Chapter 12

Bio-Based Hydrogels

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This chapter examines the development and wide range of uses for hydrogels based on biomaterials, emphasizing their special qualities and contributions to the biomedical domain. Hydrogels made from biopolymers, like collagen and chondroitin sulfate, were first created for wound healing dressings, but they have since been developed for use in tissue engineering, medication delivery, and other fields. Whether cross-linked chemically or mechanically, hydrogels' structural stability is used to manage medication release. Biocompatible and biodegradable, polysaccharide-based hydrogels are used in wound care and medication delivery. Hydrogels based on proteins, such as collagen and enzymes, show promise in the fields of flexible electronics and wound healing. This chapter covers some of the most current developments, such as genetic code engineering for artificial protein-based hydrogels and three-dimensional printing technologies for personalized meals. It goes into further detail on the Replacement, Reduction, and Refinement idea in animal research and has a strong emphasis on in vivo studies, demonstrating the many uses of hydrogels in areas including reproductive health and regenerative medicine. Hydrogels based on biomolecules are being explored, which highlights their potential as a solution to various health-care issues.

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Introduction

Hydrogels are polymer-based materials capable of expanding in water while retaining their shape over extended durations. Their unique characteristic lies in the intricacy of their three-dimensional (3D) network structures. The structural integrity and high water retention capacity of hydrogels result from the crosslinking properties inherent in each polymeric network. The pioneering work of Wichterle et al. marked the inception of hydrogel development, with the creation of the first crosslinked hydrogel. In 1989, Yannas et al. played a pioneering role in hydrogel technology by crafting materials from biopolymers like collagen and chondroitin sulfate, specifically designed for wound healing dressings. Subsequently, hydrogels derived from biopolymers have undergone significant transformations to cater to a diverse range of application needs (*1*). The remarkable characteristics of hydrogels, including their high water content, softness, porosity, and intricate 3D topologies, enable them to mimic the structural features of natural microenvironments within living tissues. This unique set of properties has paved the way for exciting advancements in various fields such as drug delivery, peptide delivery, protein delivery, and the transport of bioactive substances to cells, all made possible by the porosity or microchannel structure of hydrogels. The chemical stability or instability of hydrogels can be attributed to the interactions or bridging between polymer chains. Secondary interactions, including hydrogen bonding, ionic forces, hydrophobic forces, van der Waals, polyelectrolyte complexation, and stereo-complexation, play a major role in the 3D networks of physically cross-linked hydrogels. These hydrogels are noteworthy for their reversibility, which allows them to dissolve in response to changes in the solution's temperature, concentration, and pH. On the other hand, hydrogels that have undergone chemical cross-linking exhibit stability and resistance to environmental fluctuations, hence being referred to as "permanent hydrogels." The chemical cross-linking can result in charged or uncharged hydrogels, contingent upon the properties of the functional groups involved (*2*).

Hydrogels are classified into groups based on the structure of their physical or chemical cross-links (*3*). Covalent bonds maintain the structure of chemically cross-linked hydrogels, while hydrophobic effects like hydrogen or ionic bonds, along with secondary forces, maintain the structure of physically cross-linked hydrogels (*4*). Due to their ability to release pharmaceuticals in biological fluids in a pH-sensitive and controlled manner, hydrogels are a valuable option for injectable drugs. When combined with nanoparticles, they form nanohydrogels. In a study investigating nanohydrogels composed of tragacanth gum and cross-linkers, the swelling behavior was examined under different pH conditions, with the optimal swelling achieved at pH 7.4 and 9 (*5*). In an Ibuprofen delivery study, chitosan zinc oxide nanocomposite hydrogels were produced, and it was observed that as the content of ZnO NPs in the hydrogels increased, the controlled drug release also increased (*6*). Among various types of hydrogels, superabsorbent hydrogels (SAP) have the capability to absorb and retain significant volumes of water or aqueous solutions (*3*). They find applications in diverse areas such as hygienic products, disposable diapers, and drug delivery systems used in agriculture (*7*). Superabsorbent hydrogels can retain up to several hundred times their own weight in liquid swelling capacity (*8*). Polymers, whether natural, semi-synthetic, or synthetic, are employed in creating SAPs. However, most synthetic polymers are not biodegradable and release harmful byproducts during decomposition (*9*). These characteristics may render them unsuitable for environmental and biomedical applications. In contrast, a significant number of naturally occurring polymers are known for their biocompatibility and biodegradability (*10*). Hydrogels are valuable in various disciplines due to the biodegradability, non-toxicity, renewable nature, and biocompatibility of proteins and carbohydrates (*7*).

