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Chitosan-based hydrogels for controlled, localized drug delivery ☆

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ABSTRACT

Hydrogels are high-water content materials prepared from cross-linked polymers that are able to provide sustained, local delivery of a variety of therapeutic agents. Use of the natural polymer, chitosan, as the scaffold material in hydrogels has been highly pursued thanks to the polymer's biocompatibility, low toxicity, and biodegradability. The advanced development of chitosan hydrogels has led to new drug delivery systems that release their payloads under varying environmental stimuli. In addition, thermosensitive hydrogel variants have been developed to form a chitosan hydrogel in situ, precluding the need for surgical implantation. The development of these intelligent drug delivery devices requires a foundation in the chemical and physical characteristics of chitosan-based hydrogels, as well as the therapeutics to be delivered. In this review, we investigate the newest developments in chitosan hydrogel preparation and define the design parameters in the development of physically and chemically cross-linked hydrogels.

Contents

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1. Introduction

Despite the discovery of a large number of active compounds that could serve as therapeutics, very few candidates have shown clinical success. Poor activity in vivo is most often attributed to their low 'bioavailability', the extent and rate at which a drug reaches and affects target tissue [1]. This metric largely depends on the route of administration and on organ physiology and metabolism [2]. When administered systemically, drug bioavailability is typically very low, and the blood plasma concentration of the drug can quickly drop below an effective level, requiring re-administration. This can lead to decreased patient compliance and increase the possibility of an overdose.

Controlled delivery systems provide an alternative approach to regulating the bioavailability of therapeutic agents. In controlled drug delivery systems (DDSs), an active therapeutic is incorporated into a polymeric network structure in such a way that the drug is released from the material in a predefined manner [3,4]. Depending on the drug delivery formulation and the application, the drug release time may be anywhere from a few hours to a month to several years [3]. A variety of synthetic and natural polymers have been studied as drug carriers [5], and DDSs have capitalized on their wide-ranging hydrophobic and hydrophilic components, and their polymer–polymer, polymer–drug, polymer–solvent, or polymer–physiological medium interactions. While there are practically limitless combinations of materials to explore, engineers are restricted by material biocompatibility, toxic byproducts, surgical removal of DDSs, and manufacturing cost.

Researchers have strived to engineer the physical and chemical properties of DDSs to specifically regulate their permeability, environmental response, surface functionality, biodegradability, and biorecognition sites to produce "intelligent" DDSs. Hydrogels represent a DDS class that has excelled at intelligent drug delivery [5,6]. These gels are entangled polymer networks that trap a large amount of water without dissolving. There are several excellent reviews of intelligent hydrogel DDSs that utilize synthetic hydrophilic polymers [1,5,7–10], but many of these are not biodegradable (poly(N-isopropyl acrylamide), poly(2-hydroxyethyl methacrylate), polyvinyl alcohol) or suffer from other issues, such as local inflammation.

Biocompatible, biodegradable hydrogels have been designed using natural polymers that are susceptible to enzymatic degradation, or using synthetic polymers that possess hydrolyzable moieties. Of these, hydrogels using the natural polymer, chitosan, have received a great deal of attention due to their well documented biocompatibility, low toxicity [11,12], and degradability by human enzymes [13]. An in-depth review on chitosan's biocompatibility and biodegradability can be found in the article by ... et al. in this theme issue. These and other positive traits (e.g. hydrophilicity, functional amino groups, and a net cationic charge) have made chitosan a suitable polymer for the intelligent delivery of macromolecular compounds, such as peptides, proteins, antigens, oligonucleotides, and genes. In this review we will summarize the various classes of chitosan-based hydrogels, examine their physical properties, and present recent advances in chitosan hydrogel development for therapeutic applications. We will also present specific examples of chitosan-based hydrogels used for cancer therapeutics, subcutaneous release, and oral delivery.

2. Hydrogels

Hydrogels are comprised of cross-linked polymer networks that have a high number of hydrophilic groups or domains. These networks have a high affinity for water, but are prevented from dissolving due to the chemical or physical bonds formed between the polymer chains. Water penetrates these networks causing swelling, giving the hydrogel its form. Fully swollen hydrogels have some physical properties common to living tissues, including a soft and rubbery consistency, and low interfacial tension with water or biological fluids [8,14,15]. The elastic nature of fully swollen or hydrated hydrogels has been found to minimize irritation to the surrounding tissues after implantation. The low interfacial tension between the hydrogel surface and body fluid minimizes protein adsorption and cell adhesion, which reduces the chances of a negative immune reaction.

In addition, hydrogels have several additive characteristics that make them excellent drug delivery vehicles. First, many polymers used in hydrogel preparations (e.g. polyacrylic acid (PAA), PHEMA, PEG, and PVA) have mucoadhesive and bioadhesive characteristics that enhance drug residence time and tissue permeability [16,17]. This adhesive property is due to inter-chain bridges between the hydrogel polymer's functional groups and the mucus glycoproteins [17], which can help enhance site-specific binding to regions, such as the colon, nose, and vagina [17,18].

The dimensions of hydrogels can also vary widely, ranging from nanometers to centimeters in width. They are also relatively deformable and readily conform to the shape of any space to which they are confined. [8,19–21]. Also, because the hydrogel's physiochemistry is similar to the native extracellular matrix, both compositionally (such as GAGs) and mechanically, hydrogels can serve as dual-propose devices, acting as a supporting material for cells during tissue regeneration as well as delivering a drug payload [22,23].

2.1. Hydrogel preparation

The major structural component of a hydrogel, the hydrophilic polymer, is based on several parameters, including (1) the amount of water the hydrogel is expected to absorb, and (2) the method of binding the polymer chains within the gel network. Hydrophilic polymers can absorb different amounts of water depending upon the density of the hydrophilic groups present on the polymer. The most widely utilized hydrophilic polymers for hydrogels include PEG, PVA, PHEMA, PAA, poly (methacrylic acid) (PMA), and polyacrylamide (PAM) [5,6,16,24]. Water absorption for these polymers can range from a fraction to several thousand times their own weight [5].

To form stabilizing linkages, hydrogel polymers have functional moieties that allow binding between the chains to prevent gel dissolution. Polymer binding is accomplished either by non-covalent physical associations, such as secondary forces (hydrogen, ionic, or hydrophobic bonding) and physical entanglements, or by covalent cross-linkages [5,25]. Both methods can sufficiently restrain hydrogel swelling, but the physical associations are reversible bonds, whereas the covalent cross-linkages between polymer chains are not. This distinction is important for the biodegradation and drug release kinetics of DDS hydrogels.

During gel hydration, the polymer chains interact with the solvent molecules and expand to the fully solvated state. While the material expands, the cross-linked structure offers the retractive force to restrain the polymer chains as described by Flory's rubber elasticity theory [26]. The counterbalance of the expanding and retracting forces reaches equilibrium in the solvent at particular temperatures. The swelling characteristics of a hydrogel is a key parameter in its use in diverse applications because the equilibrium swelling ratio (i.e.

weight ratio of swollen hydrogel over the dry hydrogel) influences the solute diffusion coefficient, surface wettability and mobility, and the optical and mechanical properties of the hydrogel [6,27,28].

The physical properties of swollen hydrogels are regulated by the molecular weight (MW) of the polymer, charges on the polymers, density of the cross-linking (covalently bonded networks), and physical associations. Each of these conditions helps define the relative amount of bonding between polymer chains. For example, high molecular weight polymers typically have multiple cross-linkages per polymer producing more robust hydrogels, while smaller polymers are required at higher concentrations to produce sufficient gel rigidity [29]. Increases in the hydrogel cross-linking results in an increase in both the moduli and stiffness [30,31]. These properties are important in the protection of encapsulated biomolecules from mechanical deformation at the transplantation site and during hydrogel migration (e.g. during oral delivery) [32]. Similarly, the most important parameters that regulate diffusion of the encapsulated therapeutics out of a hydrogel are the material's pore or mesh size and the hydrodynamic size of the drug [33,34].

2.2. Hydrogel drug loading

DDSs offer localized release of therapeutics that systemically delivered agents cannot match. By selectively placing a DDS adjacent to diseased tissue, the release of the drug leads to high bioavailability at the site of action, with low therapeutic levels in other sensitive regions of the body. Alternatively, DDSs or bare drugs injected intravascularly can be rapidly sequestered by clearance organs, which can lead to an unsafe exposure of healthy tissue to toxic drug levels.

A range of hydrogel systems have been explored for the controlled delivery of many biomolecules, ranging from small molecular weight drugs to biomolecules, such as nucleic acids, peptides, and proteins. Because of the diversity in the chemistry and size of the delivered molecules, the drug loading for controlled release in any particular hydrogel can differ widely from one application to another. The method by which the drugs are loaded directly impacts the availability of the drugs during release. Therefore, several different approaches to drug incorporation have been developed.

2.2.1. Direct addition of drugs to hydrogels

Direct therapeutic loading into the hydrogel can be accomplished by encapsulation, during which the polymer chains are cross-linked in the presence of the drug, protein, or macromolecule. Alternatively, the therapeutic can be allowed to diffuse into the pores of the hydrogel after cross-linking [35]. While both of these methods represent the easiest ways to add active agents to the hydrogel, the release of the loaded molecules is not well regulated. Typical release profiles show a rapid burst release of the drugs during initial hydrogel swelling after transfer in vivo, followed by the extended release of the remaining drugs encapsulated within the gel network. These burst releases can lead to losses of up to 70% of the therapeutic payload, but can be lowered to 10–25% with increased polymer cross-linking [36,37]. The addition of drugs before or after cross-linking the polymer also alters their release profiles.

2.2.2. Incorporation of separate release systems in the hydrogel

If the retardation of drug release using cross-linked hydrogels is not sufficient to slow the release rate for long-term applications (e.g. on the order of several weeks of sustained delivery), another release system may be incorporated into the hydrogel, such as drug containing micro- or nano-capsules [38]. For example, the release of transforming growth factor-beta1 (TGF-beta1) can be well regulated over almost a month by encapsulation of TGF-beta1-loaded gelatin microparticles within the biodegradable polymer oligo (poly(ethylene glycol) fumarate) gel [39]. PVA-based hydrogels showed loss of the dexamethasone payload over 2 weeks, but when the steroid was loaded into encapsulated microspheres, drug release was slowed to ~6%/month [40]. Researchers have also dispersed microparticle-containing drugs and non-swelling polymers from the hydrogel matrix without affecting the swelling and degradation of the existing hydrogel network. As the hydrogel degrades, the encapsulated drugs or proteins are released from the hydrogel and can slowly diffuse away from the gel into the outside medium [41].

