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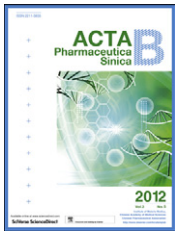

REVIEW

Modified chitosan hydrogels as drug delivery and tissue engineering systems: present status and applications

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Abstract Chitosan, a natural cationic polysaccharide, is prepared industrially by the hydrolysis of the aminoacetyl groups of chitin, a naturally available marine polymer. Chitosan is a non-toxic, biocompatible and biodegradable polymer and has attracted considerable interest in a wide range of biomedical and pharmaceutical applications including drug delivery, cosmetics, and tissue engineering. The primary hydroxyl and amine groups located on the backbone of chitosan are responsible for the reactivity of the polymer and also act as sites for chemical modification. However, chitosan has certain limitations for use in controlled drug delivery and tissue engineering. These limitations can be overcome by chemical modification. Thus, modified chitosan hydrogels have gained importance in current research on drug delivery and tissue engineering systems. This paper reviews the general properties of chitosan, various methods of modification, and applications of modified chitosan hydrogels.

Abbreviations: TPP, tripolyphosphate; GA, glutaraldehyde; PVA-g-AAm, acrylamide-grafted-poly (vinyl alcohol); CBCS, *N*-(2-carboxybenzyl) chitosan; MBA, *N*,*N*[1]-methylenebisacrylamide; CMCs, carboxymethyl chitosan; Co A, co-enzyme A; PMVC, poly (methacrylic acid-vinyl pyrrolidone)–chitosan; NVP, *N*-vinyl pyrrolidone; APS, ammonium persulfate; AAs, sodium acrylate; CMCs-g-AAs, carboxymethyl chitosan grafted with acrylate; Cs-g-PEG, chitosan grafted with poly (ethylene glycol); PEC, polyelectrolyte complex; DS, diclofenac sodium; BSA, bovine serum albumin; GP, glycerophosphate; PVP, polyvinyl pyrrolidone; HPMC, hydroxypropyl methylcellulose; TE, tissue engineering; MMH, multi-membrane hydrogel; SCS, *N*-succinyl chitosan; GOD, glucose oxidase; CNS, central nervous system; BAEC, bovine aortic endothelial cell; BASMC, bovine aortic smooth muscle cells

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1. Introduction

A hydrogel is a crosslinked network formed from a macromolecular hydrophilic polymer. It is stable upon swelling in water and capable of absorbing a large amount of water, varying from 10% to thousands of times of its own volume. The physical properties, including swelling, permeation, mechanical strength, and surface characteristics, can be modulated through structural modification. Hydrogels based on natural polymers are currently receiving a great deal of interest, and are notable for controlled delivery of bioactive molecules and tissue engineering[1–4].

Chitosan is a heteropolymer of glucosamine and *N*-acetyl glucosamine residues (Fig. 1), and is obtained by deacetylation of chitin[5]. It is a weak base, soluble in acidic solution (pH < 6.5) and insoluble in water and organic solvents. It forms a hydrogel in the presence of multi-valent anions, such as tripolyphosphate (TPP) anions by ionic interaction between the positively charged amino groups of chitosan and the negatively charged counter-ion of TPP. Due to their hydrophilic nature and greater solubility in acidic medium, chitosan hydrogels exhibit relatively low mechanical strength and limited ability to control the release of encapsulated compounds[5], thus necessitating chemical modification facilitated by its hydroxyl and amino groups.

Modified chitosan hydrogels have been proven to be a potential carrier for delivery of different drug molecules with respect to size and type[6–8]. As few reports were found on modified chitosan hydrogel, this review summarizes recent developments in its properties and applications.

2. General properties of chitosan

Chitosan is commercially obtained by hydrolysis of the aminoacetyl group of chitin, a straight chain homopolymer composed of β-(1,4)-2-acetamido-2-deoxy-D-glucose units[9]. Chitosan has one primary amino and two free hydroxyl groups for each glucose unit. The cationic amino groups react with a number of multi-valent anions to form hydrogels. In physiological environments various enzymes, such as chitosanase and lysozyme, degrade chitosan and form harmless products. Increased deacetylation enhances the biocompatibility of chitosan[10]. Entrapment of viable cartilage cells into the chitosan hydrogel does not produce a significant untoward effect[11] thereby improving the biocompatibility. Practical use of unmodified chitosan has been limited due to its poor solubility in acid solutions. Sugimoto et al.[12] reported that the water solubility of chitosan was improved by its modification with polyethylene glycol. Similarly, the properties of chitosan, such as complexation, bacteriostatic effect, absorbability and antioxidant properties, have been shown to be enhanced by its modification[13].

