. However, as a membrane material, they impart the drawback of low mechanical strength to withstand high transmembrane pressure and less chemical resistance toward wide range of pH. In order to overcome these drawbacks of CA material, the crosslinking of CA with trimesoyl chloride (TMC), glutaraldehyde, and dicarboxylic acid was carried out. The modified membrane exhibited enhanced chemical resistance against polar solvents and four times augmentation in tensile strength than those of existing CA membranes. Morphology of resultant membrane illustrates the formation of top dense layer with even pores size.

Fig. 6 The graphical representation of (a) membrane potential of CA membrane for NaCl solution and (b) the effective membrane charge density $\theta_{MD}$ and the free ion concentration within the membrane as a function of pore radius $r_p$ [19]
distribution compare to pristine CA membrane. In addition to this, substantial decrease in compaction phenomena during the filtration, due to their enhanced dense structure and mechanical strength [15]. To further enhance the membrane physicochemical properties, mechanical strength, and hydraulic permeation behavior, modification of CA membrane was carried out by adding polyethylene glycol 600 (PEG 600) as additive. The presence of different dosage of PEG 600 in CA membrane matrix resulted in significant changes in hydrophilicity, porosity, morphology, permeation, thermal stability, and other surface properties [20]. Ahmad et al. also prepared the CA/PEG-600 composite membranes via phase inversion method and further modified by in situ reduction of silver nitrate. The modification of membranes showed enhanced hydraulic permeation rate from 0.80 to 0.95 L/h. m². Moreover, presence of silver in the membrane matrix resulted in significant resistance toward the microbial growth during the prolonged period of filtration time [21]. In order to further enhance the performance, CA membrane surface was modified by using surface-modifying macromolecules (SMMs). The SMM acts as active skin layer during the process with tailored hydrophilicity, charge density, and selectivity and surface properties. For instance, SMM-modified CA membrane exhibited highest flux recovery ratio (FRR) up to 84.6% and lowest irreversible fouling ratio 15.4% compared to unmodified membrane (Fig. 7) [22].

Blending of CA with synthetic polymer like polysulfone, polyethersulfone, and polyvinylidene fluoride are also one of the important tools to enhance the mechanical strength, chemical resistance, and antifouling nature of resultant membrane. For this purpose, Sun et al. described the blending of CA membrane with polyethersulfone. The blended membrane exhibited excellent thermal stability up to 270 °C and good miscibility during the membrane preparation. The cross-sectional morphologies displayed that the blending of CA and polyethersulfone lead to the formation of larger finger-like macrovoids in the sublayer and higher surface porosity, which signified the higher hydraulic permeation and superior antifouling behavior [23]. Further, modification of CA membrane was carried out by blending with polyethyleneimine (PEI) and crosslinked through polyisocyanate. The resultant membrane showed higher mechanical strength, lower membrane hydraulic resistance, higher water flux, and better antifouling nature compared to pristine membrane. The blend membranes showed specific surface area of 12.04–24.11 m²/g and pure water flux (PWF) of 10–50 ml/cm² min with porosity of 63–75% [24]. Saljoughi and Mohammadi studied the effect of CA/polyvinylpyrrolidone (PVP) blend membrane on morphology, water uptake capacity, hydrophilicity, and permeation properties. They found that initial concentration of PVP (0–3 wt.%) in the casting solution enhances the macrovoids in sublayer and hydraulic permeation of resultant membrane. However, further increment in additive dosage (3–6 wt.%) resulted in suppression of macrovoids and decrease in permeation rate [25]. Similarly, Sikder et al. prepared the CA/polysulfone blend membrane for separation microbial cells lactic acid fermentation broth in a continuous process. The prepared membrane with CA/ polysulfone weight ratio of 75/25 yielded a fermentation broth flux of 1430 L/m² h and a pure water flux of 1830 L/m² h at around 1.5 bar TMP [26].
The blending of CA and polymer membranes was also subjected for the separation of detrimental heavy metal ions and protein from the aqueous solution. Extensive studies have been carried out by adding inorganic fillers such as bentonite kaolinite, silica, and alumina nanoparticles in to the cellulose-based membrane matrix. The composite membrane resulted in improvement in the surface properties, hydrophilicity, mechanical strength, chemical stability, hydraulic permeation, and antifouling nature. Rakhshan and Pakizeh investigated the effect of functionalized silica (SiO$_2$) nanoparticles on CA membrane morphology and separation properties. The changes in the topological properties of CA membrane are shown in Fig. 8. The nanocomposite membranes were prepared via phase separation technique by inclusion of SiO$_2$ nanoparticles in the CA casting solutions in the range of 0.01–0.1 wt.%. The membrane with 0.05 wt.% of SiO$_2$ nanoparticles dosage showed considerable increase in triazines removal and salt rejection efficiency, due to substantial changes in the morphological features, porosity, and other physico-chemical properties [27]. In another study, multiwalled carbon nanotube (MWCNT) immobilized CA nanocomposite membrane was prepared via phase separation method. The presence of small amount of randomly oriented MWCNT into CA casting solution lead to substantial changes in membrane morphology, porosity, hydrophilicity, and physicochemical properties [28]. The presence of 0.0005 wt.%,

![Fig. 7](image-url)
0.005 wt.%, and 0.01 wt.% MWCNT/CA nanocomposite membrane showed average pore size distribution of around 25–180 nm. The salt retention with differential pore volume and pore surface areas is presented in Fig. 9. Dasgupta et al. fabricated the thermally stable CA membrane by incorporating titanium silicon oxide (TiSiO₄) nanoparticles into the matrix. Nanocomposite membrane exhibited improved hydrophilicity, hydraulic permeation, dye rejection, and antifouling characteristic. The effect of TiSiO₄ membrane properties and performance is presented in Fig. 10. Hydraulic permeation rate of the membrane enhanced up to from about 55 lm⁻² h⁻¹ to 92 lm⁻² h⁻¹ with the corresponding rise in the operation temperature from 20 °C to 90 °C [29]. Similarly, Quirós et al. illustrated the fabrication of CA nanocomposite membrane comprising of copper and silver nanoparticles to improve the performance and antimicrobial properties. Incorporation of these nanoparticles offered resistance to the microbial growth during the prolonged period of filtration time [30]. The solution intercalation technique with different solvents was employed to incorporate the sodium montmorillonite as a hydrophilic additive in to CA membrane matrix. TEM and XRD studies revealed that the delamination and dispersion of the sodium montmorillonite clay are attained when the solvent exhibits positive interactions with the clay. In addition to this, nature of solvent and concentration of polymer and additive have major role in controlling surface properties and morphology of CA and CA nanocomposite membrane [31].

Similar to CA membrane, other cellulose derivatives such as cellulose acetate phthalate and cellulose acetate butyrate also have been extensively used for the
preparation of ultrafiltration and nanofiltration membranes. Fu et al. demonstrated the applicability of cellulose acetate butyrate material for the fabrication of hollow fiber membrane through both nonsolvent-induced phase separation (NIPS) and thermally induced phase separation (TIPS) methods. They stated that morphology and surface properties of hollow fiber membrane can be significantly altered by varying the composition of coagulation bath and temperature [32]. In another study, the nanostructure, mechanical strength, and thermal stability of cellulose acetate butyrate (CAB)/polylactic acid (PLA) membrane was investigated incorporating the clay nanocomposites. Inclusion of organically modified montmorillonites into the blend has considerably enhanced the mechanical properties and thermal stability. More interestingly, XRD study revealed that the interlayer spacing augmented as the affinity between the modifier and polymers enhanced. When the difference of solubility parameter (indicator of affinity) between the PLA/CAB and modifier

Fig. 9 The variation of salt retention with differential pore volume and pore surface areas for pore widths of: (a) 1.7 nm, (b) 2.7 nm, and (c) 5.5 nm [28]
was the lowest, the organoclay acted like a compatibilizer between the immiscible PLA and CAB pair [33]. Further, the extensive investigation has been carried out on interaction of cellulose acetate butyrate membrane with organic foulants such as natural organic matters (humic acids), sodium alginate, and protein molecules. The outcome shows that fouling phenomenon mainly depends on interaction between the cellulose acetate butyrate membrane and foulants during the filtration. The sodium alginate solution exhibited more hydraulic permeation decline compared to bovine serum albumin (BSA) and humic acid solutions, due to pore-plugging and subsequent cake layer formation with high-molecular-weight components with sizes exceeding 0.1 μm (Fig. 11) [34]. Chatterjee and De fabricated the flat sheet nano-composite membranes by using cellulose acetate phthalate and activated granular alumina via phase separation method. The incorporation of activated alumina significantly improved the mechanical strength and hydrophilicity. Most importantly, fluoride ion removal capacity from contaminated water enhanced up to 91% and maximum adsorption capacity for fluoride was 2.3 mg/g at room temperature [35]. Rahimpour and Madaeni fabricated the cellulose acetate phthalate/polyethersulfone blend ultrafiltration membrane via phase separation techniques. The SEM and AFM analysis showed that addition of low concentration of cellulose acetate phthalate in the casting solution results in a membrane with high surface hydrophilicity, porosity, and thin top layer with more number of macrovoids in

![Fig. 10](image-url)
sublayer. The pure water permeation and milk water permeation for the prepared membranes are improved by introducing 20% cellulose acetate phthalate in the casting solution [36].

Water-soluble derivatives of cellulose, mainly sodium salt of carboxymethyl cellulose, are used in the preparation of nanofiltration membranes. Due to its water-soluble nature, crosslinking of these polymers are necessary to prepare the membranes. Yu et al. described a facile modification of polypropylene hollow fiber microfiltration membrane to nanofiltration by dip-coating method by using carboxymethyl cellulose followed by crosslinking with AlCl3. These nanofiltration membranes showed the rejection efficiency for the electrolyte solution in the order of MgCl2 < CaCl2 < MgSO4 < NaCl < KCl < Na2SO4. This is due to the negative charge density of membrane and rejection behavior being dominated by the Donnan exclusion principle [37]. Use of carboxymethyl cellulose crosslinked with epichlorohydrin (ECH) and FeCl3 has generally been put forward for rejection of divalent ions from low-molecular-weight organics and for dye removal applications. Apart from these, frequently stated different fabrication methods, novel chemical modification of cellulose, and its derivatives are gaining momentum in the field of membrane technology. For example, immobilization of carbon nanotubes, graphene oxide, TiO2, SiO2, and other nanoparticles in to cellulose and its derivative-based membranes provides the new flat form in the membrane science [30, 33, 38, 40].