This chapter delves into the latest developments in hydrogels based on biomolecules and thoroughly examines the potential biomedical uses of these novel materials. A comprehensive approach is clearly needed to produce cost-effective and efficient hydrogels for applications in wound healing, tissue engineering, cosmetics, ophthalmology operations, and drug delivery systems, considering the various challenges that the healthcare industry faces. Due to their exceptional biological characteristics, biodegradability, biocompatibility, and manufacturability, bio-molecule-based hydrogels have attracted considerable attention. These qualities enhance the potential of these hydrogels for a range of biomedical applications. Excellent biocompatibility enables smooth integration with the body, and biodegradability reduces negative effects on the environment, providing sustainable solutions. Moreover, the remarkable biological characteristics of these hydrogels allow them to achieve outstanding outcomes in biological interactions. In this context, enhancing the industrial scalability of biomolecule-based hydrogels is intricately tied to their manufacturing capacity. The broad spectrum of applications for these cutting-edge materials expands their capability to address diverse healthcare-related needs. Hydrogels manifest in a variety of forms, encompassing nanogels (nanoparticles), microgels (microspheres), and large gels. Their classification into distinct types involves various techniques, including source, response type, and crosslinking method. This chapter aspires to provide a comprehensive understanding of the effective utilization of bio-molecule-based hydrogels, encompassing polysaccharides, proteins, and nucleic acids, in practical applications (Figure 1). Beyond the scientific comprehension of these materials, the goal is to elucidate their practical applications within the healthcare domain.

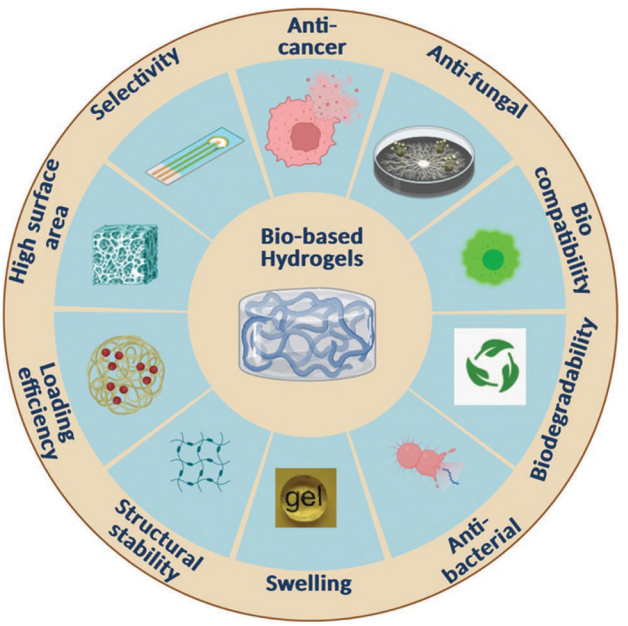


Figure 1. Special properties of bio-based hydrogels for biomedical applications.

Polysaccharide-Based Hydrogels

In recent years, the development of biomaterial-based hydrogels has gained popularity for use in biomedical applications. There is a focus on utilizing bio-based polymers derived from natural sources to reduce environmental impact and produce sustainable biomaterials. The biocompatibility and biodegradability of these polymers make them excellent candidates for *in vivo* biomedical applications. The potential of incorporating polymeric micro- or nanoparticles into hydrogels, as