3. Preparation of hydrogel by modification of chitosan

Chitosan hydrogels have limited applications in drug delivery and tissue engineering due to their hydrophilic nature and insolubility in certain physiological conditions. Modification of the polymer can change the properties of the hydrogel. Chitosan can be readily modified by reactions at the amino groups and hydroxyl groups present in the molecule.

3.1. *Modification through covalent crosslinking*

The physical properties of a hydrogel, such as crystallinity, thermal sensitivity, swelling ratio, and mechanical strength, can be improved by covalent crosslinking using glutaraldehyde (GA), oxalic acid, formaldehyde, glyoxal, and genipine.

3.1.1. *GA crosslinker*

The covalent crosslinking of natural polymers can be achieved through the reaction of their functional group with the crosslinking agent GA (Scheme 1). A pH-sensitive GA-crosslinked chitosan hydrogel system using acrylamide-grafted-poly (vinyl alcohol) (PVA-g-AAm) and hydrolyzed PVA-g-AAm has been prepared[14]. The release of drug is dependent on the amount of GA in the matrix, *i.e.*, on the extent of crosslinking. For controlled release, hydrogel microspheres loaded with 5-fluorouracil were prepared using chitosan and pluronic F-127 crosslinked with GA[15]. The release of drug can be extended up to 24 h by controlling the GA concentration. For colonic delivery of 5-fluorouracil, Lin et al.[16] prepared *N*-(2-carboxybenzyl) chitosan (CBCS)-based pH-sensitive hydrogel (Scheme 2). The release of drug was pH dependent, increasing with pH. It is assumed that the dominant carboxyl groups (COOH) in the hydrogels would dissociate with the increase of the osmotic pressure inside the hydrogel at higher pH, resulting faster swelling and consequent release of drug. Various polymers being used for the modification of chitosan hydrogel, crosslinked with GA are shown in Table 1.

3.1.2. *Genipin crosslinker*

Genipin, an aglycone derived from geniposide, is an excellent natural crosslinker for proteins, collagen, gelatin and chitosan. Beads of chitosan–alginate gel crosslinked by genipin have been employed for drug delivery[17]. The IR spectroscopic data indicates that the carboxymethyl group of genipin reacts with amino group of chitosan, resulting the formation of a secondary amide. The swelling of the prepared beads decreases with the increase of pH. It is proposed that the protonated

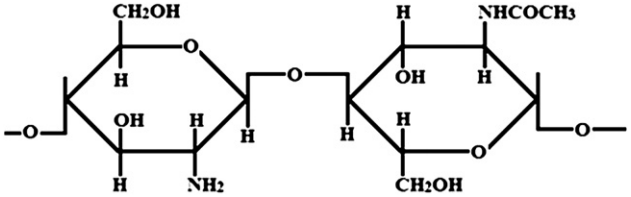
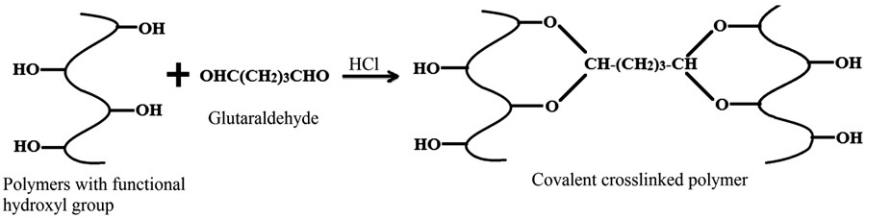
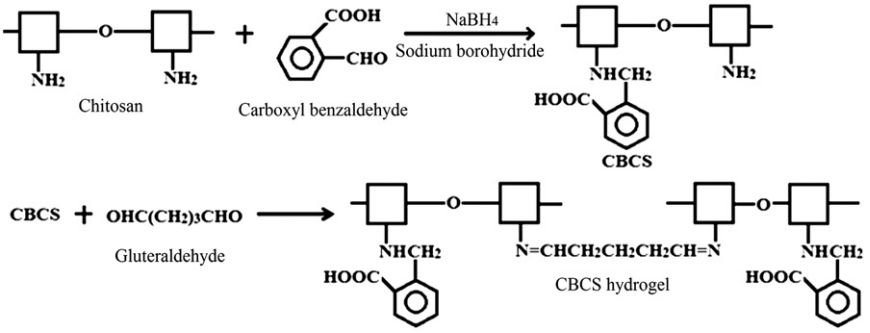


Figure 1 Chemical structure of chitosan.



Scheme 1 Covalently crosslinked chitosan hydrogel prepared using glutaraldehyde.