5.2 Heavy Metal and Dye Removal

Presence of enormous polar functional groups in cellulose-based membranes facilitates the adsorption or deposition of hazardous heavy metal ions, reactive dyes, and other contaminants from aqueous stream. Earlier, Nagendran et al. investigated the applicability of CA/sulfonated poly(ether imide) blend membranes for the rejection of detrimental heavy metal ion from wastewater. Studies were carried out to analyze...
the permeate flux and rejection capacity of heavy metal ions such as Ni (II), Zn (II), Cd (II), and Cu (II) using polyethyleneimine as the complexing ligand. The extent of rejection of metal ions follows the order Cu (II) > Ni (II) > Zn (II) > Cd (II), which depends on the complexation ability of individual metal ions with chelating agent and ligand-field stability [39]. Similarly, epoxy functionalized poly(ether-sulfone) (EPES) and CA blend membrane were also employed for the removal of hazardous chromium ions from aqueous solutions. The hydrogen bonding interaction between CA and modified EPES is presented in Fig. 12. Porosity of the CA membrane increased and mean pore radii decreased after the incorporation of EPES into membrane matrix. The percentage rejection of Cr (VI) ions at 80:20 blend composition reached up to 98.3% with reasonable hydraulic permeation rate of 48.6 l m⁻² h⁻¹ [40].

He et al. extensively investigated the rejection of salt and dye from aqueous solution using CA nanofiltration membranes. The dye rejection efficiency of membrane increased up to 99.0% and also higher rejection can be achievable with higher concentration of dyes [41]. Vijayalakshmi et al. also described the heavy metal ion rejection efficiency by CA/polycarbonates (PC) blend membranes. The effect of blend concentration on heavy metal ion rejection capacity of membrane is presented in Fig. 13. During the filtration experiment, higher percentages of rejection, up to 95%, were obtained for the proteins molecules. The toxic heavy metal ions such as Cu²⁺, Ni²⁺, Zn²⁺, and Cd²⁺ were rejected by complexing with water-soluble chelating polymer, i.e., polyethyleneimine. The modified membrane showed rejection in the order of 99%, 96%, 94%, and 90% for Cu²⁺, Ni²⁺, Zn²⁺, and Cd²⁺ ions, respectively [42]. Further, CA-immobilized polyetherimide membrane was explored.
for the separation of detrimental heavy metal ions. The modified CA membranes exhibited copper ion rejection up to 93% with steady-state hydraulic permeation rate of 32.4 l m⁻² h⁻¹ [43] incorporation of inorganic filler or blending with functional polymer into CA and its derivatives facilitate the transport of water molecules through the resultant membrane with high rejection behavior. The chemical modification of cellulose-based materials is also one of the attractive methods to improve the membrane properties for water treatment and heavy metal ion rejection applications.

5.3 Pervaporation

It is well known that a pervaporation membrane should have good mechanical strength thermal resistance, chemical stability, and hydrophilicity [44, 45]. In order to attain this, crosslinking of these materials was carried out. Cellulose and its derivatives are important class of materials for the pervaporation membranes due to the presence of abundant hydroxyl groups and other functional groups on its backbone. However, in order to achieve the good mechanical strength and chemical stability, crosslinking between these polymers are essential [13]. For this purpose, most commonly used crosslinking agents are glutaraldehyde, mixture of urea + formaldehyde + sulfuric acid, and tripolyphosphate. Cellulosic materials having the polar functional groups tend to particularly remove water due to their high affinity toward water molecules through hydrogen bonding and other secondary force of interaction with membrane surface. This results in high flux rate and selectivity [46]. Zhou et al. reported the preparation of CA and poly(vinyl alcohol) (PVA) blend membrane for the separate water–ethanol and methanol–methyl tert-butyl ether (MTBE) mixtures via pervaporation technique. They stated that as the CA concentration increases, the fractional free volume and radius of free volume cavity of the blended membranes enhance simultaneously (Fig. 14). Therefore, prepared blended membranes show higher separation performance in pervaporation.
of methanol (15 wt.%–MTBE and water (15 wt.%–ethanol mixtures. Moreover, the blended membrane exhibited enhanced permeability and selectivity compared to pristine CA and PVA membranes [47]. The influence of metal oxide particles such as Al₂O₃ and ZnO in CA membrane matrix have been investigated for pervaporation separation methanol/MTBE mixtures. When additive dosage of ZnO and Al₂O₃ was at 4 wt.% and 1.98 wt.% in CA membrane matrix, both the separation factor and permeation flux of modified membranes were improved than that of pure CA membrane. In comparison with the pristine CA membrane, the maximum flux of membrane with inclusion of ZnO and Al₂O₃ improved 111.1% and 96.5%, respectively [48].

Effect of hydrophilic additive such as poly(N-vinyl-2-pyrrolidone) (PVP) on pervaporation performance by the CA membrane was studied by Wu et al. They fabricated CA and PVP blend membrane with different composition via phase
separation method. The hydraulic permeation rate and selectivity factors are increased with content of PVP in the range of 10–15%. In addition to this, they extensively investigated the effect of feed flow rate, feed composition, and operation temperature on pervaporation efficacy. Among all the prepared blend membranes, the membrane comprising 85 wt.% CA and 15 wt.% PVP exhibited the maximum separation factor with a value of 411 and a permeation flux of 430 g m⁻² h⁻¹ for a feed composed of 20 wt.% methanol at 313 K [46]. Kusumocahyo et al. also developed the technique for modification of CA membrane via ultrathin polyelectrolyte complex (PIC) layers for effective pervaporation process. Introduction of oxygen-containing anionic groups onto the surface of the CA membrane resulted in substantial improvement of the permeation rate and selectivity [49]. Recently, Zafar et al. investigated the effect of different additives such as ethylene glycol (EG), polyethylene glycol-600 (PEG-600), and propylene glycol (PG) on CA membrane for the pervaporation of isopropanol (IPA)/water mixtures. They stated that tensile strength, thermal stability, and separation factors were considerably enhanced by the additive compared to pristine CA membrane [50].

Among the organoselective biopolymers so far reported for pervaporation applications, cellulosic esters also have been extensively utilized for membrane fabrication. They have exhibited quite outstanding properties for this particular separation as shown by the data reported by Nguyen et al. Cellulose ester appears to have a quasi-infinite selectivity toward ethanol with pervaporation ethanol content very close to 100%. However, the pervaporation flux rate of membrane achieved so far very low for an industrial application. In order to achieve this, better pervaporation flux rate cellulose propionate membrane was developed, which is almost one order magnitude higher than that of CA membrane [51]. Enrichment of hydrophobic character of membrane leads to little increase in permeation rate, but selectivity decreases sharply for cellulose butyrate membrane. For the screening of pervaporation properties of ethanol mixtures, cellulose propionate and cellulose butyrate membrane was fabricated. From these, membrane separation with interesting fluxes was obtained but substantially reduced pervaporation ethanol contents compared with CA membranes. From these outcomes, it readily gives the impression that plasticizing CA membrane would be highly stimulating for the targeted application if its selectivity could be kept in the very high range at the same time [52]. Smitha et al., Cai et al., and other researchers have reported that the cellulose esters membrane modified by the poly(meth)acrylic polymers can lead to enhanced flux rate while still keeping an excellent selectivity [53, 55, 56]. They stated that the formation of semi-interpenetrating networks between the cellulose acetate materials and crosslinked poly(meth)acrylate via photopolymerization is responsible for a slow decrease of the pervaporation features over time [54, 55]. The overview of cellulose membrane modification on pervaporation performance is presented in Table 1.

Water-soluble cellulose ethers like hydroxypropyl cellulose (HPC) and hydroxyethyl cellulose (HEC) are capable of forming blends with a vast number of other polymers. HEC was extensively investigated for pervaporation. Qu et al. illustrated the pervaporation properties of HEC for desulfurization of gasoline compounds. They have reported that 1, 6-hexanediol diacrylate was employed as crosslinking agent by using benzoyl peroxide as the initiator. The crosslinking
intensity of the membrane significantly affected the rate of sorption and transport properties [58]. In addition to this, HEC was also extensively used in pervaporation of tetrahydrofuran (THF) + water and 1, 4 dioxane + water mixtures. In view of the fact that, 1, 4 dioxane, THF are highly miscible with water in all proportions form azeotropic mixtures. Their separation is very important as they are a main class of organic solvents and find its extensive applications in pharmaceutical and chemical industries [60]. Similarly, in another study, acrylamide is grafted to HEC and then modified with sodium alginate to investigate the dehydration properties of acetic acid (AA) via pervaporation process. The modified HEC membranes were highly hydrophilic in nature, which results in effective recovery of water from acetic acid solutions up to 89%. Biopolymer HPC blended with chitosan was also used for pervaporation dehydration of isopropanol. Addition of hydrophilic nature of HPC to the hydrophilic chitosan brings about a blend having higher hydrophilicity, which enables higher degree of dehydration of the isopropanol [61]. Reports are available on the use of the sodium salt of carboxymethyl cellulose polyelectrolyte complexes for pervaporation dehydration of alcohols like isopropanol and ethanol. There is still scope for the use of other functional cellulose derivatives in the ever expanding field of pervaporation especially for dehydration application [62, 63].

### 6 Chitosan

Chitosan is a linear polysaccharide comprising of randomly distributed $\beta$-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimp. It was obtained from the

<table>
<thead>
<tr>
<th>Membranes</th>
<th>C</th>
<th>$\alpha_{PV}$</th>
<th>$J_{m}$ (Kg/m²h)</th>
<th>T (°C)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP</td>
<td>0.96</td>
<td>96.0</td>
<td>0.70</td>
<td>40</td>
<td>[53]</td>
</tr>
<tr>
<td>CAP-PAA (80–20%, w/w)</td>
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<td>79.3</td>
<td>0.87</td>
<td>40</td>
<td>[54]</td>
</tr>
<tr>
<td>CAB-CAP (10–90%, w/w)</td>
<td>0.967</td>
<td>117.2</td>
<td>0.6</td>
<td>40</td>
<td>[54]</td>
</tr>
<tr>
<td>CA-P(VP-co-VAc) (40–60%, w/w)</td>
<td>0.86</td>
<td>24.6</td>
<td>2.3</td>
<td>40</td>
<td>[57]</td>
</tr>
<tr>
<td>CA-EGDMA (50–50%, mol/mol)</td>
<td>1</td>
<td>–</td>
<td>0.02</td>
<td>40</td>
<td>[53]</td>
</tr>
<tr>
<td>CAB-EGDMA (50–50%, mol/mol)</td>
<td>0.895</td>
<td>34.1</td>
<td>4.4</td>
<td>40</td>
<td>[53]</td>
</tr>
<tr>
<td>CA-TEEMA.PEG200DMA (50 25.25%, mol/mol)</td>
<td>0.97</td>
<td>129.3</td>
<td>0.50</td>
<td>40</td>
<td>[54]</td>
</tr>
<tr>
<td>CAP-P(VP-co-AA) (95–5%, w/w)</td>
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<td>169.9</td>
<td>0.52</td>
<td>40</td>
<td>[58]</td>
</tr>
<tr>
<td>CAP-PAA (70–30%, w/w)</td>
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<td>76.0</td>
<td>0.88</td>
<td>40</td>
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<tr>
<td>CAP-P(VP-co-AA) (95–5%, w/w)</td>
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<td>169.9</td>
<td>0.52</td>
<td>40</td>
<td>[57]</td>
</tr>
<tr>
<td>CA-TEEMA.PEG600DMA (50–25.25%, mol/mol)</td>
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<td>96.0</td>
<td>0.90</td>
<td>40</td>
<td>[53]</td>
</tr>
<tr>
<td>CAP-EGDMA (50–50%, mol/mol)</td>
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<td>96.0</td>
<td>2.0</td>
<td>40</td>
<td>[53]</td>
</tr>
<tr>
<td>CA-P(VP-co-VAc) (95–5%, w/w)</td>
<td>0.98</td>
<td>196.0</td>
<td>0.3</td>
<td>40</td>
<td>[54]</td>
</tr>
<tr>
<td>CAP-P(VP-co-AA) (95–5 w/w%)</td>
<td>0.959</td>
<td>1.24</td>
<td>60</td>
<td>[59]</td>
<td></td>
</tr>
</tbody>
</table>

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alkaline N-deacetylation of chitin, which has attracted the attention of researches across the globe [64]. After cellulose, it is the second abundant naturally occurring biopolymer in the earth. It exhibits several excellent biological properties such as antimicrobial activity, biodegradability, immunological activity, and nontoxicity. More interestingly, as a membrane material, it exhibited an outstanding film-forming capacity and is highly hydrophilic in nature [65]. Even though basic building block consists of abundant polar functional groups, chitosan is insoluble in most of the common organic solvents and aqueous solution. Therefore, derivatization or functionalization of chitosan is necessary to enhance the solubility and other physicochemical properties. The modified chitosan derivatives have acquired enormous applications in the membrane technology as well as biomedical field [66].