well as integrating cellulose fiber nanomaterials into bio-based hybrid hydrogels, can be explored for various biomedical applications. Biobased polymers include chitosan, alginate, hyaluronic acid, collagen, gelatin, and gellan gum, representing biomass polymers (*11*). By exploiting the intrinsic features of polysaccharides, Zhao et al.'s study constituted a substantial advancement in hemostatic drugs (*12*). Impressive mechanical and hemostatic capabilities were displayed by the hydrogel, which was made up of oxidized dextran (OD), modified carboxymethyl chitosan (CMCS-MA), and tannic acid (TA)-doped hydrogen bond networks with additional halloysite nanotubes (HNTs). In addition to maintaining low hemolysis and encouraging cell proliferation, it demonstrated improved tissue adhesion and compressive strength. Fast wound adherence and efficient hemostasis were demonstrated by *in vivo* application. With interesting potential applications in wound care and hemostasis, this work successfully produced a stable, mechanically resilient bio-adhesive hydrogel based on polysaccharides. In another study, Xiao et al. addressed the drawbacks of conductive hydrogels in a different study by putting out a bio-based substitute (*13*). The hydrogel, which was crosslinked with borax in a glycerol-water solvent, demonstrated resilience and compressive strength using aloe polysaccharides as the matrix and degradable polyvinyl alcohol. As a wearable sensor, the aloe-polysaccharide hydrogel worked well, detecting humidities with a rapid response time during regular breathing. By overcoming earlier constraints, this study constituted a substantial leap in the field of wearable sensors. In 2023, the importance of polysaccharide-based hydrogels in a variety of applications was highlighted by Wang et al.'s work (*14*). Their findings shed light on the role these carbohydrate polymers play in the complex systems of plants, animals, and microbes, highlighting their ability to take in and hold onto water, which is a necessary component of all living things. Hydrogels' distinct structures and adjustable mechanical characteristics were brought to light by the emphasis on their mechanical characteristics, particularly those of synthetic polymeric double-network (DN) hydrogels. Natural functional polysaccharides, which are known for their abundance, unique structures, cytocompatibility, biodegradability, and variety of bioactivities, have garnered a lot of attention. The study observed that over the last ten years, there has been an increasing interest in using these naturally occurring polysaccharides as crucial ingredients in the creation of strong polysaccharide-based DN hydrogels. In order to improve the duration and efficacy of benzocaine, a local anesthetic, Elmotasem et al. developed a novel topical delivery method. The method was to encapsulate the medication in nano-mesoporous silica, which was subsequently added to different hydrogels based on natural biopolysaccharides. The process of creating these hydrogels by grafting polyacrylic acid onto naturally occurring polysaccharides was thoroughly verified by the investigation. Benzocaine was encapsulated using mesoporous silica nanoparticles (MSNs), and the drug-loaded nanoparticles were then incorporated into the hydrogels. An anesthetic topical bandage was made by applying these composites on cotton gauze. In addition to conducting *in vitro*-release and *ex vivo* drug permeation investigations, the study evaluated the treated gauze's mechanical qualities and resistance to microbiological growth. A potential advancement in benzocaine delivery was demonstrated by the results, showing significantly improved and sustained skin permeability over a one-day period compared to the free drug aqueous suspension. In summary, these investigations collectively contribute to the increasing significance of polysaccharide-based hydrogels in various biomedical applications (*15*).

Protein-Based Hydrogels

Actin, collagen, enzymes, and other macromolecules exemplify the extensive array of important proteins, characterized by amino acid monomers linked through peptide bonds. Enzymes play a crucial role in accelerating biochemical reactions and promoting wound healing, essential for tissue