2.2.3. Covalent attachment to the hydrogel-forming polymer

The porous structure of the hydrogel allows drugs loaded into the DDS through physical mixing to be rapidly eluted from the hydrogel. In order to better control drug release, drug payloads can also be covalently bonded to the hydrogel matrix in such a way that their release is primarily controlled by the rate of chemical or enzymatic cleavage of the polymer–drug bond [42]. This has been done with a number of small molecular weight drugs, such as paclitaxel (a chemotherapeutic), dexamethasone (an anti-inflammatory and immunosuppressant), and fluvastatin (a cholesterol-lowering medicine) [43–46]. Covalent conjugation has extended drug release from weeks to months. For instance, a thermosensitive polyphosphazene–paclitaxel conjugate gel showed the sustained release of paclitaxel for up to a month. In addition, dexamethasone was conjugated to a photoreactive mono-acrylated polyethylene glycol (PEG) through a degradable lactide bond to facilitate osteogenic differentiation of human mesenchymal stem cells, and daunomycin cross-linked to poly(aldehyde guluronate) was released over periods ranging from 2 days to 6 weeks based to the hydrolysis rate of the drug–polymer covalent linkage [45]. Alternately, drug release may be regulated via hydrolysis of the polymer chains. These methods slow drug release, but are not regulated by enzymatic activities at the DDS site.

Covalently attached therapeutics are released from the hydrogel networks either due to hydrolysis of the chemical bond between drug molecules and polymer or due to degradation of gel network itself. Both processes are nonspecific, leading to relatively poor control over the drug release rate. If bonding between the therapeutic and hydrogel polymer is established by an enzyme-sensitive tether and broken by the specific enzyme produced during normal cell activity in or around the hydrogel, a “smart” DDS is created and its drug release is more specific to the target tissue. For example, a vascular endothelial growth factor (VEGF) can be covalently immobilized within a hydrogel network by enzyme-sensitive oligopeptides [47]. The release of VEGF is mediated by proteases (e.g. matrix metalloproteinases) secreted by migrating cells. The cell-demanded VEGF release matches the release profiles with the cellular activity that is critical during tissue regeneration. One major difficulty in implementing this strategy is anchoring the bound therapeutics by nonspecifically blocking the active site of the attached molecule.

2.3. Hydrogel drug release

Due to the high water content of hydrogels, their molecular release mechanisms are very different from other DDSs comprised of less hydrophilic or hydrophobic polymers. Previous modelistic studies predict that the release of an active agent from a hydrogel, as a function of time, is based on the rate-limiting step for controlled release and therefore categorized as diffusion-controlled, swelling-controlled, or chemically-controlled. Diffusion-controlled release through the hydrogel mesh is the primary mechanism of release of many drugs from hydrogels, which regulates therapeutic release [48,49]. Typical mesh sizes reported for biomedical hydrogels range from 5 to 100 nm (in their swollen state) [33], which are much larger than the size of most small molecule drugs. As a result, the diffusion of such drugs is not significantly retarded in the swollen state, whereas macromolecules like protein and peptides, due to their hydrodynamic radii, will have a sustained release unless the structure and mesh size of the swollen hydrogels are designed appropriately to obtain the desired rates of macromolecular diffusion. In case of the swelling-controlled mechanism

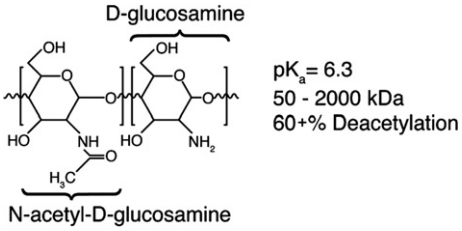


Fig. 1. Chemical structure of chitosan.

when diffusion of a drug is significantly faster than hydrogel distention, swelling is considered to be controlling the release behavior [50]. Finally, chemically-controlled release is determined by chemical reactions occurring within the gel matrix. These reactions include polymeric chain cleavage via hydrolytic or enzymatic degradation. Chemically-controlled release can be further categorized according to the type of chemical reaction occurring during drug release. Generally, the liberation of encapsulated or tethered drugs can occur through the degradation of pendent chains or during surface erosion or by bulk degradation of the polymers [51].

3. Chitosan hydrogel preparations

The development of hydrogels from a variety of synthetic materials has provided a great deal of flexibility in engineering the characteristics of the fabricated DDS. PEG, PVA, PHEMA, PAA, PMA, and PAM have all been used to form hydrogels with variable mechanical strengths and biological responses [5,6,16,24,52]. Natural polymers, such as polysaccharides and proteins, have also been used as the structural material in hydrogels. This is largely due to an interest in the intrinsic properties of these polymers including biocompatibility, low toxicity, and susceptibility to enzymatic degradation. Among these polymers, polysaccharides do not suffer some of the disadvantages of other naturally derived materials, such as immunogenicity and the potential risk of transmitting animal-originated pathogens. One such polysaccharide is chitosan. This attractive natural polysaccharide shares the benefits of other natural polymers (lysozomal degradation, etc.), but does not induce an immune response.

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units (Fig. 1). Commercially, it is produced by the exhaustive deacetylation of chitin (>60%), a structural element in the exoskeleton of crustaceans and insects, which is the second most abundant natural biopolymer after cellulose [53,54]. The most easily exploited sources of chitin are the protective shells of crabs and shrimp. The primary aliphatic amines of chitosan can be protonated under acidic conditions (amine pKa is 6.3) [55].

This polymer is distinct from other commonly available polysaccharides due to the presence of nitrogen in its molecular structure, its cationicity, and its capacity to form polyelectrolyte complexes. The cationic nature of the polymer allows it to become water-soluble after the formation of carboxylate salts, such as formate, acetate, lactate, malate, citrate, glyoxylate, pyruvate, glycolate, and ascorbate. In addition to recent monographs and review articles, valuable information on the structural, physical, and chemical properties of chitosan can be found in the American Standard Testing Materials (ASTM) standard guides and in the U.S. Pharmacopoeia (USP) [54,56].

Chitosan is an excellent excipient because it is non-toxic, stable, biodegradable, and can be sterilized. These properties also make chitosan a very versatile material with extensive application in the biomedical and biotechnological fields [53,54]. These attractive properties also make the polymer an ideal candidate for controlled release formulations. Indeed, chitosan has played a leading role in advanced biomaterial applications, including non-viral vectors for DNA-gene and drug delivery. There are many recent reviews surveying the hundreds of papers related to chitosan drug delivery systems, but little information is available on the use of this polymer in hydrogel formulations.

Chitosan hydrogels have been prepared with a variety of different shapes, geometries, and formulations that include liquid gels, powders, beads, films, tablets, capsules, microspheres, microparticles, sponges, nanofibrils, textile fibers, and inorganic composites [21]. In each preparation chitosan is either physically associated or chemically cross-linked to form the hydrogel. Our discussion below will focus on these two distinct hydrogel engineering approaches.

3.1. Physical association networks

In order to satisfy the requisite features of a hydrogel, the chitosan polymer network must satisfy two conditions: (1) inter-chain interactions must be strong enough to form semi-permanent junction points in the molecular network, and (2) the network should promote the access and residence of water molecules inside the polymer network. Gels that meet these demands may be prepared by non-covalent strategies that capitalize on electrostatic, hydrophobic, and hydrogen bonding forces between polymer chains [57,58]. Fig. 2 shows the schematics of four major physical interactions (i.e. ionic, polyelectrolyte, interpolymer complex, and hydrophobic associations) that lead to the gelation of a chitosan solution. Because the network formation by all of these interactions is purely physical, gel formation can be reversed.

Tunable gel swelling behavior can be readily achieved in a physical gel by adjusting the concentration and nature of the second component

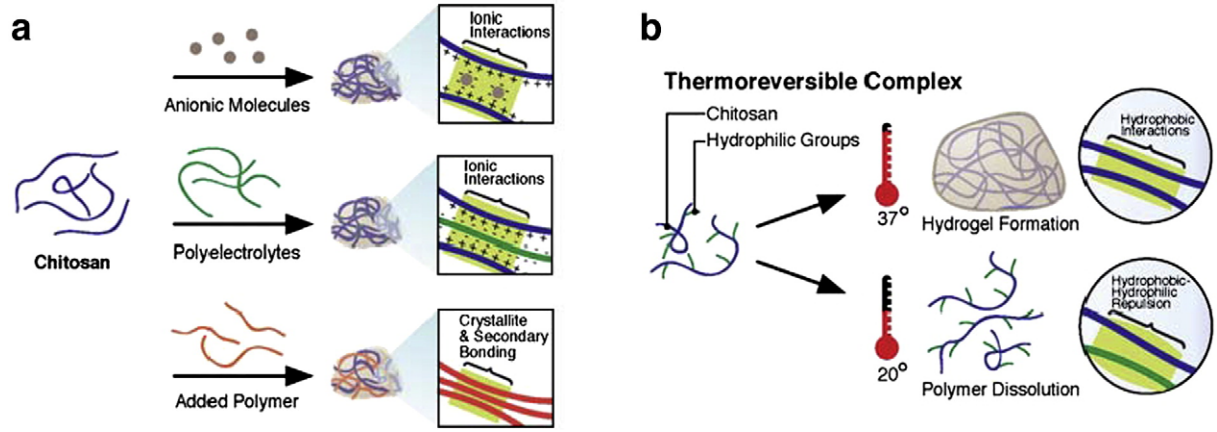


Fig. 2. Schematic representation of chitosan based hydrogel networks derived from different physical associations: (a) networks of chitosan formed with ionic molecules, polyelectrolyte polymer and neutral polymers; (b) thermoreversible networks of chitosan graft copolymer resulting semi solid gel at body temperature and liquid below room temperature.

used during the fabrication process. A chitosan-based physical gel can often be obtained by simply mixing the components that make up the gel under the appropriate conditions. These gels have a short life time in physiological media, ranging from a few days to a month. Therefore, physical gels are good for short-term drug release applications. Because the gelation does not require any toxic covalent linker molecules, it is always safe for clinical applications. However, their widespread application is limited due to the weak mechanical strength and uncontrolled dissolution [59].