Chitosan offers outstanding advantages as a membrane material since it has reactive hydroxyl and amine functionality in its backbone, which delivers abundant possibility for chemical modification (Fig. 15). In particular, amine functionality of chitosan is responsible for complexation ability and hydrophilicity of the material. The chemical modification of chitosan is essential, since it facilitates the introduction of different functionalities, which may be useful in expanding the commercial utilization of this material [67]. Advantages of chitosan membranes can be extended from separation membranes to biomedical membranes since it possesses biological properties such as antibacterial and wound healing activity. Chitosan is probably the most profoundly and widely used polysaccharide displaying a vast array of applications ranging from tissue engineering, drug delivery, heavy metal ion removal, dye adsorption, pervaporation, controlled release of fertilizers, protein separation, fuel cell application, chromatographic separations, photography, etc. [64, 66, 68].

Fig. 15 The source and chemical structure of chitosan (source: Front. Chem., 07 November 2017)
Derivative of natural chitosan offers extraordinary properties that have paved the way for the significant investigation of chitosan derivatives in the pharmaceutical, membrane technology, and biomedical fields. However, poor solubility of the chitosan limits its vast application in various fields [69]. In order to improve the solubility and other physicochemical properties, chemical modification of the chitosan has been extensively studied. Generally, modification was carried out via grafting of polymer chains; small molecules, quaternization of the amino groups, and other modification method have been extensively investigated. The chitosan has three active reactive sites for the facile chemical modification, namely one primary amine and two hydroxyl functionality (primary or secondary) [70].

Many efforts have been carried out for the chemical modification of chitosan to enhance the solubility and other properties. Sashiwa et al. reported the preparation of novel derivative of chitosan via Michael type reaction of chitosan with ethyl acrylate. The degree of substitution increases with the concentration temperature and other experimental conditions. From the intermediate N-carboxyethylchitosan ethyl ester, several substituted hydrophilic amines can also be introduced [71]. Loubaki et al. reported the chemical modification of chitosan through glycidyl trimethylammonium chloride. Structural modification was confirmed by $^1$H NMR, $^{13}$C NMR, and elemental analysis [72]. Effective chemical modification of chitosan with several heterocyclic moiety was reported by Badawy in 2008 for improvement of solubility and other properties. The modified N-[(5-methylfuran-2-yl)methyl] chitosan, N-(benzo[d][1,3]dioxol-5-ylmethyl) chitosan, and N-(methyl-4H-chromen-4-one) chitosan were showed better solubility and antimicrobial nature. These novel derivative launches new perspectives as biomedical material [73]. Huang et al. investigated the effect of modified chitosan with terminated amine groups such as tetraethylenepentamine for the removal of anionic dyes from the aqueous stream. The structural modification was confirmed by FTIR, XRD, and TGA/DTA analysis. They stated that amine-terminated chitosan fits with Langmuir model’s maximum sorption capacity of 292.4 mg/g at 298 K [74].

In acidic condition, chitosan molecules will possess positive charge density due to the protonation of the amino groups in the backbone. Therefore, increasing the amine functional groups in the chitosan via chemical modification using different types of modification agent forms new derivatives with improved physicochemical properties. Vakili et al. reported the preparation of 3-aminopropyl triethoxysilane and hexadecylamine functionalized chitosan for the treatment of contaminated water. After modification, adsorption capacity of chitosan beads increased by 1.48 times. The maximum adsorption capacity of chitosan beads increased from 317.1 mg/g to 468.8 mg/g after modification [75]. Munoz et al. reported the preparation of lauryl succinyl chitosan (LSC) through ionic gelation method. The synthesized LSC were extensively studied for physicochemical properties, cytotoxicity studies, and simulated gastrointestinal assays [76]. Hua et al. illustrated the controlled chemical modification of chitosan via reversible addition-fragmentation chain transfer (RAFT) polymerization technique. In this method, the chitosan was
initially treated with S-1-dodecyl-S-(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DDACT) to perform as RAFT agent; latter controlled graft polymerization was carried out by using the acrylic acid. The modification was confirmed by $^1$H NMR, FT-IR, XRD, transmission electron microscopy (TEM), and high performance particle sizer (HPPS) analysis [77].

Kumar et al. described the preparation of N-propylphosphonic chitosan by reacting terminal amine group with propylphosphonic anhydride (T3P), hydroxybenzotriazole (HOBt), and chitosan. The structure was confirmed by $^1$H NMR, FT-IR spectroscopy, and X-ray diffraction (XRD) analysis. The schematic representation of synthesis N-propylphosphonic chitosan is shown in Fig. 16 [78]. Tonglairoum et al. reported the synthesis of O-succinyl chitosan by protecting the amino group. After the introduction of O-succinyl group, deprotection was carried out by using hydrazine hydrate. O-succinyl-chitosan exhibits much higher solubility in aqueous solution, and the investigation of enzymatic degradation showed that the O-succinyl-chitosan was of low susceptibility to lysozyme [79]. Heras et al. stated that the synthesis of chitosan derivatives comprises phosphonic and surfactant carrying alkyl groups. These derivatives showed higher solubility in water and other organic solvent due to the presence of alkyl groups, which reduces the strong inter and intramolecular hydrogen bonding. In addition, it has positive impact to solubilize active hydrophobic compounds with essential roles in the cosmetic and pharmaceutical field [80]. Liu et al. modified the chitosan by coupling the linolenic acid through the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-mediated reaction. The degree of substitution was confirmed by $^1$H NMR, and it was 1.8%, i.e., 1.8 linolenic acids group per 100 anhydroglucose units [81]. Ramos et al. prepared the novel water-soluble chitosan derivatives bearing phosphonic groups via one-step reaction. The formation of N-methylene phosphonic chitosan was characterized by FT-IR spectroscopy, elemental analysis, and XRD studies. Taking advantage of the known chelating ability of the phosphonic groups, especially for calcium, this new derivative opens new perspectives as biomedical material [82]. Huacai et al. employed the microwave irradiation method to prepare the graft copolymerization of chitosan with acrylic acid. They investigated the effect of some grafting variables such as concentration, amounts of the initiator, volume of solvent, radiation time, etc. They stated that the microwave irradiation method can increase the reaction rate by eight times over the conventional method [83].

8 Application of Chitosan as Membrane Material

8.1 Nanofiltration and Ultrafiltration

Most of the commercially available nanofiltration membrane is made up of thin-film composite (TFC) membranes. The permeation and separation efficiency of the TFC membrane mainly depend on structure, porosity, charge density, and other physicochemical properties of top layer of the membrane. Thus, finding the suitable materials for preparation of stable active skin layer is the key area in current scenario.
For this purpose, chitosan and its derivatives have attracted the attention of the researchers for the past two decades. The chitosan has excellent film-forming capacity and hydrophilicity; it is nontoxic and biodegradable and possesses resistance toward the microbial growth on the membrane surface [84, 85]. In addition to this, fabricated nanocomposite membranes should be crosslinked with suitable
crosslinking agents such as glutaraldehyde, epichlorohydrin, sodium tripolyphosphate, toluene isocyanate, dicarboxylic acids, etc. Generally, crosslinking of thin film of chitosan provides higher stability, higher mechanical strength, chemical resistance, and higher hydrophilicity and alters other physicochemical properties.

Effect of crosslinking in the chitosan/poly(acrylonitrile) (PAN) composite nanofiltration membranes were extensively investigated by Musale et al. by means of different crosslinking times and crosslinking agent concentrations. After the crosslinking with glutaraldehyde, rate of hydraulic permeation and water uptake capacity of the membrane was reduced to certain extent (Fig. 17). At the same time, selectivity and rejection of electrolytes enhance with increasing glutaraldehyde concentration. This is because molecular weight cut-offs of surface-crosslinked membranes were in the range of 550–700 Da, a characteristic of nanofiltration membranes, whereas uncrosslinked membrane had cut-off of 1500 Da [86]. Similarly, the synthesis amphoteric polyelectrolyte, i.e., sulfated chitosan was prepared by homogeneous method. After the preparation, composite NF membrane was fabricated by coating the aqueous solution of sulfated chitosan onto a polyacrylonitrile (PAN) UF membrane, subsequently crosslinked with glutaraldehyde. They stated that modified NF membrane resulted excellent rejection performance with only 2 wt.% of sulfated chitosan concentration. The rejection efficiency of electrolyte solutions (1000 mg/L) NaCl, Na₂SO₄, and K₂SO₄ reached up to 50.3%, 92.1%, and 92.8% under 0.40 MPa pressure at 25 °C. In addition to this, the rejection rate of electrolytes decreased in the order of K₂SO₄, Na₂SO₄, KC1, NaCl, MgSO₄, and MgCl₂. This indicates that charge density of active surface layer, concentration of electrolyte solution, size of anion and cations mainly determines the membrane performance [87]. In another study, O-carboxymethyl chitosan was effectively employed as fabrication of TFC membrane over the PAN ultrafiltration support by crosslinking with different concentrations of epichlorohydrin. The fabrication composite membrane with 2 wt.% epichlorohydrin concentration, crosslinking time 12 h, and crosslinking temperature 50 °C offered the optimized condition. Resultant membrane showed rejection of various electrolytes in the order of Na₂SO₄ > NaCl > MgSO₄ > MgCl₂. Moreover, the hydraulic permeation and rejection behavior of composite membrane mainly depend on surface and other physicochemical properties [88].