restoration. Collagen, a vital component in various bodily systems, including the circulatory system, skin, tendons, and bone structure, provides crucial support and is indispensable for wound healing. Actin contributes to the regeneration and reconstruction of muscle tissue. Additionally, the incorporation of short peptide sequences into hydrogels serves to deliver bioactive signals to cells, enhancing connections between the material and living cells, thereby influencing their responses. Numerous effective hydrogel design methodologies have surfaced in recent years for probiotic delivery, offering new avenues for the development of biomaterial medicine. The increased momentum in this sector is attested to by the recent spike in hydrogel-loaded probiotic-mediated medicinal systems. In 2022, Huang et al. explored bioinspired methods for creating multifunctional hydrogels, concentrating on adhesives that were modeled after the adhesion mechanisms of mussels (*16*). Despite the fact that sticky hydrogels have been thoroughly researched, many of them have weak mechanical qualities, transient adherence, and little antibacterial activity. The researchers developed a sticky hydrogel made of acrylamide (AM), soy protein isolate (SPI), and tannic acid (TA) in response to these difficulties. Due to the development of a hybrid double network (DN), the resultant hydrogel showed amazing mechanical properties, such as a significant tensile strain, high tensile strength, and remarkable toughness. Strong adherence to a variety of substrates, antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, self-healing qualities, and biocompatibility were all displayed by this adhesive hydrogel. The hydrogel's potential use as a wound dressing was highlighted in the study's conclusion, along with its capacity to hasten skin tissue regeneration and wound healing. All things considered, Huang et al.'s work demonstrated the potential use of SPI in biomedical materials and offered a practical method for creating multifunctional hydrogels. In a different study, Song et al. used covalent and ionic cross-linking to create a sodium alginate/krill protein/polyacrylamide (SA/AKP/PAM) hydrogel with a multi-network structure (*17*). The study examined the effects of various ion species on the structure and characteristics of the hydrogel, and the results showed a considerable increase in mechanical strength, especially in Fe^{3+} cross-linked hydrogels. These hydrogels demonstrated remarkable ionic conductivity and sensitivity to external forces, allowing their application as a capacitive pen for mobile phone activities. Their compressive strength was 16.13 times more than that of non-ionic crosslinked hydrogels. The hydrogels showed good biocompatibility, an amazing shape memory, and a unique three-dimensional porous structure. Cross-linked hydrogels containing Fe^{3+}, Ba^{2+}, Sr^{2+}, and Ca^{2+} shown no toxicity and were able to promote Schwann cell adhesion and proliferation, indicating potential uses in flexible devices, biosensors, electronic skins, and tissue design. In 2023, Parvaresh et al. used alginate to create a redox- and pH-responsive magnetic hydrogel that was intended to administer doxorubicin (DOX) in a targeted manner (*18*). Using a double crosslinking technique, Fe_3O_4 nanoparticles with changed surfaces were added to the alginate-based hydrogel. The hydrogel showed effective drug loading and encapsulation, followed by release behavior that was pH- and redox-dependent. Strong contact was found in interaction studies with human serum albumin (HSA), which were corroborated by endothermic and spontaneous thermodynamic variables. After 72 hours, the developed magnetic drug delivery system (DDS) showed improved anti-cancer effectiveness against MCF7 cells, suggesting the possibility of gradual, controlled drug release. This work advances the use of responsive hydrogels in targeted and efficient drug delivery applications.

By creating semi-interpenetrating networks between peanut protein and polysaccharide, Lin et al. investigated the possibilities of 3D printing technology for customized food (*19*). The researchers improved gelling and printing qualities of hydrogels by varying the amounts of carrageenan and gellan gum. The findings emphasized that fracture stress and storage modulus rose as polysaccharide quantities increased, reaching remarkable levels with 1.5% addition. Using the thermal reversibility

of gellan gum gels and carrageenan, researchers produced edible inks that are printable and recyclable for use in 3D food printing. The hydrogels' ability to change color in response to pH variations was another feature that made them suitable for use in 4D food printing applications. This study offers insightful information about creating bio-based edible inks that can be used to create customized dishes with distinctive qualities. In order to create 3D hydrogels, Meganathan et al.'s study explored the use of genetic code engineering to create proteins encoded with unusual amino acids (*20*). By using genipin as a cross-linker to promote intermolecular cross-linking and the creation of a hydrogel, hydrogelation was accomplished. The scientists created a synthetic protein-based, biocompatible, 3D adjustable hydrogel by customizing synthetic collagen using unusual chemistry. This next-generation tunable hydrogel was notable for having larger pores, which were especially noticeable when the hydrogel took on a fibrillar shape. The comprehension and possible uses of artificial protein-based hydrogels with improved tunability and distinctive structural properties have advanced as a result of this work. Carbodiimide hydrochloride/*N*-hydroxysuccinimide was utilized in Yan et al.'s investigation to facilitate the grafting of gallic acid (GA) with soybean protein isolate (SPI) in order to generate SPI-GA conjugates and hydrogels (*21*). The materials that were altered, which were mainly connected by C-N bonds, demonstrated exceptional antioxidant qualities. Spectral analysis showed that the grafting of GA resulted in more flexibility in the SPI structure. Schiff base, Michael addition, and hydrogen bonding are examples of covalent and non-covalent cross-linking processes that were used to create the SPI-GA hydrogel. The hydrogel's microstructure, rheological characteristics, thermal stability, and textural attributes were all impacted by the amount of GA grafted; 0.015 weight percent was shown to yield excellent results. Furthermore, SPI-GA's strongly cross-linked structure stopped pepsin from breaking down the protein too soon. To sum up, these results create a lot of opportunities for the creation of active and multipurpose drug delivery systems.