3.1.1. Ionic complexes

Thanks to the cationic amino groups of chitosan, ionic interactions can occur between chitosan and negatively charged molecules and anions. Ionic complexation of mixed charge systems can be formed between chitosan and small anionic molecules, such as sulfates, citrates, and phosphates [60,61] or anions of metals like Pt (II), Pd (II), and Mo (VI) [62,63]. These interactions can yield hydrogels with varying material properties that depend upon the charge density and size of the anionic agents, as well as the degree of deacetylation and concentration of the chitosan polymer.

Both anions and small molecules bind chitosan via its protonated amino groups, but metal ions form coordinate–covalent bonds with the polymer instead of electrostatic interactions [62,63]. These bonds are stronger than those found between anionic molecules and the polycation. In addition, the charge density of metal anions is pH independent, whereas the global charge density of small molecules and chitosan is influenced by environmental pH and the materials' respective pKa's [64]. With a pKa ~6.3, chitosan has little or no charge above pH 6, limiting its ability to form ionic complexes, and subsequently, reducing its use under physiological conditions. Similarly, anionic molecules that retain a high charge density must be chosen to ensure strong ionic interactions and have a small enough MW to freely diffuse throughout the polymer matrix and quickly form electrostatic bonds.

Ionic complexation can be accompanied by other secondary interchain interactions including hydrogen bonding between chitosan's hydroxyl groups and the ionic molecules, or interactions between deacetylated chitosan chains after neutralization of their cationic charge [62,65]. These interactions can enhance the physical properties of the hydrogel, and can be modulated to express unique material properties, such as pH sensitivity.

3.1.2. Polyelectrolyte complexes (PECs)

While polyelectrolytes form electrostatic interactions with chitosan, they are different from the ions or ionic molecules used in ionic complexation in that they are larger molecules with a broad MW range, such as polysaccharides, proteins and synthetic polymers (Fig. 2). The associations between the chitosan polymer and polyelectrolytes are stronger than other secondary binding interactions like hydrogen bonding or van der Waals interactions. The advantages of this type of complex are significant. They are complexed without the use of organic precursors, catalysts, or reactive agents, alleviating the concern about safety in the body or cross-reactions with a therapeutic payload. In addition, because PECs consist of only chitosan and the polyelectrolyte, their complexation is straightforward and reversible.

Chitosan-based PEC networks have been produced by water-soluble anionic macromolecules like DNA, anionic polysaccharides (e.g. alginate, GAGs (chondroitin sulfate, hyaluronic acid, or heparin), carboxymethyl cellulose, pectin, dextran sulfate, xanthan, etc.), proteins (e.g. gelatin, albumin, fibroin, keratin, and collagen), and anionic synthetic polymers (e.g. polyacrylic acid). The stability of these compounds is dependent on charge density, solvent, ionic strength, pH, and temperature [66,67].

The choice of the anionic molecule for PEC formation is highly dependent upon its charge under physiological conditions because the pH of the hydrogel environment modulates ionic interactions and, subsequently, PEC hydrogel properties. If the electrostatic interactions of the polymer are strong enough, the physical associations between the polymers at physiological pH can be maintained.

3.1.3. Physical mixtures and secondary bonding

In addition to the specific physical interactions described, hydrogels can be formed by polymer blends between chitosan and other water-soluble nonionic polymers, such as PVA. These polymer mixtures form junction points in the form of crystallites and interpolymer complexation after lyophilization or after a series of freeze–thaw cycles [57,68]. The chain–chain interactions act as cross-linking sites of the hydrogel. In the case of chitosan–PVA polymer blends, increasing the chitosan content negatively affects the formation of PVA crystallites, leading to the formation of hydrogels with less ordered structures.

Recently, a new hydrogel consisting of a polymer blend of chitosan and polyethylenimine (PEI) was prepared [69]. PEI is a polycationic material that has been extensively used as a gene transfection agent [70]. By mixing the polymer with chitosan, a 3D hydrogel was formed within 5 min that was stable under cell culture conditions and could support the growth of primary human fetal skeletal cells. It is posited that the gel structure is held together by chitosan–chitosan interactions. When the polymer mixture is prepared at pH 7.5, chitosan is insoluble, possibly leading to crystallite formation between its chains. Chitosan alone can also be prepared to form a hydrogel without the addition of any other polymer or complexing molecule. This was demonstrated by Ladet et al. using a hydro-alcohol method of gel formation that relied upon the neutralization of chitosan's amino groups using a sodium hydroxide solution [20]. This prevented ionic repulsion between the polymer chains, allowing for the formation of hydrogen bonds, hydrophobic interactions, and chitosan crystallites. Using this technique, hydrogels on the order of cubic centimeters could be prepared. Macroscopic shrinkage of the hydrogel during neutralization and gel depletion with the increase in the concentration of neutralizing agent was observed (Fig. 3). Interestingly, an interrupted gelation method was used that led to the preparation of multilayered, “onion-like” hydrogels (Fig. 3), which could be used to encapsulate drugs for the co-delivery of multiple therapeutics or pulse-like delivery of a given payload [71,72].

3.1.4. Thermoreversible hydrogels and hydrophobic associations

Researchers have engineered a class of hydrogel systems called thermoreversible gels that form transient gel or liquid states depending upon the environmental temperature. These polymers take advantage of hydrophobic interactions or secondary bonding to form junctions between chains that yield a semi-rigid gel from a flowable liquid solution. Specifically, when system temperatures pass a lower critical solution temperature (LCST), the material undergoes a hydrophilic–hydrophobic transition. The importance of a polymer solution that has a low viscosity at room temperature, but forms a gel above a LCST is significant for its use in biomedical applications. These materials can be injected into the body as a liquid, forming a gel in situ where the body temperature is above the LCST, offering the potential to serve as carrier matrices for a wide range of biomedical and pharmaceutical applications [24,73]. These injectable, gelling systems can be introduced into the body without the need for invasive surgeries, and deliver the bioactive agents to a defect site without significant negative effects (local heating, use of organic solvents, formation of toxic byproducts, etc.). In clinical applications, injectable hydrogels are also especially suitable for treating irregularly shaped tissue sites, eliminating the need for custom produced scaffold designs.

Hydrogels prepared by aggregation of chitosan-based co-polymers or by neutralization with polyol salts show promising thermoreversible gelation properties in aqueous media [74–78]. One such engineering strategy used the temperature-sensitive behavior of a physical mixture of glycerol phosphate disodium salt (GP) and chitosan. The phosphates of the GP salt are believed to neutralize the ammonium groups of

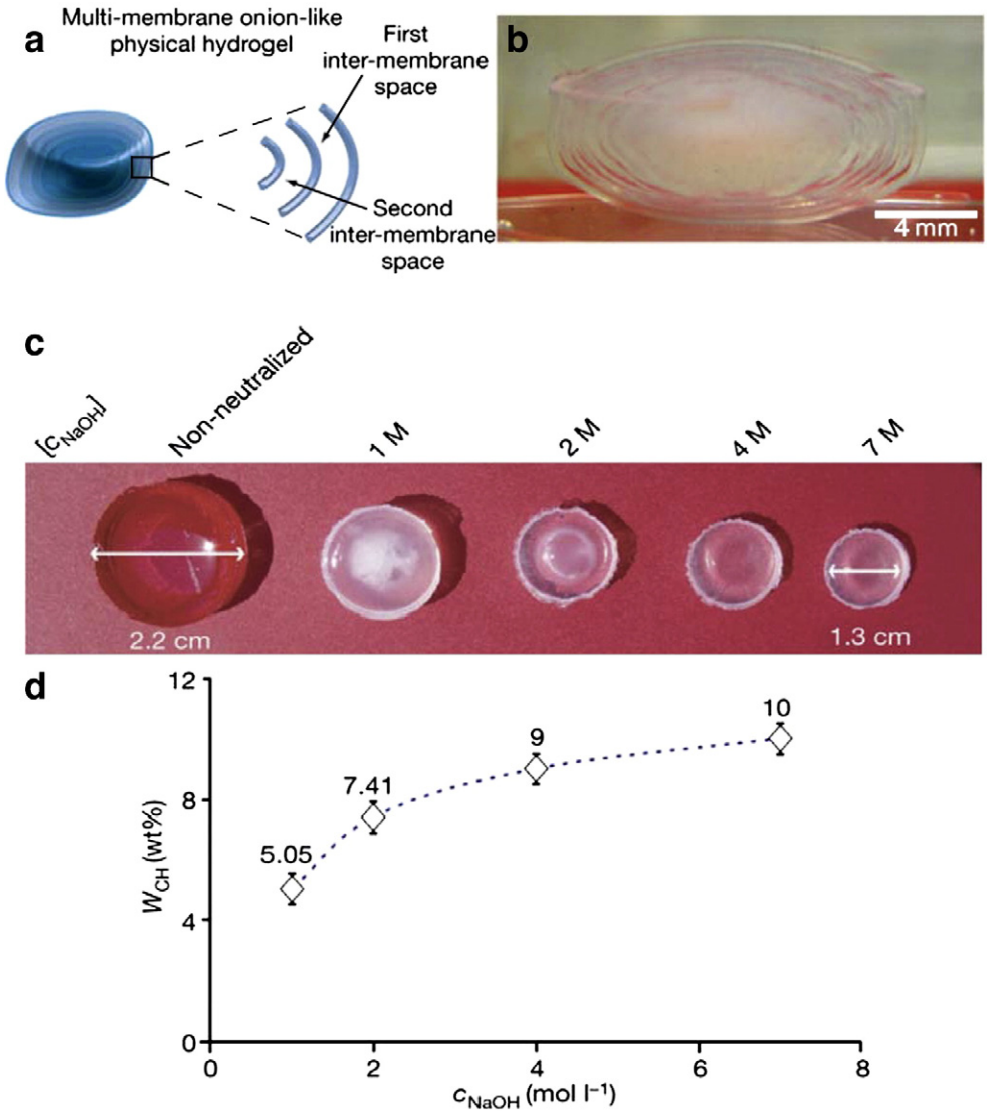


Fig. 3. Top: (a) Schematic diagram of the multi-membrane onion-like structures; (b) multimembrane biomaterial with 'onion-like' structure based on chitosan hydrogel. Bottom: parameters influencing the polymer mass fraction of physical gels based on chitosan. (c) Variation of hydrogel shrinkage during neutralization as a function of the concentration of sodium hydroxide. The initial polymer concentration in the non-neutralized alcohol gel is constant and close to 4.5 wt.% in each case. (d) Evolution of the chitosan mass fraction in the gel (W_{CH}) at different steps of the hydrogel neutralization as a function of the NaOH concentration in the neutralization bath. [20]. Reproduced and adapted with permission from Nature Publishing Group.

chitosan, allowing increased hydrophobic and hydrogen bonding between the chitosan chains at elevated temperatures. The mixture remains a clear liquid at room temperature and gels at 37 °C [78]. The chitosan/GP gel showed promise as a biotherapeutic system capable of delivering a bioactive bone protein (an osteogenic mixture of TGFβ family members), and as a cell matrix whereby chondrocytes were implanted in vivo and showed normal cartilage formation over 3 weeks.