Novel positively charged composite NF membrane was fabricated by using PAN ultrafiltration membrane as support layer. For the preparation of active surface layer, 2-hydroxypropyltrimethyl ammonium chloride chitosan (HACC) (quaternized chitosan) is used for the formation thin film with diisocyanate as crosslinking reagent. They stated that a membrane from 2.0 wt.% HACC casting solution, cured at 50 °C for 2 h, crosslinked at 50 °C for 9 h with toluene diisocyanate (TDI) and hexamethylene diisocyanate (HDI) (TDI/HDI(w/w) = 0.3 g/0.3 g) in 50 g ethanol, and heat-treated at 50 °C for 20 min showed optimum performance [89]. In the same way, Zinadini et al. reported the fabrication of antifouling polyethersulfone NF membrane comprising carboxymethyl chitosan coated Fe₃O₄ nanoparticles (Fig. 18). The modified membranes had significantly improved hydrophilicity,
higher pure water permeation rate, and antifouling nature with flux recovery of 91.7% compared to the unmodified membrane [90]. Further, N,O-carboxymethyl chitosan/cellulose acetate blend NF membranes were developed for the effective treatment of hazardous heavy metal ions from aqueous stream. The blend membrane showed the rejection of copper and chromium ion from aqueous solutions up to 72.60% and 83.40%, respectively, at 1 MPa transmembrane pressure. In another study, Huang et al. prepared the composite membrane with quaternized chitosan active layer over the PAN support membrane by using anhydride mixture as crosslinker. Composite membrane showed considerable higher rejection of electrolytes in the order of $\text{CaCl}_2 < \text{MgCl}_2 < \text{NaCl} < \text{KCl} < \text{MgSO}_4 < \text{Na}_2\text{SO}_4 < \text{K}_2\text{SO}_4$ [91]. The positively charged composite NF membrane is fabricated by grafting graft copolymer of trimethylallyl ammonium chloride onto chitosan by toluene diisocyanate crosslinking. As concentration of diisocyanate crosslinking agent increased from...
0.25–1.25 wt.%, the decrease in permeate flux and increase in the rejection was observed [92].

In order to improve the selectivity and separation characteristic of NF membranes, Zhu et al. developed chitosan-modified clay/polymer nanocomposite membrane. The resultant membrane showed considerable enhancement in the mechanical strength, hydrophilicity, thermal stability, etc. compared to pristine polymer membrane. The schematic representation of interaction of chitosan and clay is shown in Fig. 19. They stated that with increase in the additive dosage from 0 to 1.0 wt.%, the hydraulic permeation rate of nanocomposite membrane increases from 32 \( \text{L m}^{-2} \text{h}^{-1} \) to 68.82 \( \text{L m}^{-2} \text{h}^{-1} \) [93]. The fabrication of polysulfone and chitosan blend membrane was carried out by incorporating titanium dioxide nanotubes into the
membrane matrix. The modified membrane showed improved water permeation rate with higher flux recovery ration of 76% [94]. The variation in the hydraulic permeation of modified membrane is shown in Fig. 20. The modified membrane showed rejection of 46% NaCl solution at pH −5. They stated that in acidic condition, higher percentage of swelling and partial dissolution of protonated chitosan in the membrane resulted in efficient solvent diffusion due to chain relaxation and porosity. In the same way, at basic environment, there will be less amount of the protonated positively charged functionality in chitosan molecules. This gives less repulsive force of interaction between chitosan molecules, thereby increasing polymer aggregation. Similarly, Shenvi et al. prepared the composite membrane by using chitosan as selective thin film over the poly(1,4-phenylene ether ether sulfone) (PPEES) support membrane and it was ionically crosslinked with sodium tripolyphosphate (TPP) (Fig. 21). The changes in surface properties and other topological characteristic features of membrane is due to the formation of ionically crosslinked chitosan layer PPEES, which was confirmed by analyzing contact angle, water uptake capacity, and hydraulic permeation studies. The membranes exhibited rejection up to 21% and 55% toward NaCl and MgSO₄, respectively, at pH = 5 and a flux recovery ratio of 73% [95].

Musale et al. prepared the PAN/chitosan ultrafiltration membrane via formation of thin film on PAN membrane. Incorporation of chitosan in to membrane showed reduction in the negative zeta potential, enhanced hydrophilicity, morphological changes, and other physicochemical properties compared to unmodified PAN membrane. Moreover, composite membrane showed high resistance in both acidic and basic environment [96]. To further investigate, polysulfone–chitosan blend
ultrafiltration membranes was fabricated through phase separation method. The variation of casting solution after the addition of chitosan during the membrane preparation is shown in Fig. 22. The enhanced hydrophilicity, water uptake capacity, water permeation, and antifouling properties were reported for the blend membrane [97]. Zhao et al. described modification of polyvinylidene fluoride (PVDF) ultrafiltration membrane through hydrophilic chitosan layer formation of the active layer of the membrane. The modified membrane exhibited higher operating range and showed comparatively good hydraulic permeation rate during the prolonged period of filtration process. Also membrane exhibited enhanced protein rejection efficacy up to 90% with water permeation rate of 70.5 L/m² h during bovine serum albumin (BSA) filtration [98]. The hydrophilic modification of PVDF membrane was carried out by chitosan solution for the mitigation of protein fouling during the filtration. Presence of chitosan in the membrane matrix showed higher antifouling properties by reducing irreversible membrane fouling. This was because the hydrophilic polar
functionality of chitosan has strong affinity toward water molecules to form strong hydration sphere on active layer of the membrane, thereby reducing the interaction of protein interaction with membrane [99].

8.2 Heavy Metal and Dye Removal

One of the most detrimental problems affecting the people across the world is contaminated water by toxic heavy metal ions, hazardous dyes, and other materials. The most important sources of heavy metal and dye pollutants are various industrial activities comprising metal plating, mining, electronic device manufacturing, printing, oil refining, and the manufacture of chemicals, paper dyes, textiles, paints, fertilizers, leather pesticides petrochemicals, etc. Most importantly, heavy metal ions such as chromium, mercury, nickel, copper, zinc, lead, and cadmium are nonbiodegradable toxic, carcinogenic, and tend to accumulate in living organisms leading to several fatal diseases [100–102]. The most frequently used methods for the removal of heavy metals and dyes are chemical precipitation, ion exchange, evaporation, complexation, liquid–liquid extraction, electrodeposition, advanced oxidation processes, biological treatment, electrolysis, and membrane separation. Due to high efficiency, easy operation and space saving by membrane filtration, playing major role for past few decades in removal of heavy metal and dye from aqueous solution [103, 104]. As discussed earlier, the functional biopolymer chitosan has abundant polar functional groups having the ability to form complex with heavy metal ions. Figure 23 represents the chelating ability of the chitosan with heavy metal ions. The presence of anime and hydroxyl groups have capacity to sorb heavy metal ions and dyes through several mechanism comprising hydrogen bonding, electrostatic interactions, chemical interactions, ion exchange, or the formation of ion pairs, etc. [105].

It is generally well known that chitosan-based NF and UF membranes are commonly employed for removal of heavy metal ions and dyes from aqueous solutions. Liu and Bai prepared the highly porous hollow fiber membrane comprising chitosan and cellulose acetate for the removal of Cu (II) ions from aqueous solution. The resultant membranes showed adsorption capacities of up to 35.3–48.2 mg/g for Cu (II) ions at pH 5 and offered adsorption equilibrium times
less than 20–70 min [106]. Juang et al. extensively investigated the removal of divalent metal ions such as Ni(II), Cu(II), Zn(II), and Co(II) from contaminated water via chitosan-enhanced membrane filtration. The rejection efficiency of metal ions acidic solutions (pH < 6) could be enhanced by 6–10 times in presence of chitosan [107]. The removal of chromium ions from aqueous solutions was conducted by using natural and crosslinked chitosan membranes through batch adsorption experiments. The highest removal capacity of about 1400 mg g⁻¹ was obtained for epichlorohydrine-crosslinked chitosan at pH 6.0 [108]. The adsorption and desorption mechanism of heavy metal ions were explore by using the crosslinked chitosan membranes. The result showed that adsorption and desorption results related to both electrostatic attraction and chelation interaction with mercury [109]. Similarly, removal of copper (II) ions from contaminated water was carried out through alumina/chitosan composite membranes. The resultant composite membranes diminished a 50 mg/l concentration of Cu²⁺ solution to a level below 1 mg/l. [110]. Shariful et al. fabricated the chitosan/poly(ethylene oxide) nanofibers through electrospinning process. Chitosan/PEO composition that produced beadless fibers tend to possess higher hydrophilicity and maximum specific surface area. The maximum adsorption capacity of the beadless fibers for Cu (II), Zn (II), and Pb (II) ions were found to be 120, 117, and 108 mg g⁻¹, respectively [111].

The heavy metal rejection behavior of polysulfone blend membrane with different chitosan derivatives such as N-propylphosphonyl chitosan, N-succinyl chitosan was studied by Kumar et al. The blend membranes showed higher hydrophilicity, water uptake capacity, better water flux, and antifouling nature. For N-succinyl chitosan modified membrane, maximum of rejection of 78% of Cu, 73% of Ni and 68% of Cd was achieved (Fig. 24). The rejection rate of 75% of Cu, 71% of Ni and 66% of Cd for N-propylphosphonyl chitosan membrane and 76% of Cu, 69% of Ni and 66% of Cd rejection for chitosan modified membrane [112]. Kanagaraj et al. also investigated the influence of N-phthaloyl chitosan for the rejection of heavy metal ions for poly(ether imide) ultrafiltration membranes. The schematic representation of
preparation of N-phthaloyl chitosan is shown in Fig. 25. Blend membrane with 2 wt. % N-phthaloyl chitosan showed lower hydraulic resistance (3 kPa/l m⁻² h⁻¹), higher pure water flux (112.21 m⁻² h⁻¹), and higher water content (63.4%). The membrane showed good higher rejections for toxic heavy metal ions such as Cr (III), Zn (II), Cd (II), and Pb (II) (Fig. 26) [113]. The influence of macroporous chitosan membrane on removal of hazardous heavy metals such as Cu (II) and Ni (II) ions were investigated via adsorption process. For this purpose, batch adsorption experiments were conducted with various concentration of heavy metal ions, temperature, mono and binary component solutions, and contact time for the analysis of rejection behavior. In mono-component adsorption, the Cu (II) ions showed adsorption efficacy of 19.87 mg/g, which was higher than those of the Ni (II) ions (i.e., 5.21 mg/g). Comparing to mono adsorption, the amount of adsorption for individual component in bicomponent mixtures showed a decrease. That is due to competitive adsorption and coordination site limitation [114]. Salehi et al. prepared amine functionalized multiwalled carbon nanotubes (CNTs) and employed to fabricate novel chitosan/ polyvinyl alcohol thin adsorptive membranes for the removal of Cu (II) ions from aqueous solution. The adsorption of Cu (II) ion was predominantly high with higher content of functionalized CNTs in the membrane matrix as well as temperature. The adsorption ability of nanocomposite membrane with 2 wt.% of functionalized CNTs (20.1 mg/g at 40 °C) was almost twice as large as that of neat membrane (11.1 mg/g) [115]. These experimental results clearly indicate the heavy metal ion rejection capacity of chitosan-based membrane due to strong affinity toward heavy metal ions and its complexation ability.