In summary, proteins—such as actin, collagen, and enzymes—are diverse and important biological molecules that are involved in a wide range of biological activities. Because they can quicken biological reactions, enzymes are crucial for the repair of damaged tissue and the healing of wounds. Actin aids in the regeneration of muscle tissue, whereas collagen, a basic component of biological systems, offers essential support and is essential for efficient wound healing. Short peptide sequences are included into hydrogels to improve cell-material interactions and affect biological responses. Recent developments in hydrogel design techniques highlight the expanding significance of biomaterial medicine, especially in the administration of probiotics. Research shows how to develop multifunctional hydrogels for a range of uses, such as targeted medication administration, flexible electronics, and wound healing.

Nucleic Acid-Based Hydrogels

An important development in the field was made by Wang et al.'s investigation of switchable stiffness in hydrogels through redox and light responsiveness (*22*). Shape memory and self-healing features of redox-responsive hydrogels were revealed by the research, showcasing their remarkable potential. The study also explored the field of light-responsive hydrogels, demonstrating how they can perform self-healing and shape-memory tasks. Notably, the study expanded on its conclusions to incorporate the usefulness of these hydrogels in switchable drug release, which significantly increased their functionality. Moreover, adding gold nanoparticles or nanorods to DNA hydrogels revealed exciting new possibilities by emphasizing thermoplasmonic stiffness qualities that are light-switchable. Wang et al.'s thorough investigation of these responsive hydrogels has provided insightful information that will help design dynamic, flexible materials with a wide range of possible uses. In 2022, Zhong et al. addressed the ongoing problem of chronic inflammation in diabetic foot ulcers

(DFU) that are recalcitrant (*23*). Acknowledging that increased extracellular cell-free nucleic acid (cfDNA) levels play a critical role in promoting inflammation, the scientists developed cationic hydrogels based on polyacrylamide. In a diabetic rat model, these hydrogels were topically applied to the ulcers. The study's conclusions showed a significant decrease in cfDNA levels in the wound area after hydrogel application, which resulted in the removal of inflammation. Crucially, systemic toxicity was not brought on by this action. The noteworthy reduction in inflammation and the noteworthy enhancement of wound closure highlight the effectiveness of cationic hydrogels as a potentially effective treatment for uncontrollable activation in DFU.

Assessing the Biocompatibility and Future Prospects: In-Depth Analysis of Bio-Hydrogels through *In Vivo* Trials

The use of animals in scientific procedures has been a subject of debate in recent years due to ethical and scientific concerns. To address these issues, corrective studies are conducted based on principles aimed at modifying, decreasing, and advancing animal use. Despite these efforts, animals continue to be utilized not only in *in vivo* experiments with animal models but also in cell and tissue culture studies, as well as in the production of animal products for cell cultures. The Replacement, Reduction, and Refinement (3R) concept, first introduced in 1959, advocates for replacing animal experiments (*24*). The European Union actively promotes the development of alternative approaches for scientific studies involving animals. Furthermore, studies delve into the utilization of examples such as in silico models, *in vitro* analyses, and alternative models to diminish reliance on animals. *In vivo* animal models demonstrate the wide range of uses for biomaterial-based hydrogels, which provide special benefits for drug administration, tissue engineering, wound healing, cancer research, biosensing, orthopedics, and neurological implants. These hydrogels, which are made of hydrophilic polymers, can be shaped to resemble tissues' extracellular matrix, giving cells a favorable habitat. They reduce side effects by enabling the focused and controlled release of therapeutic ingredients in drug delivery. Hydrogels are used in tissue engineering as scaffolds to promote cell proliferation and regeneration. By fostering a supportive environment, they aid in wound healing, and by simulating tumors, they aid in cancer research. Hydrogels are also utilized in orthopedics for minimally invasive operations and in biosensors for real-time monitoring. Before bringing these applications into clinical settings, however, comprehensive preclinical research and careful consideration of biocompatibility are crucial. However, the adoption of these alternatives raises concerns related to data reliability, reproducibility, and the development of non-animal products.