Bhattarai et al. developed an injectable, thermoreversible gel that utilized chitosan chain interactions for gelation. The gel was formed by a chitosan-PEG co-polymer (chitosan-*g*-PEG) that was produced by chemically grafting monohydroxy PEG onto the chitosan backbone using Schiff base and sodium cyanoborohydride chemistry [75]. By optimizing the polymer's PEG content (45–55 wt.%) and PEG MW (i.e. the balance of the ratio of hydrophilic and hydrophobic groups), the resultant co-polymer underwent a thermoreversible transition from an injectable solution at room temperature to a gel at body temperature. Fig. 4 illustrates the temperature-dependent solution-to-gel transition; the abrupt increase in the viscosity of the hydrogel at approximately 25 °C marks the onset of gelation. The solution could be injected through a 22G needle below the transition temperature, and transformed into a transparent gel above the transition temperature (Fig. 4B). It is believed that at low temperatures the hydrogen bonding between the PEG and water molecules dominate, while at high temperatures the hydrophobic interactions between the polymer chains prevail [79,80]. This hydrophilic–hydrophobic transition results in gel formation. This type of thermosensitive gelation has also been observed in other cellulose derivatives grafted with hydrophilic moieties [59].

Recently, researchers have developed other hydrogels using chitosan co-polymers in combination with poly(N-isopropyl acrylamide) (PNiPAM) and poloxamers whose hydrophobic group interactions dominate at elevated temperatures. These polymers have been recognized as good candidates for in situ, reversible hydrogel formation [5].

PNiPAM solutions characteristically precipitate above 32 °C, while at lower temperatures the hydrogen bonding between polymer polar groups and water molecules leads to polymer dissolution. Gel formation at higher temperatures is caused by dehydration of the hydrophobic isopropyl groups during the coil-to-globule transition. PNiPAM has been modified with chitosan, collagen, and other natural polymers to adjust the gelation temperature closer to physiological temperatures, increase its mechanical strength, and improve hydrogel biocompatibility [81,82].

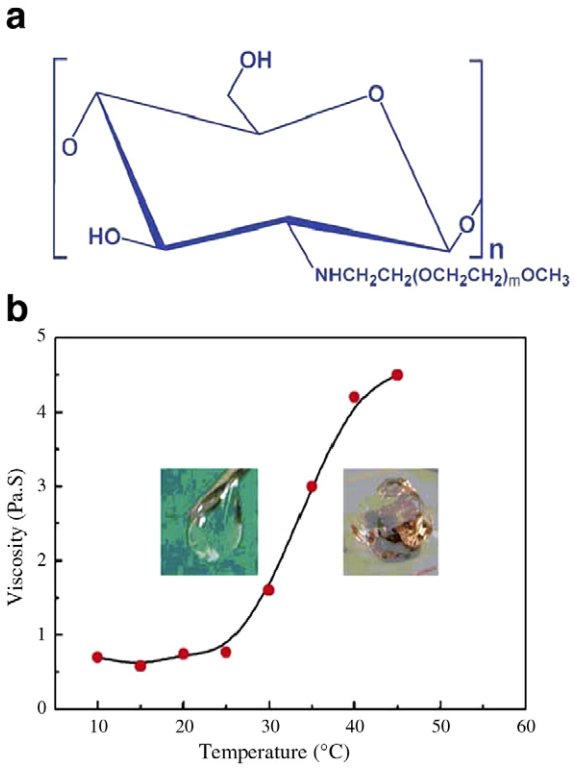


Fig. 4. Thermoreversible hydrogels of PEG grafted chitosan (PEG-*g*-chitosan): (a) Chemical structure of chitosan-PEG. (b) Temperature-dependence of viscosity of PEG-*g*-chitosan solution (PEG wt.% was 55 and polymer concentration was 3 wt.%) [74]. Reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA.

These formulations could be used for the delivery of drugs, peptides, proteins, and cells by mixing these agents with the dissolved polymer and quickly injecting them into the body. The gel formed after injection could provide a controlled release system.

Poloxamers, amphiphilic triblock copolymers including PEO-PPO-PEO, are characterized by a center hydrophobic segment adjoined by two hydrophilic regions. These polymers are known to undergo hydrogel formation when polymer concentrations are maintained above a critical value, and the temperature is above the polymeric LCST [83]. By grafting chitosan to the terminal groups of this polymer, this thermosensitive chitosan–poloxamer hydrogel showed improved stability, biocompatibility, and mechanical properties. The aqueous chitosan–poloxamer solution had a solution-gel transition at ~25 °C and demonstrated its possible use as an injectable delivery vehicle for cartilage regeneration [84].

3.2. Cross-linked networks

While physically bonded hydrogels have the advantage of gel formation without the use of cross-linking entities, they have limitations. It is also difficult to precisely control the physical gel pore size, chemical functionalization, and degradation or dissolution, leading to inconsistent performance in vivo.

Alternatively, robust chitosan hydrogels can be produced using irreversible networks. Polymeric chains of these hydrogels are covalently bonded together either by using small cross-linker molecules, secondary polymerizations, or irradiation chemistry. Fig. 5 shows several common cross-linker molecules that have been used to make covalent hydrogels of chitosan, their possible linking chemistry, and reaction conditions. Most of these linker molecules react with the primary amines of chitosan and form irreversible inter- or intramolecular bridges among the chitosan chains. Covalently cross-linked hydrogels are also obtained by attaching photo-reactive or enzyme-sensitive molecules on the chitosan, followed by their subsequent exposure to UV or sensitive enzymes, respectively. The properties of cross-linked hydrogels depend mainly on their cross-linking density and the ratio of moles of cross-linker molecules to the moles of polymer repeating units [85]. The following sections describe the different ways of making irreversible chitosan hydrogels.

3.2.1. Chemical cross-linking

Chemical cross-linking is a straightforward method to produce permanent hydrogel networks using covalent bonding between polymer chains. Cross-linked chitosan networks can be prepared using the available $-NH_2$ and –OH chemical handles and cross-linkers that can form a number of linkage chemistries, including amine-carboxylic acid bonding and Schiff base formation [45,65,86]. Specifically, these networks can be formed by using small molecule cross-linkers, polymer–polymer reactions between activated functional groups, as well as photosensitive agents or enzyme-catalyzed reactions.

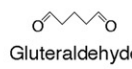
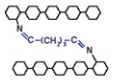
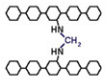
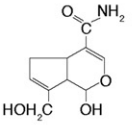
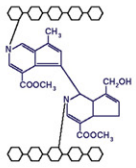
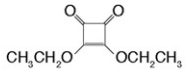
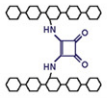
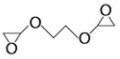
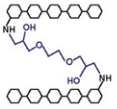
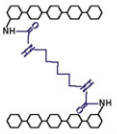
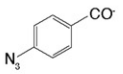
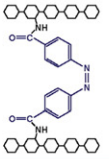
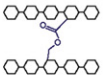
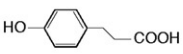
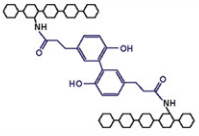
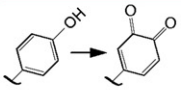
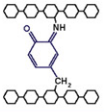
3.2.1.1. Small molecule cross-linkers. Many bifunctional small molecules have been used to cross-link chitosan polymers, including glutaraldehyde, diglycidyl ether, diisocyanate, diacrylate, and others shown in Fig. 5 [45,65,86,87]. The structural properties of these hydrogels with specific reference to their cross-linkers and pharmaceutical applications have been reviewed by Berger et al. [65]. In general, these hydrogels have improved mechanical properties when compared to physical hydrogels. Importantly, PECs and the other polymers described in Section 3.1 can be reinforced with the addition of chemical cross-linkers.

These hydrogels can offer desirable properties, but the biocompatibilities of many cross-linkers are unknown, while others have been found to be relatively toxic. In addition, the fate of many of these molecules in the body has not been established. To prevent trace amounts of unreacted cross-linker agents in vivo, synthesized hydrogels must undergo stringent purifications prior to administration. Moreover, cross-linking agents can react with the hydrogel payload, deactivating or limiting therapeutic release. For the moment, the choice of safe, biocompatible covalent cross-linkers is limited, representing the main drawback of these hydrogels.

The new cross-linking agent, genipin, is a naturally derived chemical from the gardenia that has been shown to be one such biocompatible cross-linking agent [88]. Genipin has been reported to bind biological tissues [89] and biopolymers, such as chitosan and gelatin, leading to covalent coupling. It works as an effective cross-linking agent for polymers containing amino groups and is much less cytotoxic than glutaraldehyde [90]. In addition, genipin cross-linked chitosan membranes exhibit a slower degradation rate than their glutaraldehyde cross-linked counterpart [91]. Use of genipin also showed extended drug release by chitosan hydrogels cross-linked in situ [75,92]. Even though genipin shows good biocompatibility, it is still liable to negatively interact with encapsulated drugs, an unavoidable problem for gelation in the presence of a therapeutic.

3.2.1.2. Polymer–polymer cross-linking. In order to eliminate the use of cross-linker molecules during gelation, researchers have pre-functionalized polymer chains with reactive functional groups. Importantly, this approach can be used to form covalently bonded hydrogels in situ. Several types of covalent linkages can be formed depending on the desired speed of cross-linking and selection of targeted reactive functional groups.