One more significant applicability of chitosan-based membranes was explored for the removal of hazardous dye from aqueous solution. For this purpose, the thin film composite membrane comprising organoclay/chitosan nanocomposite coated on the PVDF microfiltration membrane was fabricated. The resultant membrane showed good rejection of dye such as methylene blue with 2 wt.% of organoclay/chitosan
**Fig. 25** The chemical structure of N-phthaloyl chitosan [113]

**Fig. 26** The effect of modified chitosan content on (a) rejection of heavy metal ions (b) permeate flux (initial feed concentration 1000 ppm) [113]
nanocomposite [116]. In addition to this, dye rejection efficiency and hydraulic permeation was enhanced by incorporating O-carboxymethyl chitosan/Fe$_3$O$_4$ into polyethersulfone (PES) nanocomposite membrane. The modified membranes exhibited reasonably high water permeation rate compared to unmodified PES membrane. They stated that rejection of Direct Red 16 for the pristine PES membrane was around 80%. This was altered to about 99.0%, 99.0%, and 98.5% for 0.1, 0.5, and 1 wt.% of the O-carboxymethyl chitosan/Fe$_3$O$_4$ embedded membranes, respectively (Fig. 27). The dye rejection behavior of the membrane is based on Donnan exclusion mechanism. Membranes in contact with an aqueous solution get an electric charge by dissociation of surface functional groups, causing electrostatic repulsion of the dye [90]. Further, chitosan-based hollow fiber membrane was
fabricated via dry-wet spinning method for the effective removal of hazardous reactive dyes. The hollow fiber membrane showed more than 454.5 mg/g adsorption capacity for the reactive blue 19. The interaction and rejection mechanisms were explained by pseudo-second-order kinetics and Freundlich equation [117]. Zhu et al. developed new chitosan–Montmorillonite (CS–MMT) nanosheets for the preparation of nanocomposite membrane via phase separation method. The CS–MMT incorporated membrane showed higher rejection for Reactive Red 49 and Reactive Black 5 up to 94.0%. Donnan exclusion mechanism played a significant role in rejection of lower molecular weight organic, thus resulting in higher rejection efficiency of hybrid membranes than that of pristine membrane [93]. The nanoporous cellulose membrane comprising chitosan as functional entities were developed for the dye-removal applications with improved surface and other physicochemical properties. The chitosan (10 wt.%) bound the cellulose to form nanoporous and stable membrane structure with thickness of 250–270 μm, which was further stabilized by crosslinking with glutaraldehyde vapors. The resultant membrane showed the membranes successfully removed 70%, 84%, and 98% of positively charged dyes like Rhodamine 6G, Methyl Violet 2B, and Victoria Blue 2B, respectively [118].

8.3 Pervaporation

Chitosan and its derivatives are able to form strong hydrogen bonds with water molecules owing to the close proximity of solubility parameter value to that of water. This significant characteristic properties of chitosan is generally exploited for dehydration of alcohol solutions and pervaporation. Also, it provides good film-forming capabilities and high hydrophilicity [119]. The initial experiments on pervaporation dehydration of organic/water mixtures was conducted through poly(vinyl alcohol)–chitosan composite membranes. The presence of chitosan in the membrane matrix alters surface hydrophilicity, physicochemical properties, and microstructure of resultant membrane. They observed that chitosan plays a vital role in enhancing the flux rate of the membrane, the chitosan content with 60 wt.%, and the composite membrane exhibited high performance without a decrease in separation factor (Fig. 28) [120]. Further, pervaporation and dehydration properties of the enhanced by inclusion of mussel adhesive mimic molecule for chitosan/polyacrylonitrile composite membrane. The composite membrane was prepared by layer-by-layer technique, in which carbopol was used as an intermediate layer bridging the polyacrylonitrile support layer and the chitosan active layer. When the concentration of carbopol was 0.5 wt.%, composite membrane showed the permeation flux of 1247 g/m² h and separation factor of 256 for ethanol dehydration at 353 K. The permeation rate and selectivity factor of the membrane is presented in Fig. 29 [121]. The dense chitosan membranes were developed via ionically crosslinking with phosphoric acid for the effective pervaporation process. The modified membrane of 2 h crosslinking interval exhibited high mechanical strength. The resultant membrane showed the substantial azeotrope breaking of 95.58 wt. % ethanol with water permeation rate of
0.58 kg/m² h and pervaporation selectivity of 213 [122]. Similarly, chitosan blended with the hydroxyethylcellulose and further crosslinked through urea–formaldehyde–sulfuric acid mixture forms better pervaporation membrane with high selectivity of 10,491 when the hydroxyethylcellulose content is 50 wt.%.

Likewise, for pervaporation through ethanol, dehydration was carried out by fabricating chitosan crosslinking membrane by using 3-aminopropyltriethoxysilane as crosslinking agent. After incorporation of silica particles into chitosan membrane matrix, the hydrophilicity, morphology, and other physicochemical properties were improved with maximum content of 10 wt.% crosslinking agent. The modified membrane showed higher separation factor and permeation rate for ethanol dehydration; this was due to low swelling behavior and strong covalent bond as well as hydrogen bond interaction of crosslinker with chitosan membrane [123]. Zhang et al. improved the pervaporation capacity by crosslinking chitosan membrane with

Fig. 28 The effect of (a) temperature and (b) feed water content of dehydration of ester/water mixtures [120]
glutaraldehyde. The surface-modified membrane displayed higher separation factor of 500 with permeation rate of 400 g/m² h for the mixture of 90% ethanol/water at 60 °C. According to literature, crosslinking forms amorphous regions of the membrane more rigid and offers less space or higher hydraulic resistance to transport of feed solution through membrane. In order to substantiate this, pervaporation of ethanol and other alcohol/water mixtures has been extensively investigated with PVA membranes crosslinked with glutaraldehyde [124]. At 30 °C, the permeation rate was 50 g/m² h for ethanol of 10 wt.% water content and 130 g/m² h observed when the water content was 20 wt.%. The corresponding separation factors were 180 and 150. The permeation rate was improved with increasing water content in the mixture, while the reverse was the case with the separation factor.

Modification of chitosan membranes has been carried out by the inclusion of functional nanoparticles into membrane matrix to enhance the pervaporation efficiency. For this, multiwalled carbon nanotubes (MWCNT) was functionalized via poly(3-hydroxybutyrate) and immobilized in to chitosan membrane. The resultant nanocomposite membrane exhibited relatively higher selectivity and permeation rate toward the water molecules in pervaporation of 1,4-dioxane dehydration compared to pristine membrane [125]. Similarly, Han et al. explored the effective surface modification of membrane via 4-isocyanato-40-(3,30-dimethyl-2,4-dioxo-azetidino)diphenylmethane (IDD) as a coupling agent to form effective chemical linkages between the chitosan chains and polybenzoimidazole membrane surface (Fig. 30). The modified membrane showed higher affinity toward the water molecules, thereby increasing the permeation rate and selectivity factor pervaporation dehydration on isopropanol aqueous solutions up to concentration range from 30 to 90 wt.% [126]. The facile and versatile modification chitosan membrane was carried out by grafting polyaniline via oxidative-radical copolymerization with ammonium persulfate ((NH₄)₂S₂O₈) as an initiator. The modified membrane was extensively
investigated for separation water–isopropanol mixtures by pervaporation with temperature range of 30–50 °C. They stated that membrane with 1:3 grafting rate displayed the higher hydraulic permeation flux of 1.19 × 10⁻² kg/m² with selectivity factor of 2092 at 30 °C for 10 mass % of water in the feed [127]. Dogan and Hilmioglu illustrated the preparation of coating chitosan on zeolite and incorporated in cellulose membrane for pervaporation of ethylene glycol/water mixtures. After

**Table 2** The pervaporation properties of some chitosan-based membranes

<table>
<thead>
<tr>
<th>Chitosan modification</th>
<th>Flux, g/m²h</th>
<th>Separation factor</th>
<th>Water (Wt.%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncrosslinked</td>
<td>7</td>
<td>202</td>
<td>–</td>
<td>[129]</td>
</tr>
<tr>
<td>Phosphorylated</td>
<td>180</td>
<td>541</td>
<td>10</td>
<td>[130]</td>
</tr>
<tr>
<td>Hydroxyethylcellulose blend</td>
<td>112</td>
<td>10,491</td>
<td>10</td>
<td>[131]</td>
</tr>
<tr>
<td>GA crosslinked</td>
<td>4</td>
<td>2208</td>
<td>4</td>
<td>[129]</td>
</tr>
<tr>
<td>Acetate salt</td>
<td>142</td>
<td>242</td>
<td>–</td>
<td>[132]</td>
</tr>
<tr>
<td>3-Aminopropyl-triethoxysilane</td>
<td>887</td>
<td>597</td>
<td>15</td>
<td>[123]</td>
</tr>
<tr>
<td>Carboxyethylated</td>
<td>30</td>
<td>301</td>
<td>–</td>
<td>[132]</td>
</tr>
<tr>
<td>Carboxymethylated</td>
<td>36</td>
<td>1294</td>
<td>–</td>
<td>[132]</td>
</tr>
<tr>
<td>99% Deacetylated</td>
<td>840</td>
<td>5.4</td>
<td>5</td>
<td>[133]</td>
</tr>
<tr>
<td>98% Deacetylated-HOAc</td>
<td>400</td>
<td>123</td>
<td>10</td>
<td>[133]</td>
</tr>
<tr>
<td>98% Deacetylated-H₂SO₄</td>
<td>160</td>
<td>1040</td>
<td>10</td>
<td>[133]</td>
</tr>
<tr>
<td>73% Deacetylated</td>
<td>6</td>
<td>1240</td>
<td>8</td>
<td>[132]</td>
</tr>
<tr>
<td>Cyanoethylated</td>
<td>80</td>
<td>52</td>
<td>–</td>
<td>[133]</td>
</tr>
</tbody>
</table>

Fig. 30 Reaction route for surface modification of PBI membrane with chitosan chains [126]
modification, membrane offers high separation factor because presence of zeolite in the matrix nonselective voids. In addition to this, new approach for pervaporation was developed by incorporating different mass % of NaY zeolite diisocyanate in crosslinked chitosan membrane. The membrane containing 40 mass % of NaY zeolite exhibited the highest separation selectivity of 11,241 with a flux of $11.37 \times 10^{-2}$ kg/m² h for 10 mass% of water in the feed [128]. The overview of pervaporation efficiency of chitosan-based membranes is presented in Table 2.

9 Conclusions

The most abundant biopolymers such as cellulose and chitosan are the important class of membrane materials for water purification, desalination, pervaporation, dye removal, and heavy metal ion rejection. Versatile applicability of these biopolymers in separation process put forward new site for researchers in membrane technology. Exploration of these biopolymers and its derivatives can easily be altered via chemical modification to improve the permeation and selectivity resultant membrane. The positive transformation of these membranes offers excellent potential to understand the sustainable development of contemporary membrane technology. However, more detailed experimental and simulation investigations are necessary for the sustainable implementation of membrane technology by considering social, environment, and economic point of view.