Bioengineering applications, such as hydrogels, play a crucial role in human medicine. Ongoing studies focus on the development of *in vitro* models for various cases, including regeneration in endometrial tissue, uterine incision repair (*25*), regulation of fertility, ovarian functions, follicle growth, and hormone sensitivity. Consequently, these applications span a broad spectrum within the human reproductive system. The applications extend to the development of artificial ovaries for fertility preservation, *in vitro* and *in vivo* models for addressing reproductive disorders, organoid cultures capable of mimicking the physiology and pathology of reproductive tissues, and the advancement of reproductive organs. In the work by Franc et al. (*26*), numerous reproductive applications were explored, ranging from ovarian tissue regeneration and follicle encapsulation to uterine wall regeneration and endometrial repair. Biomaterials, including hydrogels, decellularized cell wall matrix (dECM), and polymer scaffolds, were employed in these applications. The study also delved into the development of organoids and *in vitro* models for research on reproductive organ cancers.

In recent years, studies have commenced on the utilization of bio-based nanomaterials in cancer treatment. Traditional cancer treatments, including surgery, chemotherapy, and radiotherapy, occasionally face challenges in effectively addressing the disease due to the complex pathophysiological structure of tumors (*27*). Consequently, the exploration of nanomaterials for tumor treatment has gained prominence. Bio-based nanomaterials possess advantageous features such as autologous pharmaceutical properties, synergistic effects, biocompatibility, biodegradation, and biosafety. The rise in cancer-related deaths underscores the imperative to develop innovative methods in cancer treatment. In recent years, studies have been initiated to explore the use of nanocomposite hydrogels in cancer treatment. It is emphasized that nanocomposite hydrogels may exert a therapeutic effect when applied in treatment methods (*28*). These hydrogels are formed by dispersing nanoparticles in hydrogels, combining the softness and wetting capacity of hydrogels with the stiffness, dimensional stability, and thermal stability of nanomaterials.

The preservation of bioactive chemicals is achievable due to the 3D structure and appropriate mechanical and biological properties of hydrogels. However, conventional hydrogels made through chemical crosslinking pose challenges for future use because of their poor mechanical qualities and limited functionality. These constraints have prompted the consideration of combining nanoparticles with hydrogels (*7*). Studies on the use of injectable nanocomposite hydrogels in the management of various types of cancer, including ovarian, colorectal, and breast cancer, have been conducted. Data indicate that nanocomposite hydrogels show promise in targeted synergistic therapy, drug delivery, and the prevention of cancer recurrence (*7*). Colorectal cancer (CRC) stands as one of the leading causes of cancer-related mortality globally. Additionally, the liver is a common site for metastatic lesions due to its proximity to the portal drainage area of the colon. Consequently, new studies are underway to explore treatments and drug delivery systems for liver metastases in colorectal cancer. The total resection of the tumor is deemed the most appropriate method for treating malignant lesions in the large intestine. Radiation therapy proves effective in reducing pelvic recurrences. In the context of liver metastases, direct chemotherapy infusion into the hepatic artery or portal vein enhances the treatment's effectiveness (*29*). Studies (*30*) underscore the effectiveness of drug delivery systems, such as biocompatible polymeric structures, against colorectal cancer. The utilization of biocompatible polymeric agents, encompassing hydrogels and nanoparticles, as drug delivery systems suggests a range of alternatives for delivering chemotherapeutic agents. These systems exhibit targeted distribution and controlled drug release in cancerous tissue, thereby enhancing drug effectiveness while reducing side effects. Biocompatible polymeric agents hold potential for obtaining therapeutic agents, including chemotherapeutics, proteins, and agents used in gene therapy, paving the way for innovative alternatives in cancer treatment. In another study, Kono et al.(*31*) demonstrated that β-cyclodextrin-grafted carboxymethyl chitosan hydrogels (CD-*g*-CMCs) serve as a potential drug bio-carrier capable of releasing aspirin under regulated conditions. This is attributed to hydrogels having a high concentration of functional groups, making them suitable for bio-conjugation with cell-targeting drugs as well as crosslinking with additional functional crosslinkers. In a study on the encapsulation of caprine follicles with different concentrations of alginate (ALG) hydrogels *in vitro*, higher growth rates, steroid hormone production, and meiotic resumption of the oocytes were observed in the follicles. In the field of biomedical research, Thuwanut et al.(*32*) reported that biodegradable hydrogels, such as fibrin-thrombin clot, fibrin-collagen, alginate, and 3D-printed microporous hydrogel scaffolds, can be used for follicle culture with fibrin thrombin at different concentrations. The objective was to delineate the effects of encapsulating ovarian tissue with varying fibrinogen concentrations on the outcomes of cryopreservation. The study also sought to determine how ovarian tissue and follicle integrity