A biodegradable hydrogel comprised of chitosan and hyaluronic acid polymers was produced by in situ polymer–polymer bonding. Schiff bases were formed between the polymers when *N*-succinylated chitosan and aldehyde-terminated hyaluronic acid were mixed together at physiological pH for 1–4 min [93]. The hydrogel was stable for at least four weeks and could be loaded with chondrocytes, highlighting its mild reaction conditions. Schiff bases were also used in the preparation of a hydrogel formed with oxidized dextran polysaccharides used to crosslink chitosan

	Agent	Target Functional Groups	Reaction Conditions	Cross-linkage	Comments
Small Molecule	 Gluteraldehyde	Primary amines & aldehydes	Reaction favors basic & neutral pH		Reaction completes within 1 h; Difficult to remove trace gluteraldehyde
	 Formaldehyde	Primary amines & aldehydes	Basic & neutral pH		Reaction completes within 1 h; Difficult to remove trace formaldehyde
	 Genepin	Primary amines & aldehydes	Independent of pH		Nontoxic linker; Can undergo self polymerization
	 Diethyl Squarate (DES)	Primary amines	pH 4.5-5.5; Solution precipitates at higher pH; Reaction favors elevated temperatures		Reacts under mild conditions & is nontoxic; Long reaction time precludes use for in situ gelation
	 Ethyleneglycol diglycidylether (EGDE)	Primary amines and oxiranes	Basic pH; Reaction favors elevated temperatures		Difficult to remove EDGE traces; Long reaction times & basic pH can yield hydrogel beads
	 Blocked Diisocyanate	Primary amines	Basic pH; Reaction requires elevated temperatures		Long reaction times & basic pH requirements preclude in situ gelation; Can produce hydrogel beads
Photo-Sensitive	 Functional azides	Primary amines	Independent of pH		Multi step crosslinker; Suitable for injectable hydrogel hydrogel preparation
	 Functional acrylates	Other acrylic acids	Independent of pH		Multi step crosslinker; Suitable for injectable hydrogel hydrogel preparation
Enzymatic	 Phloretic acid	Primary amines	Physiological pH		Fast gelation; Suitable for in situ gelation
	 Activated Quinone	Primary amines	pH 5.8-6 at 35°C		Gelation occurs under 2 hr; Suitable for in situ gelation

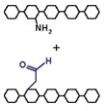
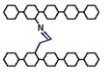
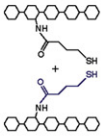
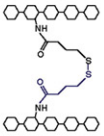
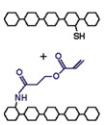
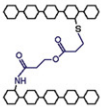
	Reaction	Reaction Conditions	Reactive Polymer Groups	Cross-linkage	Comments
Polymer-Polymer	Schiff Base Formation	Neutral pH			Good candidate for in situ gel formation; Hydrogel formation in around 10 min
	Disulfide Bonding	Neutral pH			Good candidate for in situ gel formation; Hydrogels have enhanced mucoadhesive properties
	Michael Addition	Weak base; Catalysts required			Good candidate for in situ gel formation; Hydrogels have enhanced mucoadhesive properties

Fig. 5. Examples of various linker molecules and their network structures in covalently crosslinked chitosan hydrogels.

chains [94]. Similar approaches have also been adapted in other hydrogel systems like cellulose and alginate [95,96].

Chitosan hydrogels have also used Michael addition reactions to form polymer–polymer linkages. Here, a nucleophile (such as chitosan's primary amino groups) reacts with the vinyl group on another polymer. This approach has been popular for hydrogel preparation due to its rapid reaction time, ability to form different types of bonds, and relatively benign reactivity with biomolecules [97,98]. Chitosan hydrogel engineers have prepared a chitosan-poly (ethylene oxide) (PEO) hydrogel by reacting acrylated chitosan with thiolated PEO [99]. It has been noted that the preparation of polymers using active thiols, including those prepared for Michael addition reactions, benefits from enhanced mucoadhesive properties, which can assist in the oral delivery of therapeutics.

While polymer–polymer systems have many advantages, they require multi-step preparation and purification processes. In addition, a chitosan polymer functionalized with a reactive group might be cytotoxic, even if the parent chitosan polymer is highly biocompatible. Extra care is warranted in the selection of bio-friendly precursors and extensive purification of the modified chitosan after functionalization.

3.2.1.3. Photo-cross-linking. Like polymer–polymer cross-linking, researchers have been able to develop polymer mixtures that can form hydrogels in situ using photo-sensitive functional groups. By adding these reactive moieties to chitosan, the polymer can form cross-linkages upon irradiation with UV light. This technique offers other considerable advantages (ease of formation, speed, safety, low cost, etc.) over the conventional chemical methods, which generally require the participation of different reactive species, initiators, or catalysts.

A photo-cross-linked chitosan hydrogel with in situ properties has been developed by One et al. They were able to prepare a photo-sensitive chitosan hydrogel that could be formed in situ by functionalizing the polymer with azide groups ($-N_3$) [100]. After UV irradiation, the azide is converted into a reactive nitrene group that binds chitosan's free amino groups, causing gelation within 60 s. The chitosan hydrogel showed the ability of controlled release of various growth factors, serving as a novel carrier inducing neovascularization in vivo [101,102].

A thermo-sensitive, chitosan-pluronic hydrogel was also produced by UV photo-cross-linking [103]. The chitosan and pluronic groups were functionalized with photosensitive acrylate groups that were cross-linked by UV exposure. The resultant polymers could then form a physical network at temperatures above the LCST. The hydrogel showed the sustained release of encapsulated human growth hormone (hGH) and plasmid DNA, demonstrating its potential application for different types of drugs [103,104].

Another chitosan-PEG-based hydrogel was prepared using a photoreactive azidobenzoic acid and acryloyl-PEG [105]. The PEG was functionalized with a RGD peptide that could help target the injured myocardium for the delivery of growth factors and cells transported within the hydrogel. The hydrogel showed good formation after UV exposure, as well as active delivery of the payload.

3.2.1.4. Enzymatic cross-linking. Photosensitive polymers represent a promising class of materials for in situ-forming hydrogels, but still suffer from drawbacks. For instance, photo-cross-linking can require a photosensitizer and prolonged irradiation, which could also lead to local temperature increases, subsequently damaging neighboring cells and tissue [106]. A new, mild approach to in-situ hydrogel formation uses enzyme-catalyzed cross-linking reactions. This technique has shown great potential for biomedical applications, such as tissue engineering and drug or protein delivery [107,108]. Horseradish peroxidase (HRP), a single-chain β-type hemoprotein that catalyzes the coupling of phenol or aniline derivatives via the decomposition of hydrogen peroxide, has been used to catalyze cross-linking reactions [109,110]. Fig. 6 shows the formation of an HRP-catalyzed in situ gel of chitosan.

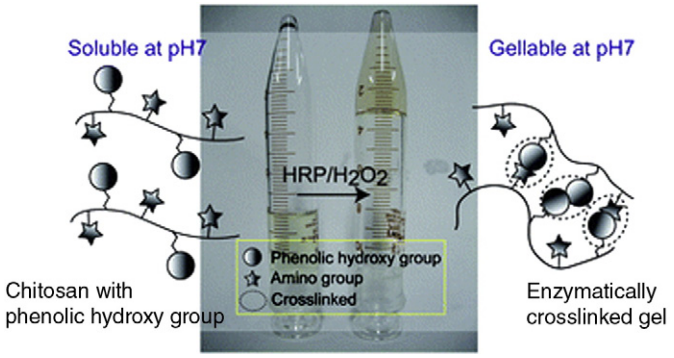


Fig. 6. Photograph of enzymatically crosslinked hydrogel. Chitosan-3-(*p*-hydroxyphenyl) propionic acid conjugate was dissolved in 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer solution (pH 7.0) at 1.0% (w/v) before (left) and after (right) adding Horseradish peroxidase (HRP) and H_2O_2. The network structure shows the gelation scheme of before and after enzyme treatment [110]. Reproduced by permission of The Royal Society of Chemistry.

Recently, Jin et al. developed an injectable chitosan-based hydrogel from water-soluble chitosan derivatives, chitosan-*graft*-glycolic acid (GA), and phloretic acid (PA) (CH-GA/PA) through enzymatic cross-linking with horseradish peroxidase (HRP) and H_2O_2 [111]. Gelation times can be varied from 4 min to 10 s by increasing the polymer concentration from 1 to 3 wt.%. The gel content, water uptake, enzymatic degradation by lysozyme, and mechanical properties could be adjusted by varying the initial polymer concentration. Tyrosinase, an oxidizing enzyme found in animal and plant tissues, has also been used to cross-link chitosan with gelatin to form a hydrogel in situ. Specifically, tyroxinase oxidizes the tyrosyl residues of gelatin, forming quinone residues that react with chitosan's amino groups and form intermolecular cross-linkages [112,113]. Cross-linking between chitosan and gelatin occurs on the order of 30 min, making it suitable for in situ gel applications [113].

3.2.2. Interpenetrating networks (IPNs)

Entangled polymer networks can be further strengthened by interlacing secondary polymers within the cross-linked networks. Here, a cross-linked chitosan network is allowed to swell in an aqueous solution of polymer monomers. These monomers are then polymerized, forming a physically entangled polymer mesh called an interpenetrating network. There are also semi-IPNs where only one of the polymer networks is cross-linked, while the second polymer remains in its linear state. If the second polymer is also cross-linked, a full-IPN is formed. There are several chitosan-based semi-IPNs (prepared with polyether [114,115], silk [116], PEO[117], and PVP [118]) and full-IPNs (prepared with PNIPAM [119]).

This technique allows for the specific selection of polymers that can complement the deficiencies of one another. For instance, a hydrophilic polymer can be chosen to enhance the structural characteristics of the hydrogel, while a biocompatible polymer may limit the immunological response. Although the cross-linking density, hydrogel porosity, and gel stiffness can be adjusted in IPN-based hydrogels according to the target application, they have difficulty encapsulating a wide variety of therapeutic agents, especially sensitive biomolecules. In addition, IPN preparation requires the use of toxic agents to initiate or catalyze the polymerization or to catalyze the cross-linking. Complete removal of these materials from the hydrogel is challenging, making the clinical application problematic.