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Inulin Type Fructan: A Versatile Functional Material for Food and Healthcare

Muhammad Ajaz Hussain, Muhammad Tahir Haseeb, Gulzar Muhammad, and Muhammad Nawaz Tahir

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Abstract
Inulin type fructan (ITF), a plant derived polysaccharide, is a bio-inspired, versatile, and functional biomaterial. Chemically, ITF is glucopyranosyl-fructofuranoside polymer. ITF is very well-established prebiotics due to its ability to facilitate the growth of beneficial bacteria, i.e., bifidobacteria, lactobacilli, and bacteroides in colon. The detail study of prebiotic activity has been discussed. Furthermore, the ability of ITF as hypolipidemic agent and antioxidant with major applications in gastrointestinal disorders, especially, inflammatory bowel disease, Crohn’s syndrome, and constipation, is the focus of this chapter. Additionally, hepatoprotective, anticancer, and immunomodulatory effects of ITF have made it an important functional food ingredient. Due to these diverse properties, ITF is an important and useful biomaterial for future medicine and pharmaceuticals.

1 Introduction
Biomaterials are designed to interact with living cells under physiological conditions. These materials possess inherent physical properties compatible to physiological conditions, therefore, stand as top rank choice in biomedical applications. Among different biopolymers, inulin type fructans (ITF) which are primarily linear or branched oligosaccharides or polymers containing fructose with mostly fructosyl-fructose (β-(2 → 1)) glycosidic linkages have attracted the vigil eye of researchers due to their numerous biomedical applications. Fructans can have at least one hydrolysable fructosyl-glucose glycosidic linkage at the start of the polymeric chain, which is similar to that of sucrose and gives free glucose units by enzyme sucrase secreted from epithelial villi of small intestine.

On the basis of structure, ITF can be classified into five types: (i) ITF (1-kestose), (ii) levan-type fructans (6-kestose), (iii) fructans of the inulin neoseries (neokestose), (iv) mixed-type levans (bifurcose), and (v) fructans of the levan neoseries also called mixed-type levans. ITF containing only fructose units are indigestible and are resistant to hydrolysis because of the presence of β-configuration at anomeric carbon. Actually, enzymes present in gastrointestinal tract are specific for α-glycosidic linkages [1–4].

These natural products can be obtained from cereals, fruits, and vegetables, such as wheat, leek, garlic, banana, and onion. They are stored as carbohydrates in leaves, roots, stems, or branches. As an example, inulin type fructans are extracted from fresh roots of Cichorium intybus ( chicory), Helianthus tuberosus, Polymnia sonchifolia, and Dahlia pinnata. ITF possess exceptional physical and chemical properties, e.g., high molecular weight, viscosity, glass transition, melting temperatures, different glycosidic linkages, and low solubility in water. These unique characteristics make ITF a very
useful material for many different applications. The ITF are very good dietary fibers, resistant to hydrolysis in small intestine and undergo fermentation in colon by microorganisms. Therefore, ITF are prebiotics that help to absorb minerals and fermentation end products. ITF improve endocrine functions by gastrointestinal peptides and protect from translocation and multiplication of pathogenic bacteria. These carbohydrates are dietary substitutes of fats and sugars with low calories. They are used as inactive pharmaceutical excipients in colon-targeted release of drugs, thus protect the stomach from adverse effects of the drugs and promote the growth of beneficial bacteria such as bifidobacteria [5–9]. Prebiotics are useful in declining the frequency of diarrhea, respiratory infections, fever, wheezing, allergy, and atopic diseases in infants. They also speed up gastrointestinal transit time, improve stool consistency, and increase stool frequency. Inulin-type prebiotics regulate the blood sugar levels and reduce the proliferation of cancerous cells in colon in model animals. They lower the triglycerides, HDL-cholesterol, and LDL-cholesterol levels in blood of individuals with hyperlipidemia and lessen the weight gain in human due to decrease in fats accumulation [10–14]. They are functional macromolecules which cause changes in the composition and activity of microflora (Bifidobacteria and Lactobacilli species) in intestine benefiting health and growth of human beings.

Keeping in view the immense importance of ITF (polysaccharides of natural origin), our aim is to review the potentials of this versatile biopolymeric material in the development of functional food and advanced drug delivery systems. To the best of our knowledge, there is no comprehensive review presenting the broad-spectrum properties such as their use in functional food and medicinal applications. Therefore, this compilation will be highly valuable for the people working in these areas. Due to broad spectrum of applications of ITF, we will focus on its general characteristics, isolation methodologies, chemical structure, and its applications in functional food, medicinal, and pharmaceutical sectors. Present review will bridge the knowledge gaps of food chemists, phytochemists, and pharmaceutical chemists. Contents of this compilation will attract and be beneficial for the audience from food and pharma industries as well as from academia. We managed the content in the way that common people may get information about beneficial health effects of ITF as a food.

2 Structure of ITF

A compound having one or more fructosyl-fructose linkages are classified as fructan [15]. Fructan is either a cyclic or a branched molecule which is also known as polyfructosyl-fructose [16]. Chemically, fructans are linear chain of \( \beta-D-\text{glucopyranosyl}[-\beta-D-\text{fructofuranosyl}]_{n-1}\beta-D-\text{fructofuranoside} \) (ITF) or a \( \beta-D-\text{fructopyranosyl}[-\beta-D-\text{fructofuranosyl}]_{n-1}\beta-D-\text{fructofuranoside} \) (inulin) [15]. It is noted from literature that fructans isolated from all dicotyledons and some monocotyledons are ITF [17].

Moreover, it is important to mention here that other structurally related polysaccharides based on fructosyl-fructose linkages are inulin which were discovered from plant, Inula helenium, and may confuse the reader. A wide range of plant
species (approximately 45,000, i.e., 15% of flowering plants) contain inulin as major carbohydrate storage [18]. Therefore, some basic structural information about these closely related polymers, inulin and ITF, are being reported in this chapter. Inulin has $\beta-(2 \rightarrow 1)$ fructosyl-fructose linkage (straight chain, indigestible), and if inulin contains a terminal glucose unit in the chain, then it is called ITF [15, 19–21] (Fig. 1). ITF is also straight chain fructan having indigestible fructose backbone ($\beta$-linked) similar to inulin but ITF has a digestible terminal glucose unit in gastrointestinal tract (GIT). Permethylation analysis of inulin revealed small degree of branching as well [22]. Cyclic forms of inulin have also been reported that contain 6, 7, or 8 fructofuranose rings [23].

3 Isolation Strategies for ITF

The ITF are normally isolated using polar solvents, e.g., water. The factors that influence the quality and yield are temperature, solvent compositions, and number of extraction cycles. As an example, ITF was isolated from the roots of Pfaffia

![Fig. 1 Structures of fructans (ITF and inulin)](image-url)
glomerata Pedersen when dried roots were soaked in distilled water (1:15 w/v) for 2 h at 100 °C. The procedure was repeated thrice to ensure maximum extraction. The aqueous extract was filtered, concentrated, and precipitated using ethanol. The precipitates were separated and lyophilized to get the final product [24].

Recently, ITF was also isolated from roots of Codonopsis pilosula. Sample was dried at 50 °C and grind to get fine powder. After subsequent extraction employing a mixture of ethanol (96%) and distilled water (4%), the liquid extract was filtered, concentrated, and lyophilized. The optimized extraction conditions were established through response surface methodology using Box-Behnken factorial design. The optimized temperature, extraction time, and solvent to material ratio for maximum yield of ITF (20.6 ± 0.2%) are 100 °C, 2.5 h, and 40 mL/g, respectively [25].

Low (<3 kDa) and high (>3.5 kDa) molecular weight ITF were isolated from raw garlic and aged (20 months) garlic extracts. Raw garlic (100 g) was homogenized with Tris-HCl buffer (10 mM) of pH 8. The mixture was centrifuged and filtrate was lyophilized to get powdered form of fructans [26]. Aged garlic extract was collected by storing fresh garlic for a period of 20 months in 25% ethanol. Decanted solution was lyophilized to collect powder form of fructans.

Solid-liquid extraction method was also employed to isolate fructans from Agave pines using distilled water. Agave pines were homogenized with water in a blender at room temperature and pH 5.5. The extract was filtered, centrifuged, and fructans were separated by concentrating the supernatant. Different extraction conditions, i.e., water to raw material ratio, extraction time, and temperature of the solvent were optimized. The maximum fructans yield of 83.3% was achieved at 5.13 mL/g, 1.48 h, and 79 °C, respectively [27].

In a study carried out in Thailand, ITF was isolated from different varieties of plants to optimize the yield. ITF was isolated using hot water extraction method. High levels of ITF were isolated from Chinese garlic, great headed garlic, Jerusalem artichoke, and common garlic. Moreover, medium level ITF were present in red onion and shallot. Great headed garlic has the highest level (29.2 ± 5.62 g/100 g fresh weight of sample) of ITF among all varieties [28].

4 Functional Food and Medicinal Applications

ITF is an important plant derived biomaterial and widely used in many different medicinal and functional food applications. Chemically, ITF is a polysaccharide which is biocompatible and can be isolated from different parts of plants in abundant amount. It has been used as prebiotic due to its beneficial effects on microflora of gastro-intestinal tract (GIT). The ITF also exhibited hypolipidemic effect, blood sugar regulator, colon-specific drug delivery attribute, and eradication of colon specific diseases such as diarrhea, constipation, irritable bowel syndrome, and ulcerative colitis. Although ITF qualifies as potential candidate for many different applications, due to its immense use in functional food and medicine, we will restrict our discussion in these two areas.
4.1 Sugar Replacer

In food industry, low molecular weight fructans like short chain oligofructose and fructooligosaccharide are favored as sugar replacers owing to their greater solubility. Functional characteristics of such polysaccharides are similar to that of sugar or glucose syrup and they possess almost 30–50% sweetness as compared to table sugar. To balance sweetness, artificial sweeteners such as aspartame are mixed with ITF which mask the effect of sweeteners and improve the customer satisfaction [29, 30].

4.2 Prebiotics

Prebiotics is defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [31]. Many oligosaccharides, ITF, and dietary fibers (polysaccharides) have been used for their prebiotic activities [32], though all carbohydrates are not prebiotics [33]. Scientific literature shows that only three products meet the criteria of prebiotics which include ITF, (trans)-galactooligosaccharides, and lactulose [33]. We also noted from the literature that inulin itself is a proved prebiotic [34–36].