and quality were affected by static and non-static culture methods after encapsulating and cryogenic preservation. They found that the novel technology improved cryogenic preservation, *in vitro* growth, and ovarian tissue encapsulation when they used a non-static system in a cat model. Alginate, either alone or in combination with other materials, is the most commonly used hydrogel-based method for encapsulating follicles and tissue fragments in ovarian bioengineering. In studies assessing hydrogels, mouse, dog, goat, ovine, primate, and human ovarian follicles and tissues were employed for *in vitro* cultures. However, natural and synthetic hydrogels, such as laminin, collagen, or polyethylene glycol (PEG), are less frequently utilized in these types of studies (*26*).

Excipients play crucial roles in the stability, release, and bioavailability of pharmaceuticals, making their use essential in the pharmaceutical business. Bio-based materials are fundamental components for drug delivery systems due to their stability, renewability, and low toxicity (*33*, *34*). Composite hydrogels, with improved mechanical properties, resistance to degradation, and controlled drug delivery, have demonstrated greater advantages in biomedical applications compared to single-component hydrogels (*35*). Despite these benefits, it is essential to acknowledge the difficulties and limitations associated with the application of composite hydrogels. Challenges such as the long-term biodegradability of therapeutic effects, toxicity, accumulation, and the evaluation of foreign body response *in vivo* have been emphasized (*36*). In a study by Yadollahi et al. (*37*), the fabrication of hydrogel beads containing an antibacterial chitosan/silver bio-nanocomposite for use as a drug delivery system was investigated. Silver nanoparticles were employed, using sodium tripolyphosphate as a cross-linker, and physical crosslinking occurred during the fabrication of chitosan hydrogel beads. The objective of the study was to demonstrate the use of these nanocomposite beads in drug delivery systems. The nanocomposite hydrogels exhibited a significant antibacterial effect against bacteria such as *Escherichia coli* and *Staphylococcus aureus*, with an increased capacity attributed to the presence of silver nanoparticles in the environment.

In tissue engineering, the utilization of biobased composite hydrogels containing cellulose nanocrystals or nanofibrils has exhibited promising biocompatibility. This compatibility renders bioadhesives suitable for potential applications in intervertebral disc regeneration, wound closure, and healing. Furthermore, the presence of nanocrystals or nanofibrils underscores the potential of nanocellulose-containing bio-based hydrogels in controlled drug delivery systems, as well as in the sustained release of therapeutic agents. Studies on the application of bio-based composite hydrogels for biomedical purposes are not only promising but also shed light on the environmental and biocompatibility benefits of employing natural macromolecules.

In studies on tissue engineering, the 3D printing of alginate bioinks cross-linked with calcium ions and loaded with human chondrocytes has been investigated (*38–40*). Beyond assessing cell cytotoxicity, bioink has demonstrated its ability to support cell viability in composite hydrogels, promote cell proliferation, induce chondrocyte differentiation, and facilitate the neo-synthesis of cartilage-specific extracellular matrix components (*39*, *40*). The synthesis of chitosan/cellulose nanocrystal hydrogels for theophylline regulated delivery was investigated in a different work. It was found that the release of the medication decreased in proportion to the quantity of dialdehyde nanocellulose present in the blends. The potential of composite hydrogels in gastric medication delivery systems is highlighted by this discovery (*41*).

Preantral follicles cultured *in vitro* is an essential technique for researching the mechanisms controlling the initial stages of follicle development. This process optimizes the amount of oocytes available for cloning, oocyte production, and *in vitro* embryo creation (*42*). There are several methods available for *in vitro* follicle culture. Using the connected follicle method, follicles can spread out across a two-dimensional (2D) surface by connecting with one another. The follicles

maintain their inherent 3D structure using the second technique (*43*). Hydrogels made of alginate, a biocompatible polysaccharide, have been used in numerous investigations to encapsulate and cultivate isolated oocyte follicles in 3D networks. To maximize circumstances for sufficient follicular development, these parameters are chosen based on the physical characteristics and concentration of alginate (*44*). An encapsulated 3D culture method with varying alginate concentrations was examined in a particular study to improve the growth and survival of goat secondary follicles and oocytes. In comparison to lesser concentrations, the study found that a higher alginate content increased the rate of morphologically normal follicles. The study's overall findings suggested that the 3D alginate culture method might somewhat replicate the *in vivo* environment (*45*).