3.3. Drug loading and release triggers

3.3.1. Drug loading and release

The performance of a hydrogel as a DDS depends upon both the physical and chemical properties of the gel as well as the therapeutic itself. In fact, the choice of hydrogel materials, network conformation,

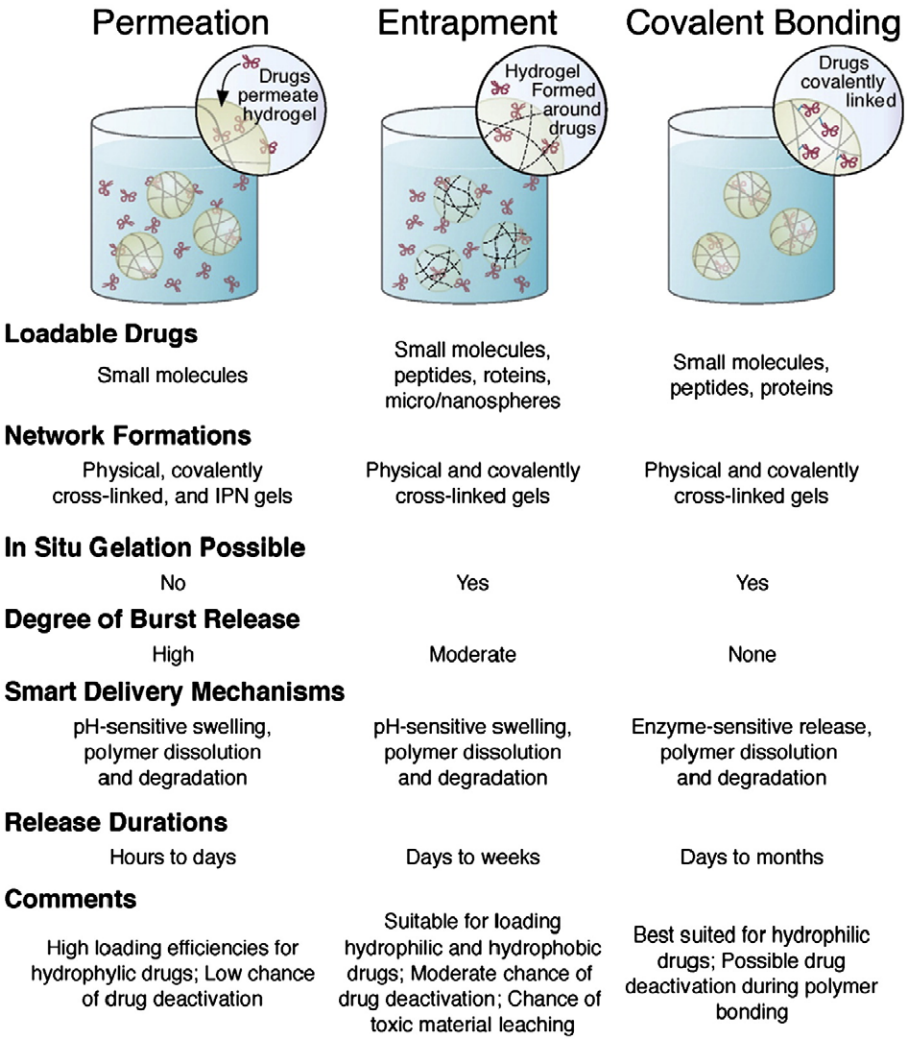


Fig. 7. Three different drug loading strategies for chitosan hydrogels.

and drug loading mechanism must be made to complement the properties of the drug (e.g. hydrophobilicity, charge) and its mechanism of action (sustained drug release versus rapid, high exposure). In Fig. 7, three major approaches to drug loading are illustrated: diffusion, entrapment, and tethering [23,39,42,47,120]. Each method bears specific advantages and disadvantages and should be selected after taking into consideration the hydrogel network used as well as the nature of the drug.

The easiest drug loading method is to place the fully formed hydrogel into medium saturated with the therapeutic [35,121]. Depending upon the porosity of the hydrogel, the size of the drug, and the chemical properties of each, the drug will slowly diffuse into the gel. When placed in vivo, the drug will then freely diffuse back out of the hydrogel into the neighboring tissue. This approach is effective for loading small molecules, but larger therapeutics — peptides and proteins in particular — are not readily able to migrate through the small pores of the hydrogel. In addition, this drug loading process can take time long amounts of time.

In the case of larger drugs and bioligands, the payload must be entrapped during the gelation process. Here, the drug is mixed with the polymer solution, and the cross-linking or complexation agent is added. It is important to consider the chemistry of the drug molecule to prevent unwanted cross-linking or deactivation of the therapeutic during gelation. A number of examples of encapsulated drug strategies are covered in Section 2.2 and also in recent reviews [34,45]. Both diffusion and entrapment allow for free movement of the therapeutic out of the hydrogel network. This can lead to an initial burst release after implantation of the hydrogel in vivo due to the concentration gradient formed between the gel and the surrounding environment. In order to limit the loss of the therapeutic reserve (and the risk of toxic exposure), drugs can be covalently or physically linked to the polymer chains prior to gelation. This tethering method limits tissue exposure to the agent to only when the hydrogel breaks down or the molecular tether is broken [45]. Linkages between the drug and polymer that are susceptible to environmental enzymes have been used to control the speed and timing of release.

Drug loading is also complicated by molecules that have the opposite hydrophilicity or the same charge as the constituent polymer. For instance, hydrophobic molecules like paclitaxel must be complexed with amphiphilic additives before the hydrogel and payload will blend in solution [122,123]. This has been accomplished by binding the drug to albumin (Abraxane) or by mixing it in an aqueous citric acid/glyceryl monooleate solution prior to hydrogel loading [124]. Therapeutics have also been loaded into small secondary release vehicles (e.g. microparticles, microgels, liposomes, and micelles) prior to hydrogel encapsulation [125,126]. In addition to modifying the drug prior to encapsulation, the hydrogel polymer can be functionalized with small binding domains. This was demonstrated in a chitosan hydrogel that was loaded with the hydrophobic drug, denbufylline, by attaching small hydrophobic moieties to the polymer prior to loading and gelation [127,128].

Release of loaded therapeutics from a hydrogel can occur by one of three different modes: diffusion, chemical/environmental stimulation, and enzyme-specific stimulation [129]. As discussed, diffusion is regulated by movement through the polymer matrix or by bulk erosion of the hydrogel as it breaks down in vivo. Environmentally responsive hydrogels — gels that swell in response to external cues like pH and temperature — effectively open their pores for enhanced diffusion of the entrapped therapeutic under predetermined conditions. This type of controlled release can be used to limit drug release outside of the effective range of the diseased tissue. Environmental cues are specific to limited regions within the body, but better specificity is being investigated with new release mechanisms that release a drug payload only when triggered by local enzymatic cues. These biochemically stimulated responses occur by tethering drugs to the hydrogel via labile domains that are susceptible to matrix-remodeling enzymes or using polymers that are targeted by enzymes [130]. This method has received the least amount of attention, but offers selective, sustained release mechanisms that are beginning to receive attention from chitosan hydrogel engineers.

3.3.2. Drug release triggers

Local drug release by diffusion provides a basic mechanism for non-specific drug release, but alternatively, chemically and biologically stimulated release triggers offer finer tuned control for selective treatment. This is important when hydrogel particles are administered orally or intravenously, and interact with significant amounts of healthy tissue before encountering the diseased target site. Release triggers can also moderate the speed of drug release to maintain effective drug levels at the local site of action without raising exposure to toxic levels.

Environmental or enzymatic triggers can induce three types of hydrogel conformational changes, swelling, dissolution or degradation, which facilitate drug payload release. Swelling of the hydrogel opens the "pores" of the polymer network, which allows for faster diffusion of entrapped materials out of the hydrogel. Dissolution and degradation, on the other hand, are the physical breakup of the hydrogel. When the cross-linkages or binding molecules between the polymer chains break, the hydrogel is said to dissolve, subsequently allowing release of the drug. Degradation is the destruction of the polymer chains themselves (e.g. by enzymes), causing therapeutic release. Each of these mechanisms can be executed with a number of different hydrogel preparation strategies. A number of them that have been applied to chitosan-based materials will be described here.

3.3.2.1. pH responsive release. Oral drug delivery represents approximately 90% of all therapeutics used, thanks to the fact that it is noninvasive and easy to administer. Unfortunately, due to adverse environmental factors that include extreme acidic pH and high enzymatic activity, there can be significant variability in the effectiveness of the treatment. DDSs offer a way to limit both the exposure to harsh environmental conditions and the possibility of selective medicine release within the gastrointestinal (GI) tract.

The most attractive route of controlled release is by pH triggering. The pH gradient in the human GI tract ranges from 1 to 7.5 (saliva, 5–6; stomach, 1–3; small intestine 6.6–7.5; and colon 6.4–7.0) [131]. Hydrogels can react to changes in ionic strength, which causes a volume phase transition (e.g. swelling leading to drug release). Here, the chemical properties of the constituent polymer are critical. Neutral or anionic polymers do not experience significant pH-sensitive behavior under acidic conditions, while the cationic chitosan (one of the few positively-charged natural polymers) is responsive at low pH. Specifically, chitosan exhibits the pH-sensitive behavior of a weak polybase, dissolving easily at low pH, but remaining insoluble at higher pHs. To take advantage of this phenomenon, PEC hydrogels that dissolve in the stomach have been prepared. Swelling of the PEC complexes is due to a change in the charge balance inside of the hydrogel. If the charge density of either the polymer or polyelectrolyte is no longer sufficiently high enough to ensure complexation, then the polyelectrolyte "glue" can no longer hold the polymer chains together, leading to swelling or dissolution [132,133]. By selecting polymers or polyelectrolytes based on their isoelectric points (pI), PEC swelling and dissolution can be tailored for drug release at varying pHs. In addition, cross-linking the PEC structure can make the polymer network stable in certain pH environments, but unstable under more extreme conditions. For instance, hydrogel swelling is limited in the stomach, but triggered in the intestine due to a further change in pH. In one example, ionically cross-linked succinyl chitosan(Suc–Chi)/alginate hydrogel beads showed the release of as little as 11.6% of its nifedipine payload at pH 1.5 (3 h — the typical residence time in the stomach), and as much as 76% after 15 h at pH 7.4 [134]. This particular polyelectrolyte complex was made sensitive to the higher pH due to the large number of carboxylated groups in the networks. This result clearly suggested that the Suc–Chi/alginate hydrogel bead may be a potential polymeric carrier for drug delivery in the intestinal area. A number of different chitosan-based PEC systems have been reviewed by Berger et al. [57].

Swelling in IPN hydrogels is moderated by the amount of hydrophobic/hydrophilic groups and the cross-linking density [135,136]. In most cases, the contribution of the primary amines from chitosan is minimal. As a result, hydrogel networks were obtained with lesser swelling at an acidic pH and higher swelling at a pH close to neutral. pH sensitive chitosan-acrylamide-grafted hydroxyethylcellulose (AAm-g-HEC)-based semi-IPN hydrogel microspheres have been prepared for pH sensitive drug release, i.e. slower release at pH 1.2 and faster at pH 7.4, illustrating their potential drug release application in the GI tract [136]. Drug release from such an IPN is further affected by temperature if the hydrogel expresses thermoresponsive behavior (e.g. PNiPAM-chitosan) [135].