In a study, prebiotic effect of fructans, i.e., inulin and oligofructose, was observed by simulating the human intestinal microbial ecosystem at a dose of 2.5 g/day and monitoring the microbial composition and fermentation activity in the colon. Inulin showed a pronounced prebiotic effect as compared to oligofructose and having positive influence on the microbial growth in different parts of colon [37]. Fermentation of ITF mainly depends on the strain of bifidobacteria rather than the species [38]. Prebiotic effects are also attributed to the production of bacterial metabolites through immune regulation which leads to increased gut microbiota, e.g., bifidobacteria, lactobacilli, and bacteroides [39, 40]. The prebiotic effect is also associated with the modulation of biomarkers and stimulation of the immune system [40]. Low dose of ITF is considered as prebiotic which reduces the symptoms of inflammatory bowel syndrome, whereas high dose has negative or neutral effect on such symptoms [39].

High protein intake may lead to the production of toxic end products in the colon as a result of bacterial proteolysis. Prebiotics have the potential to reverse the side effects of bacterial protein fermentation. After administration of ITF, an increase in bifidobacteria and decrease in the concentration of branched chain fatty acid (BCFA) and ammonia (protein fermentation metabolites) was observed. In vivo investigation in 43 volunteers was carried out to confirm the ITF benefits with high intake protein diet. Results of this study indicated that ITF supplementation has inhibitory effects on colonic bacterial proteolysis and potentially stimulate the growth of beneficial bacteria [41].

In a double-blind cross-over study, 34 persons were given low dietary fiber (LDF) and high dietary fiber (HDF) to observe the colonic microbiota response to ITF. After
3 weeks study, an increase concentration of Bifidobacterium and Faecalibacterium was observed in HDF group which indicated the greater response of gut microbiota to HDF in the presence of ITF than LDF [42].

Effect of prebiotic (ITF) on bile acid, fecal microbiota, and metabolites were studied in overweight beagle dogs. Results indicated that ITF are modulator of gut microbiota, bile acid, and metabolites [43].

A study was designed to ascertain the prophylactic effect of ITF on reoccurrence and frequency of infectious diseases in children. ITF supplement (6 g/day) was given to the children (aged from 3 to 6 year) for 24 weeks. During this period, stool consistency, stool microbiota analysis, disease symptoms, dietary habits, and kindergarten absenteeism were determined. At the end of study, the abundance of Bifidobacterium and Lactobacillus were 19.9% and 7.8% higher as compared to control. Additionally, stool sample was soft in the children taking ITF. The incidence of sinusitis and febrile episodes are also reduced from 0.06 to 0.01 and 0.9 to 0.65, respectively. Therefore, ITF supplement exerted beneficial effects on intestinal microbiota and protection against some infectious diseases [32].

4.3 Fat Replacer

Due to structural, nutritional, and physiological aspects related to ITF, their presence in daily diet is important for the growth of beneficial gut bacteria. They are not hydrolyzed by enzymes in upper GIT, but they undergo fermentation in colon and produce hydrogen gas, carbon dioxide, short-chain fatty acids, and lactate. In this way, ITF regulate the bowel, as a result fat deposition will be avoided in body. People with balance food habits such as in Europe and North America ingest 3–11 and 1–4 g ITF daily, respectively [21]. ITF are GRAS (generally recognized as safe) listed item [44] therefore, a number of food materials are supplemented with ITF to enhance fibrous content of food which are generally responsible for fat uptake [14, 36]. Trend of the use of ITF in food industry is growing day by day because they do not affect appearance, color, texture, or taste of nutritional substances. ITF with degree of polymerization greater than 10 are considered the best-known fat replacer in water-based foods without compromising the taste and texture [14]. Hydrophobic long chains of ITF reduce solubility and make smooth and creamy spreads with low fat contents [29]. Therefore, ITF supplemented food is valuable to address obesity issue.

4.4 Peptide Modulator

Diets supplemented with ITF are used to control the food intake by modulating gastrointestinal peptides such as glucagon-like peptide-I and ghrelin in Wistar rats during 3 weeks study. Fructans-based diet when fermented in caecum and
proximal colon reduced the energy intake and fat mass in epididymis owing to increment in glucagon-like peptide-I and proglucagon mRNA concentrations. The diet also decreased the ghrelin concentrations in plasma of treated rats. Thus fructans fibers controlled food and energy intake by producing incretins and anorexigenic–orexigenic peptides [45].

Endothelial dysfunction is the indication of oncoming cardiovascular diseases. Microbiota present in gut played significant role in preventing endothelial dysfunction-related metabolic diseases through initiating various mechanisms. Reversal of endothelial dysfunction was observed in mesenteric and carotid arteries of mice fed with n-3 polyunsaturated fatty acid (PUFA)-depleted diet supplemented with ITF in 12 weeks study. It was due to the activation of nitric acid synthase/NO pathway, which replenished the endothelium lining by promoting growth of bacteria, Akkermansia, and decreasing the population of bacterial taxa in secondary bile acid synthesis. Fructans-based diet preserved the functions of endothelium by increasing concentration of glucagon-like peptide-I and causing changes in expression of gut and liver genes. Thus, diet enriched with ITF improved endothelial function by changing the gut microbiota and peptides [46].

4.5 Obesity Reducing Potential
Accumulation of fats in adipose tissues leads to obesity which may also be due to the environmental and genetic factors. ITF increase fermentation and modify gut microbiota to lessen obesity (Fig. 2). Fat-rich diet results in the production of large adipocytes which are further described by insulin sensitivity. Fat rich diet also stimulates the proliferation of peroxisome activated gamma receptors and G protein coupled receptors. Diet supplemented with ITF enhanced fermentation in the gut, which is depicted by increased weight of gut in mice. It also decreased the expression of G protein coupled receptors and peroxisome-activated receptors induced by fatty diet. Fructans-based diet also decreased the size of adipocytes [47] (Fig. 3).

4.6 Free Radical Scavenging Agent
ITF protects from oxidative stress caused by lipopolysaccharides when studied on mucosa of human colon using proteomic approach. ITF not only prevent the alteration of proteins such as myosin light chain kinase and myosin regulatory subunit but also restore their appropriate level and improve contraction of smooth muscles of intestine. This is due to free radical scavenging and bacterial growth promoting potential of ITF. They also decrease the upregulation of proteins involved in free radical oxidative stress and increase level of detoxifying enzymes such as the metallothionein-2 MT2A, the glutathione–S-transferase K GSTk, and two UDP glucuronosyltransferases, UGT2B4 and UGT2B17 [48].
Fig. 2  Metabolic and histological analysis of subcutaneous adipose tissue in mice fed a CT diet, an HF diet or an HF-ITF diet after 4 weeks of treatment. (A) Subcutaneous adipose tissue mass. (B) Mean adipocytes size. (C) Histological pictures in hematoxylin and eosin stain (bar =100 µm). (D) Free fatty acids
4.7 Anticancer/Anti-Tumor Potential

ITF displayed anticancerous potential in various animal models. ITF decreased abnormal crypt foci and metastasis of tumor cells which were induced by chemicals in large intestine of mice and rats. It was noted that ITF also reduced the incidence of tumor in abovementioned animal models [11]. Fructooligosaccharide (10.0 g) was given to 74 subjects on daily basis for 3 months which was divided into three groups suffering from small colorectal adenoma, large adenoma, and without adenoma. After 3 months study, fecal pH, crypt cell proliferation, and blood parameters were not affected by fructooligosaccharide. However, fructooligosaccharide may affect the colonic environment to prevent the colorectal neoplasia [49]. Thus it can be said that ITF have potential to lessen cell proliferation at infected sites, but results in human beings are not convincing. Extensive research in human beings is still demanding to investigate the potential of ITF in preventing/curing cancer.

Another recent research work has shown the anti-tumor potential of ITF isolated from Atractylodes chinensis rhizome. The anti-tumor activity (in vitro) of ITF was carefully evaluated on four different cancer cell lines (human), i.e., ovarian carcinoma cell line (Skov3), liver hepatocellular carcinoma (HepG2 and 7721), and cervical cancer cell line (Hela) [50] (Fig. 4). From this study, it was inferred that ITF significantly reduced cell proliferation in all cell lines whereas 87.40% proliferation inhibition rate was noted for hepatocellular carcinoma cell lines (HepG2). However, further investigation and clinical trials are required to establish this potential.

![Fig. 3](image-url) Expression of GPR43 and aP2 in explants of subcutaneous adipose tissue incubated in a control medium (CT) or with different pharmacological agents: a PPARγ agonist (TZD), a PPARγ antagonist (GW9662), or a combination of both during 24 h. The CT group is set at 1 value. Data are mean ± SEM. Data with different superscript letters are significantly different at P<0.05, according to the post hoc ANOVA statistical analysis. (Reprinted from [47] with permission from Elsevier)
4.8 Gastrointestinal Disorders

ITF are fibrous indigestible biomaterials, which may affect the bowel transit time, stool frequency, and consistency in various subjects. Bottle-fed 56 preterm infants of just 2 weeks received a formula augmented with inulin (oligofructose) or maltodextrin for 2 weeks. Stool frequency was increased in infants fed with oligofructose which softened the stool and altered the stool consistency [51]. Whereas, 56 healthy children with age ranging from 16 to 46 weeks were provided with fructooligosaccharide (0.74 g per day) supplemented diet for 28 days. Alongside, placebo group received normal routine diet. Stool became softened in treated group with increase in mean number of stools per day as compared to placebo group. Infants fed with fructooligosaccharide have fewer days without stool than

Fig. 4 Inhibitory effects on the proliferation of cancer cells. (A–C) Trypan blue exclusion assay and (D) MTS assay. Data are expressed as mean ± SD of triplicate analyses. Asterisks indicate significant difference versus the control group (*P < 0.05, **P < 0.01, ***P < 0.001). (Reprinted from [50] with permission from Elsevier)
placebo [52]. Recently, it has also been reported that ITF profoundly modifies the composition of caecal microbiota in mice models [46].

Cow’s milk with or without oligofructose fed to full term infants of 2–6 weeks age increased the frequency of stool. Oligofructose augmented diet also made the stool loose. Frequency and consistency of the stool became more significant with increase in concentration of oligofructose in milk [53]. ITF have the potential to manage gastrointestinal disorder such as acute pancreatitis by modulating gut immune and supporting fermentation. Diet supplemented with long chain ITF helps to control caerulin-induced acute pancreatitis in mice as it is evident from decreased levels of serum amylase and lipase, and activity of pancreatic myeloperoxidase. The ITF-based diet also restored the dysfunction caused by acute pancreatitis by upregulating modulatory proteins and microbicidal peptides, and downregulated the pancreatitis initiated up-regulation of IL-1 receptor-associated kinase 4 and phosphor-c-Jun N-terminal kinase. This diet also prevented the inflammation and damage caused to pancreas [54].

Irritable bowel syndrome (IBS) is a chronic disease having symptoms of gastroenteritis, low grade inflammation, dysbiosis, and alteration in colonic gas production. A decrease level of bifidobacteria was observed in IBS and to increase its level, prebiotic stimulated growth ingredients have shown positive effects [39]. Another study on animals showed the effectiveness of ITF in Crohn’s disease and possible mechanism is through increasing the number of beneficial gut microbiota [55].