Recently, smart polymer-based hydrogels have gained attention for drug delivery systems, especially for targets that are not easily accessible. These hydrogels have the ability to transform into a liquid form at suitable temperatures, pH levels, and ionic strengths in the surrounding media. Among the preferred options is the viscoelastic compound gelatin, suitable for use in the body (*46*). In a particular study, chitosan and poloxamers (Pluronic^{TM}) were employed to create mucoadhesive thermosensitive hydrogels. In order to create thermosensitive hydrogels, the study assessed the adhesive qualities of combinations of samples of CS·HCl and Pl F-127, both with and without the addition of D-AscA. These hydrogels, which have a high aminopolysaccharide content and strong antibacterial activity, gel at physiological temperatures. These hydrogels are meant to be used in the management of postoperative *serous-catarrhal vaginitis* in cows (*47*).

Chitosan-based biopolymers and zinc oxide nanoparticles (ZnO NPs) are utilized in the development of nanocomposite hydrogels for drug delivery systems. *In vitro* drug release tests have demonstrated that chitosan particles containing ZnO NPs provide a long-term and controlled drug release. The addition of ZnO NPs to the chitosan matrix has been observed to influence the surface morphology. Chitosan/ZnO nanocomposite hydrogels are promising for biomedical applications due to their biocompatibility, environmental friendliness, and cost-effectiveness (*37*).

Hydrogels designed to mimic the extracellular matrix (ECM) find numerous applications in biomedicine. Studies have explored the use of the electrospinning technique to produce these hydrogels. Electrospinning employs hydrophilic and biocompatible polymer hydrogels to create fibrous structures. These fibrous hydrogels have versatile applications, including serving as platforms for 2- and 3-dimensional cell cultures, implanted scaffolds for tissue regeneration, wound dressings, and biosensors. The extensive use of hydrogels in healthcare opens up possibilities for treating many previously untreatable diseases and introducing new therapeutic agents to the medical field. Hydrogels are poised to play a crucial role in the development of novel treatment procedures, particularly in the effective treatment of cancer, one of the most significant diseases today.

Conclusion

In conclusion, this chapter has provided a thorough review of the various uses and advancements in hydrogels made of biomaterials. The emphasis on biopolymers—such as collagen and chondroitin sulfate—has illustrated their progression from dressings for wound healing to flexible materials for tissue engineering, medication administration, and other applications. The ability to manage drug release through the structural stability of hydrogels—whether cross-linked chemically or mechanically—demonstrates the versatility of these materials in medication administration. The investigation of hydrogels based on polysaccharides has highlighted the importance of using bio-based polymers obtained from natural sources. These hydrogels, containing polymers like chitosan, alginate, hyaluronic acid, collagen, gelatin, and gellan gum, are biocompatible and biodegradable, making them excellent choices for a range of biomedical applications. Current research on

carbohydrate polymers emphasizes the crucial role of naturally occurring functional polysaccharides in developing robust hydrogels used in medication delivery and wound care. Protein-based hydrogels, containing enzymes, collagen, and actin, have shown promise in various applications, including flexible electronics and wound healing. Protein-based hydrogels have achieved significant advancements due to creative methods like bioinspired adhesives and 3D printing technologies. The promise of protein-based hydrogels in furthering therapeutic applications is exemplified by noteworthy studies on redox- and pH-responsive magnetic hydrogels for targeted drug delivery. The issue of nucleic acid-based hydrogels is expanding in the field of biomaterials research. The use of nucleic acids in hydrogel production and the incorporation of genetic code engineering for artificial protein-based hydrogels represent a novel approach to developing structurally different and customizable hydrogels. These developments create new opportunities for customized hydrogel applications, such as the research on 3D hydrogels using genetic code engineering. In conclusion, the ongoing research and development of hydrogels based on biomaterials emphasize their importance in tackling a range of healthcare issues. These hydrogels provide a flexible platform with significant promise for future breakthroughs and applications in biomedical research and technology, ranging from wound care to drug delivery.

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