In addition to PEC and IPN networks, polyblend-based hydrogels also show pH sensitivity. If the blend component is neutral and hydrophilic, such as PEO and poly(vinyl pyrrolidone) (PVP), under acidic conditions, the primary amines of the chitosan are protonated causing chain–chain repulsion and subsequent swelling or dissolution [118,137]. Freeze-dried chitosan-PEO hydrogels were shown to selectively release antibiotics at low pH, indicating their possible use in localized therapeutic release in the stomach. Amoxicillin and metronidazole demonstrated hydrogel swelling in enzyme-free simulated gastric fluid (pH 1.2), allowing the release of most of the amoxicillin (65%) and metronidazole (59%), but showed limited release in simulated intestinal fluid (pH 7.2) after 2 h [137].

3.3.2.2. Enzyme responsive release. Better control of drug release has been demonstrated by targeting enzymes that are localized to different areas of the body. For example, hydrogels prepared with chitosan (and several other polysaccharides) have been used for colon-specific drug delivery because of the active enzymes present there. These polymers are excellent targets for degradation within the gastrointestinal tract because of the large variety of bacteria in the intestine that secrete enzymes used in polysaccharide processing (e.g. glucosidase, galactosidase, amylase, pectinase, xylanase, xylosidase, dextranase, etc) [138,139]. As such, chitosan polymers are readily cleaved when exposed to these local enzymes, leading to the release of the entrapped drug molecules. These chitosan-based hydrogel capsules have demonstrated success in locally releasing insulin [140]. The chitosan capsules containing insulin (5(6)-carboxyfluorescein (CF)), with or without protease inhibitors or absorption enhancers, were used. The surfaces of these capsules were coated with hydroxypropyl methylcellulose phthalate. Colon-specific delivery of insulin after oral administration was found to be stable in the stomach and small intestine of Male Wistar rats. However, they were specifically degraded by microorganisms in the cecal contents of rats when they reached the colon, and showed insulin absorption.

In addition to the direct degradation of the polymer network, enzymes can cause local pH fluctuations in the hydrogel microenvironment, which may affect drug release. For instance, insulin-loaded hydrogels prepared

with immobilized glucose oxidase produced a pH drop after the enzymes converted glucose to gluconic acid. Acidic environments are known to increase insulin solubility, so the lower pH may lead to higher permeation of the drug out of the porous hydrogel.

3.3.2.3. Electro-sensitive release. The electro-sensitive release method is an external trigger for drug release [141]. This triggering method was originally developed for hydrogels other than chitosan. A typical scenario would involve the subcutaneous implantation of a biodegradable hydrogel-drug formulation containing electro-sensitive moieties (e.g. polyelectrolyte hydrogel). When drug release is desired, an electro-conduction patch is applied on the skin directly over the gel. The electric field stimulates the drug carrier situated under the skin and in response the drug carrier releases the drug. The electro-conducting patch is removed when the required amount of drug has been released. The main mechanisms of drug release from the electro-responsive hydrogels include ejection of the drug solution during deswelling, diffusion, electrophoresis of charged drugs, and electro-induced gel erosion [141]. Similarly, a typical magnetic field-sensitive hydrogel can be prepared by incorporating the magnetic particles into the cross-linked gelatin hydrogel.

Recently, a chitosan-based nanocomposite hydrogel composed of chitosan and montmorillonite (MMT), a nanohydrogel exhibiting an exfoliated nanostructure, has been prepared and its drug release by electrostimulation has been studied [142]. At a certain MMT concentration in the gel, the release kinetics of vitamin B12 from the gel showed a pseudo-zero-order release, and the release mechanism changed from a diffusion-controlled mode to a swelling-controlled mode under electrostimulation. An increase in MMT content reduced both the diffusion exponent n and the responsiveness of the nanohydrogel to electrostimulation. In addition, a consecutively repeated "on" and "off" operation showed that the electroresponsiveness of the nanohydrogel containing higher MMT concentrations was reduced, but its anti-fatigue behavior was considerably improved. In this same work, the 2 wt.% MMT nanohydrogel achieved a mechanically reliable and practically desirable pulsatile release profile and excellent anti-fatigue behavior when compared with that of the pure chitosan.

There are several examples of chitosan-based IPN hydrogel systems that exhibit either swelling/shrinking or bending behaviors in response to an electric stimuli [143–145]. These properties of chitosan hydrogels can be applied to develop controlled drug release systems similar to other hydrogel systems [146,147]. Although electro-sensitive chitosan hydrogels can be used to controlled drug release by modulating an electric field, achieving the desired release rate under physiological conditions remains challenging.

4. Applications

By selectively implementing the release mechanisms described in the previous sections, chitosan-based hydrogels have yielded excellent controlled release devices. In addition, the biochemical properties of chitosan make it an excellent bioadhesive material, which excels at drug administration through subcutaneous, oral, ocular, and transdermal delivery [53,54].

4.1. Subcutaneous delivery

The ability of chitosan-based hydrogel DDSs to selectively gel and release a therapeutic payload within the body has made chitosan a popular material within the field of subcutaneous delivery and implantable therapeutics. Chitosan is also a preferred material due to its lack of immunogenicity and inflammation, which have resulted from many other subcutaneously implanted materials. Most of the research on these chitosan hydrogels has focused on the use of biodegradable systems that require no follow-up surgical intervention [148]. These hydrogels have used a variety of binding chemistries and physical interactions to load therapeutic materials.

4.1.1. Growth factor delivery

One of the major areas of implantable delivery device usage has been in the development of cartilage, bone, and nerve tissues via supplementation with growth factors or glycosaminogylcan (GAG) molecules. Chitosan hydrogels coupled with BMP-7 have shown the ability to enhance lesion repair [149]. For instance, to enhance cartilage formation, chondroitin sulfate, a GAG molecule found in cartilage, has been immobilized in chitosan hydrogels [150]. Platelet-derived growth factor has also been loaded into chitosan gels to enhance osteoinduction by release of the growth factor as the hydrogel degraded at the defect site [151,152]. Chitosan–alginate hydrogels loaded with BMP-2 and mesenchymal stem cells (MSCs) were shown to induce subcutaneous bone formation [153]. Chitosan–laminin nerve guides loaded with glial cell line-derived nerve growth factor (GDNF) enhanced both the functional and sensory nerve recovery by releasing GDNF in the early stage of implantation [154].

Treatment with some growth factors that have short therapeutic half-lives, such as endothelial growth factor, require frequent administration to maintain an effective concentration. Chitosan–albumin hydrogel microspheres have shown continuous release for over 3 weeks after subcutaneous implantation in rats, indicating possible success in vivo [155].

4.1.2. Cancer therapy

The principal modes of cancer management are surgery, radiotherapy, and chemotherapy. Hydrogel DDSs can be used in the latter two treatment approaches. The implantation of radiotherapeutics adjacent to the target tissue is called brachytherapy, or sealed source radiotherapy. This technique provides high doses of radiotherapy to the target site, but can be complicated by the invasive placement and removal procedures of the brachytherapy devices. Chitosan hydrogels have provided matrices within which radioisotopes have been loaded for controlled exposure, but can also gel in vivo, thus limiting their invasive nature. Azab et al. developed a chitosan-based hydrogel cross-linked with glutaraldehyde [156,157] and loaded with ^{131}I-norcholesterol (^{131}I-NC), and tested the hydrogel in a breast cancer xenograft mouse model. This hydrogel showed a reduction in the progression rate of the tumor, and prevented 69% of tumor recurrence and metastatic spread. Importantly, there was little or no systemic distribution of the radioisotope after hydrogel implantation.

Local chemotherapeutic delivery has been a popular area of interest for chitosan hydrogel-based delivery. DDSs are particularly important in cancer management because many of the effective anti-cancer drugs are highly toxic, but poorly specific to cancer cells. Localized delivery of chemotherapeutics has emerged as a method of reducing systemic toxicity with the advent of novel biodegradable polymers [158]. Typically, hydrolytically degradable matrices have been applied for localized cancer treatment. There are several prominent examples including Gliadel, which is reaching the clinic for the treatment of brain tumors [159]. Most local biodegradable devices release drug in the local region of the tumor at a rate solely determined by the polymer system chosen.

Traditional DDSs, prepared as microspheres, microcapsules, or gel systems, are typically loaded by passive absorption, which limits the material loading capacity. In addition, most of these materials require surgical placement near the tumor site, which has focused much of the current research on the investigation of in situ gel formulations. Chitosan-based in situ gel systems excel in this area, and have been prepared for controlled chemotherapeutic delivery [122,123,160–162].

Photoresponsive and thermoreversible systems have been used for in situ chitosan gel formation. For instance, a chitosan solution mixed with paclitaxel was shown to form an insoluble hydrogel after in situ UV-irradiation [123]. This hydrogel inhibited the growth of subcutaneously

grown Lewis lung cancer (3LL) cells and limited angiogenesis. A thermoreversible chitosan–GP formulation, also loaded with paclitaxel, was found to be an effective treatment for localized solid tumors [122]. Here the hydrogel was injected intratumorally into EMT-6 tumors and showed a similar effectiveness as Taxol injections, but with limited toxicity.

Recently, a novel in situ gelling chitosan/dipotassium orthophosphate hydrogel system was designed for the delivery of doxorubicin [162]. The incorporation of doxorubicin in the hydrogel not only significantly inhibited the growth of primary and secondary osteosarcoma, osteolysis, and lung metastasis, but also reduced the side effects of doxorubicin in mice when compared to its conventional administration.

4.2. Oral drug delivery

The oral administration of therapeutics leads to internalization by the body at the mouth (oral cavity), stomach, small intestine, or colon. Using DDSs, clinicians can specifically target these different tissue systems for local drug action within the gastrointestinal (GI) tract, or achieve drug delivery to the vasculature through the expansive capillary beds of the small intestine.

Chitosan and chitosan-based hydrogels have two advantageous characteristics that enhance DDSs: pH sensitivity and mucoadhesive properties. The fluctuation in pH through the GI tract is significant, ranging from 1 to 7.5 [131]. This offers significant drug release targeting based on pH shifts by regulating the hydrogel swelling response. Mucoadhesion, the ability of a material to bind to the mucus lining of the GI tract, is regulated by the affinity of the DDS for the mucin glycoproteins of the mucus. Polysaccharides are very good mucoadhesives due to their non-toxic nature, and can be made to bind to mucins through either electrostatic or hydrophobic interactions. The amine and hydroxyl groups of chitosan have been implicated in the polysaccharide's excellent mucoadhesive properties, leading to prolonged residence time in the GI tract.