ITF changed the composition of microbiota present in rat intestine by promoting beneficial bacteria such as bifidobacteria and lactobacilli. Fructans also improved health of model animals by reducing the population of pathogenic bacteria such as Salmonella enterica. Fructans also modified the composition of mucosa in the colon by exciting multiplication in crypts and mucin release. Fructans-based diet also modifies the mucin components in goblet cells and epithelial mucous layer [56].

Owing to fibrous nature of ITF, they may be used to relieve constipation. ITF were ingested by 10 elderly females for 19 days to get rid of hard stool consistency. During first 8 days, dose of ITF was 20 g, which was continuously increased to 40 g from day 9 to 11. After that, dose remained 40 g till the end of study. After ITF intake, bowel consistency was increased with the softening of stool. Bowel frequency improved from 1–2 to 8–9 per week in dose independent fashion in seven female subjects. Other three female subjects showed lesser increase in stool frequency and consistency [57]. Effect of ITF fermentation on the fecal metabolites and the composition of colon bacterial ecosystem were investigated. Change in the composition of fecal metabolites, condition of stool, and occurrence of constipation were observed during the study. The decrease in Bilophila is indicated by the softness of stool. Modest effects on microbiota composition were also observed by abundance in Anaerostipes, Bilophila, and Bifidobacterium [58].

Effect of oligofructose on incidence and severity of diarrhea in 282 infants (6–12 months) were studied for 6 months. Infants received regular diet or diet
mixed with oligofructose (0.55 g/15 g cereal). Incidence of diarrhea occurred after 10.3 and 9.8 days in placebo and oligofructose supplemented diets, respectively. However, prebiotic supplementation has not shown any significant effect on breastfeed infants and young children [59].

ITF enriched diet is used to relieve functional bowel disorder. Functional bowel disorder is similar in symptoms to irritable bowel syndrome and is associated with abdominal fluctuation, rumbling, abdominal pain, bloating and alternating chances of constipation and diarrhea. Fructooligosaccharide (5.0 g twice a day) decreased the symptoms of functional bowel disorder in participants as compared to placebo group which received mixture of sucrose (50%) and maltodextrins (50%). The 6 weeks study was completed by 97 participants [60].

ITF have potential to cure acute ulcerative colitis. Subjects with mild to moderate colitis randomly received high molecular weight inulin (12 g) or maltodextrin along with regular medication using mesalazine (3 g per day) for 2 weeks. Out of 15 subjects who completed trial, 14 showed reduction in disease activity. Decrease in calprotectin, protein biomarker of inflammation in intestine was observed showing that inflammation was reduced with lesser chances of colitis incidence [61]. Ethanol induced gastric ulcer was treated by ITF at two different doses (25 and 50 mg/kg body weight of mice) and results indicated that mucosa ulcer index decreased significantly as compared to control group [62]. Patients suffering from active ileocolonic or colonic Crohn’s disease received inulin oligofructose (15 g) for 3 weeks. These prebiotics increased interleukin-10 positive CD11c + dendritic cells, toll-like receptor 2 (TLR2), and TLR4 expression which indicated the reduction in inflammation by commencing cytoprotective mechanisms in colonic cells [63].

4.9 Regulation of Blood Sugar

ITF do not regulate blood sugar in normoglycemic and hyperglycemic subjects as it is reported in various studies. Twelve healthy individuals divided into four groups were provided diet containing ITF for 84 days. There was investigated insignificant difference in insulin response and glucose tolerance tests [64]. Similar results were observed when 10 g high molecular weight inulin divided into two doses was provided to eight healthy subjects for 3 weeks [65]. Fructooligosaccharide (10.6 g) was used by 30 participants with mild hyperlipidemia in tea or coffee for 2 months. Placebo was provided with 15.0 g maltodextrin with aspartame. There was no statistical difference in fasting blood glucose or insulin. Postprandial plasma glucose level was also same in both groups. However, postprandial insulin was decreased in fructooligosaccharide group than placebo group [66].

Twelve individuals with type 2 diabetes were given fructooligisaccharide (20 g per day) while keeping their medical therapies as per routine. Placebo group was provided with sucrose. Blood glucose concentration was same in treated and placebo groups. Indicators of long-term sugar control such as hemoglobin A1c and fructosamine were not affected in all individuals. Insulin levels
were also remained unaffected [67]. Thus it can be concluded that diet augmented with ITF cannot improve blood glucose levels in normoglycemic subjects and have no clinical advantage in improving metabolism in hyperglycemic subjects. ITF promoted modulatory T-cell response and cytokine production in colon, spleen, and pancreas. Hence, protect the body from autoimmune diabetes through modulation of gut-pancreatic immunity, microbiota homeostasis, and barrier function [68].

4.10 Immunological Properties

ITF have shown immune modulating effect in mice and human through direct and indirect mechanism [69]. In direct mechanism, ITF can be detected by gut dendritic cells through receptor ligation of pathogen recognition receptors and eventually inducing pro- and anti-inflammatory cytokines. Indirect mechanism involves the stimulation and growth of lactic acid bacteria which leads to the production of fermentation products. Being a potent immunomodulator, ITF can be used for prevention of many diseases. Numerous gut immune markers are modulated by ITF as is evident from increased fecal IgA and IL-10 levels, and IFN-γ expressed in Peyer’s patches. ITF-based diet increased immune cell activity in spleen cells as well [69, 70].

4.11 Hypolipidemic Activity

Various reports involving healthy subjects showed insignificant differences in triglyceride, HDL-cholesterol and LDL-cholesterol levels among treated and placebo groups [66, 71, 72]. Studies carried out in hyperlipidemic individuals revealed the lipid lowering potential of ITF, while some studies reported insignificant hypolipidemic potential of ITF. Thirty persons were selected for the evaluation of lipid metabolism having mild hypercholesterolaemia. After stabilizing on standard diet for 1 month, subjects were given ITF (10.6 g/day) for 1 month. Fasting lipoprotein, triglyceride, and cholesterol were measured, and slight decrease in cholesterol level was observed [66].

High molecular weight inulin (10.0 g) decreased significantly plasma triglyceride levels in healthy individuals. However, diet did not affect total, HDL, and LDL cholesterol levels [66]. Twelve obese subjects with high triglyceride and cholesterol received inulin (7.0 g/day) for 4 weeks. Inulin enriched diet decreased their total cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglyceride levels from 248.7 to 194.3, 36.0 to 113.0, 45.9 to 31.6, and 235.5 to 171.1 mg/dL, respectively [10]. Oligofructose-rich high molecular weight inulin caused significant reduction in body mass index of 89 adolescent subjects. Average difference in body mass index between treated and controlled groups was investigated to be 0.52 kg/m². Similarly, average gain in body weight (0.84 kg) due to fat accumulation was less than placebo group [73]. Various mechanisms are
established to improve the lipid profile and reduce the risk of cardiovascular diseases. Such mechanisms are to increase the activity of muscle lipoprotein lipase enzyme, altering blood sugar, insulinemia, and production of polyamines, and increase in the bile salt excretion, cholesterol, and Bifidobacterium population [74].

4.12 Liver Protection

Infusion of *Artemisia vulgaris* is prepared to test its effects on liver in a recent study [75]. This *A. vulgaris* infusion (VI) of aerial parts showed hepatoprotective effects on animal model which were credited to the presence of 40% carbohydrate contents (i.e., ITF) along with antioxidant and immunomodulatory properties of VI. The mechanism of liver protection was studied and it was noted from this work that VI prevents necrosis, raises the reduced glutathione (GSH) levels, and it also reduces the levels of tumor necrosis factor alpha (TNF-α) in the liver (Fig. 5). This study unveiled the protective effect of ITF on liver.

4.13 Arthritis Preventing Effects

ITF has been rarely studied for its anti-arthritic potentials. In a recent study, ITF isolated, purified from *Artemisia japonica* showed molecular weight of 3.1 kDa. Oral administration of this water soluble ITF (200 mg/kg) significantly decreased the clinical parameters of collagen-induced arthritis (CIA) in DBA/1 mice compared with untreated mice [76] (Fig. 6). It was also noted from histopathological analysis of ITF-treated mice that there is less joint damage and inflammatory cell infiltration and reduced serum levels of the interleukins IL-17A and IL-6 which indicate that ITF is a functional food supplement with anti-arthritic properties. Further studies on cartilage repair and its clinical results will insure the said potential.

5 Pharmaceutical Applications

Being a biocompatible polysaccharide, ITF was introduced as a novel drug-delivery system for ibuprofen which belongs to nonsteroidal anti-inflammatory drugs (NSAIDs). Fructans isolated and purified from *Agave tequilana* Weber var. *azul* juice was modified to synthesize acetylated derivative for its application as excipient in sustained drug release systems. Microspheres prepared through coacervation were loaded with ibuprofen, anti-inflammatory drug, and showed sustained drug release in media containing *Bifidobacterium animalis*. With increase in degree of acetylation, solubility of fructans in water decreases. Fructans are nutrient biomaterials providing carbon to bacteria. It delays the hydrolysis of fructans by bifidobacteria due to acetyl group and release of ibuprofen. Maximum amount of drug was released after 12 h of fermentation, which corresponds to optimum growth of bacteria. Thus, acetylated
fructans is novel biomaterial, which improved bioavailability and decreased dosage frequency of the drug with lesser side effects [77]. As well as, ITF have many other biochemical and pharmaceutical technological aspects. It is considered as a renewable material for the production of fructose syrup, bioethanol, single-cell oil, single-cell protein, and many other important products [78].

Fig. 5 Photomicrographs of liver sections stained with hematoxylin–eosin from mice representing the following groups: (A) control (ultrapure water + corn oil); (B) CCl₄ (ultrapure water + CCl₄ 2%, 5 mL/kg); (C) VI: A. vulgaris infusion 4 mg/kg + CCl₄; (D) VPI (10 mg/kg + CCl₄); (E) VPI (30 mg/kg + CCl₄); (F) VPI (100 mg/kg + CCl₄). Symbols: black arrow: centrilobular necrosis; brackets ([]): areas with tumefaction and microvesicular steatosis; asterisks (*): centrilobular inflammatory infiltrate (CV): centrilobular veins; (PV): portal veins. Scale bar = 100 μm. (Reprinted from [75] with permission from Elsevier)
6 Conclusion and Future Prospective

Inulin type fructans (ITF) appeared a highly valuable functional food supplement especially due to their prebiotic and anti-obese properties. Additionally, ITF has been potentially used in targeted delivery of ibuprofen. ITF is degraded in the colon due to the presence of inulinase; therefore, ITF-based drug delivery system may release the active ingredients in colon, hence areas are of commercial importance. Based on literature presented, it can be inferred that ITF, a polysaccharide material, could be very useful for effective delivery of nucleic acid, amino acid, and protein drugs in colon passing safely through harsh gastric pH. ITF is also used to get rid of GIT disorders and can act as a sugar replacer, antioxidant, immune modulator, and hypolipidemic agent. Moreover, acetylated derivatives of ITF through chemical modification have been used in development of novel drug delivery system. ITF is recommended functional food, pharmaceutical ingredient for various biomedical applications, and is placed in generally recognized as safe (GRAS)-listed materials.

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