Several reactions with the thiolated chitosan have been designed [53,163]. This approach has great potential for the design of mucoadhesive hydrogels as future drug delivery vehicles by forming disulfide bonds between the thiomers and mucus glycoproteins (mucins) [164]. Mucoadhesive properties make the gels useful for vaginal, nasal, ocular, and oral delivery. The great advantage of the use of thiolated polysaccharides in gel formulations has been seen not only in the mucoadhesive, but also in the in situ gelling properties [164,165].

4.2.1. Drug delivery in the oral cavity

The local delivery of therapeutics to the mouth can be used to treat a number of diseases, such as periodontal disease, stomatitis, fungal and viral infections, and oral cavity cancers. In addition, drug administration through the buccal mucosa in the mouth provides some unique advantages, including avoidance of the hepatic first-pass metabolism and the acidity and proteolytic activity of the rest of the GI tract. Unless an excipient is used, intraorally administered therapeutic uptake is typically low.

Due to their mucoadhesive properties, chitosan-based hydrogels have been recognized as excellent candidates for oral delivery DDSs. Indeed, these materials have enhanced drug penetration within the mouth, improving therapeutic efficacy by maintaining high levels of antimicrobial agents in the crevicular fluid with minimal systemic uptake [166]. Chitosan hydrogels have excellent paracellular permeability of the mucosal epithelia, which has led to effective peptide drug transport of the transforming growth factor-β (TGF-β) across a porcine oral mucosa system tested in vitro.

In another delivery system, chitosan integrated into bilayered films and tablets with the oral drugs nifedipine and propranolol hydrochloride showed effective buccal membrane adhesion. These complexes were used with and without PEC forming polymers, such as polycarbophil, sodium alginate, and gellan gum [167]. In addition, bioadhesive tablets of nicotine containing 0–50% w/w glycol chitosan produced good bioadhesion [168].

Chitosan hydrogels have been developed for the local release of a number of other drugs in the oral cavity. In addition to the released drugs, the chitosan polymer itself has shown antifungal activity. For instance, chitosan hydrogels and films were able to limit adhesion of the common pathogen *Candida albicans* to human buccal cells. These DDSs were also able to sustain drug release (chlorhexidine gluconate) from a hydrogel as well as film formulations [169]. Chitosan hydrogels were also able to deliver ipriflavone, a lipophilic drug that promotes bone density, into the periodontal pockets. For this purpose, mono and multi layer composite systems consisting of chitosan and PLGA were designed and were shown to prolong drug release for 20 days in vitro [170].

4.2.2. Drug delivery in the GI tract

Successful, localized administration of therapeutic drugs within the GI tract faces several formidable barriers. A highly acidic environment, destructive enzymes, and low residence times can all limit therapeutic efficacy. Regardless, targeted drug delivery to the stomach and colon is extremely important for the treatment of local maladies, such as Crohn's disease, inflammation, ulcerative colitis, infection, and carcinomas [171]. Selective drug release reduces the necessary dosage as well as the side effects that these drugs can produce through exposure to non-targeted tissue. Thankfully, the wide-ranging pH microenvironments of the GI tract allow selective delivery by DDSs that exhibit pH-sensitive swelling. In this context, chitosan hydrogels can be prepared with pH-sensitive or enzyme-specific release triggers, making their use as oral DDSs ideal.

Delivery of therapeutics to the stomach has been demonstrated by using chitosan networks that rapidly swell in acidic environments. This is important due to the fast gastric emptying time and subsequent short residency time of therapeutics in the stomach. As described previously, Patel et al. [137] demonstrated this selective release mechanism in vivo using a chitosan-PEO semi-IPN hydrogel. This work showed the localized delivery of the amoxicillin and metronidazole antibiotics to the stomach.

In addition, chitosan-based bioadhesive PEC hydrogels have been prepared that can bypass the acidic environment of the stomach and release the loaded drug into the intestine. For example, PEC networks loaded with 5-fluorouracil (effective against colon carcinomas) and insulin (effective against diabetes mellitus) showed selective release in the intestine [172]. In another example, a chitosan-alginate hydrogel microcapsule containing nitrofurantoin (NF) showed selective drug release in an intestinal medium compared to a gastric medium, due to the pH-dependent swelling properties of the PEC hydrogel [173].

In addition to the physical protection provided by the chitosan hydrogel, peptide payloads have also been protected from degradation by intestinal peptidases by the attachment of enzyme inhibitors to the chitosan polymer. Serine proteases and metallopeptidases have been inhibited by the covalent attachment of competitive inhibitors, like the Bowman–Birk inhibitor, and chelating moieties, such as EDTA [174].

Proteolytic activity in the colon is lower than in the small intestinal region. Therefore, the large intestine has been considered to be a safe absorption site for orally delivered peptides and proteins. Chitosan-based hydrogel DDSs loaded with acetaminophen, mesalazine (5-ASA), sodium diclofenac, and insulin showed satisfactory uptake within the colon [140,175–177]. The chitosan polymer itself was found to be degraded by the microflora of the colon, offering a degradation mechanism that leads to controlled drug release.

4.3. Ophthalmic delivery

The development of novel liquid-based delivery formulations has led to a growing interest in ocular drug delivery [178]. Conventional

systems (e.g. eye drops) tend to be eliminated rapidly from the eye, and the drugs administered exhibit limited absorption, leading to poor ophthalmic bioavailability. This has initiated the development of new materials that prolong drug retention and enhance drug penetration using bioadhesive polymers and penetration enhancers [179]. Chitosan-based hydrogels hold great promise thanks to their adhesive and penetration-enhancing properties [180,181].

Chitosan hydrogels have shown higher corneal residence times when compared with commercial drug solutions [182]. Specifically, the entire radioactive payload of the commercial drug was flushed into the lachrymal duct after 10 min. The chitosan DDS, however, showed $25 \pm 5\%$ payload residency in the cornea.

Although micro and nanoparticle delivery systems have been investigated for ocular delivery, in-situ-forming hydrogels are also an attractive delivery approach because of their ability to be administered as a liquid and their long-term retention after dosing. Cao et al. developed a chitosan-based thermosensitive in situ hydrogel for ocular drug delivery and tested it in rabbits [183]. Using the microdialysis method of analysis, the $C(\max)$ of timolol maleate released from the hydrogel was 11.2 μg/ml, two-fold higher than that of the conventional eye drop. Furthermore, the hydrogel had a greater capacity to reduce the intra-ocular pressure (IOP) than the conventional eye drop of same concentration over a period of 12 h.

4.4. Transdermal delivery

Unlike the harsh environment of the GI tract, low-molecular weight drugs can be administered by local transdermal DDSs, which benefit from sustained drug release and easy therapy interruption by removal of the DDS. Hydrogels offer attractive DDS structures because of their high water content, providing a comfortable feeling on the patient's skin, leading to better compliance over the duration of the therapy [184,185]. Glimepride, a third generation oral antidiabetic sulfonylurea drug that has encountered bioavailability problems due to poor solubility during oral administration, has shown potential for effective delivery by chitosan hydrogel release. In vivo application in mice showed consistent therapeutic efficacy over 48 h, suggesting possible effectiveness in the clinic [186]. Chitosan hydrogels were also able to deliver the berberine alkaloid (with the aid of a penetration enhancer) [187] and the active S-enantiomer of racemic propranolol [188]. The latter study used a composite membrane formed with a chitosan hydrogel reservoir containing a poloxamer for further control of the drug's release.

4.5. Wound healing

In the area of wound healing, an ideal dressing should protect the wound from bacterial infection, provide a moist and healing environment, and be biocompatible [189]. Chitosan-based materials, produced in varying formulations, have been used in a number of wound healing applications. Chitosan itself can induce faster wound healing and produce smoother scarring, possibly due to enhanced vascularization and the supply of chitooligomers at the lesion site, which have been implicated in better collagen fibril incorporation into the extracellular matrix [190,191].

While different material dressings have been used to enhance endothelial cell proliferation, the delivery of growth factors involved in the wound-healing process can improve that process [192]. Importantly, chitosan hydrogels that take advantage of the reparative nature of the polymer have been developed, and additionally deliver a therapeutic payload to the local wound. For instance, fibroblast growth factor-2 (FGF-2) stimulates angiogenesis by activating capillary endothelial cells and fibroblasts [193,194]. In order to sustain its residence at the wound site, the factor was incorporated into a high molecular weight chitosan hydrogel, formed by UV-initiated cross-linking. The growth factor remained bound tightly within the hydrogel until exposed to chitinase, after which it showed bioactivity, indicating that there was no loss of functionality during the material preparation [195].

While acute wound healing can be enhanced by chitosan alone [196,197] due to its attractive properties for neutrophils, which can incite an aggressive inflammation [198], chronic wounds must heal differently. Here, the slow release of growth factors can offer more effective treatment. Recently, Park et al. developed a chitosan hydrogel scaffold impregnated with bFGF-loaded microspheres that can accelerate wound closure in the treatment of chronic ulcers [193].

5. Conclusions

With the development of unique cross-linking mechanisms and new molecular agents that can induce physical gelation, there has been significant growth in the variety of hydrogels that can be produced. These gels can have tailored porosities, mechanical strengths, and dimensions that can be engineered to complement their area of application. Importantly, in this review we have seen how the unique cationic properties of chitosan offer biomaterial engineers even greater latitude in the types of hydrogels that can be formed and the mechanisms by which they fragment and degrade in the body.

In particular, these engineering approaches can be used in the context of localized drug delivery to selectively capture a therapeutic payload and control its release in local proximity to its target. Recently, environmental stimulation has become an intense area of research for the development of unique materials that can (1) form safely within the body negating the need for surgery, (2) trigger drug release at local sites preventing systemic toxicity, and (3) degrade in a controlled manner for effective, long-term drug release. The flexibility of chitosan as a major material component in such “smart” delivery systems is compounded by its biocompatibility and biodegradability in vivo. Indeed, chitosan has received significant attention in the development of injectable, in situ gelling systems for tumor treatment and tissue regeneration purposes and as a delivery vehicle in oral and ophthalmic delivery systems.

We anticipate that these current advancements will yield next generation delivery systems as we gain a further understanding of the dynamics of mixed chitosan chain networks. With an understanding of the fundamental loading and release criteria of varying therapeutics, we will be able to adapt delivery systems for different drug formulations, release conditions, and treatment intervals. Once these design parameters have been established, cheap, non-toxic, and efficient chitosan hydrogel drug delivery systems can move closer to clinical availability.

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