Scheme 2 Mechanistic pathway for the preparation of GA-crosslinked CBCS hydrogel[16].

Table 1 Polymers used for modification of chitosan hydrogels cross-linked with glutaraldehyde.

Polymer	Change in property after modification	Application
Gelatin	Increase in rigidity	Carriers for caffeine in the human body[17]
Starch	Flexibility and elasticity of the patch improved with good bioadhesive properties	Controlled release delivery of α-hydroxy acid contained in tamarind fruit pulp extract[18]
Hydroxyethyl cellulose	Forms rigid network	Controlled release delivery of chlorthiazide[19]
Polyethylene glycol	Improves heparin blood compatibility and anticoagulant property	Compatibility studies for various biomedical applications[20]
Polyvinyl pyrrolidone	Improves pH dependent swelling	Controlled release system for amoxicillin delivery[21]
Poly(ethylene-graft-acrylamide)	Forms rigid network	Controlled release of capecitabine[22]
Poly(acrylic acid-*co*-acrylamide)	Improves mechanical strength, mucoadhesive force and solubility	Potential muco-adhesive systems for *per oral* delivery of peptide and protein drugs[23]
Pluronic F127	Network structure becomes more rigid resulting increased retention of drug	Controlled delivery of 5-flurouracil[15]

amino group of chitosan is shielded by excess Cl^- ions, resulting the inhibition of nucleophilic attack on the dihydropyran ring of genipin.

A novel sponge hydrogel of chitosan and silk crosslinked by genipin has been prepared by Silva et al.[18]. It has been observed that these sponge hydrogels promote adhesion, proliferation, and matrix production of chondrocyte-like cells. The investigators suggested that the genipin-crosslinked chitosan-silk fibroin sponge hydrogels may be potential candidates for cartilage tissue engineering. Polymers used for preparing modified chitosan hydrogels crosslinked with genipin are presented in Table 2.

3.1.3. *N,N¹-Methylenebisacrylamide (MBA) crosslinker*

*N,N*¹-Methylenebisacrylamide, a bifunctional monomer with two identical unsaturated double bonds, is widely used as a crosslinking agent in many fields. A novel heat- and pH-sensitive hydrogel of carboxymethyl chitosan (CMCs) and poly (*N*-isopropylacrylamide) crosslinked by MBA has been reported[19]. The amount of co-enzyme A (Co A) released from the MBA crosslinked hydrogel was relatively low (22.6%) at pH 2.1, while at pH 7.4, the release of Co A increased significantly (89.1%) at 37 °C. Under the same conditions, the swelling ratio has been found to increase with an increase of pH. The specific hypothesis under study is that the release of Co A from the hydrogel was hindered at pH 2.1 by large number of H-bonds between the polar groups in Co A (–OH, $–NH_2$, $–H_2PO_4$, –SH and –NHCO) and the groups in the polymer network. However, the phosphoric groups in Co A are negatively charged at pH 7.4, and the electrostatic repulsion between phosphoric salt and $–COO^-$ facilitates the release of Co A. It has also been observed that the release rate of Co A is higher at 37 °C than that at 25 °C in buffer solution of pH 7.4.

Verapamil-loaded hydrogels, composed of chitosan and acrylic acid, were prepared by free radical polymerization using MBA as a crosslinking agent and benzoyl peroxide as a catalyst[4]. The porosity and gel fraction of the beads increased with the increasing MBA content. The release of verapamil

Table 2 Polymers used for modification of chitosan hydrogels cross-linked with genipin.

Polymer	Change in property after modification	Application
Alginate and *N,O*-carboxymethy chitosan	Electrostatic repulsion between ionized acid groups improves swelling property	Site specific protein delivery in the intestine[26]
Carboxymethyl hexanoyl chitosan	Degree of hexanoyl substitution changes the swelling ability as well as solubility	Encapsulation of poorly water soluble drug[27]
Carboxymethyl cellulose and chitosan	Formation of polyion complex provides high strength and stability	As a drug delivery carrier[28]
Chitosan and poly(vinyl alcohol)	Modification of chitosan content in the graft polymer improves cell viability	Potential use in a variety of biomedical application[29]
Chitosan and gelatin	Rigidity of the matrix increased	Biomedical application[30]
Chitosan and polyethylene amide	Decrease in drug release rate with increase in crosslinking density	Drug delivery application[31]
Chitin and chitosan	Provides favorable environment for growth of cartilage cells	Cultivation of bovine knee chondrocyte[32]
Chitosan and gelatin	Increase in porosity of the scaffolds	Articular cartilage tissue engineering[33]

depended on the ratio of chitosan to acrylic acid, the degree of crosslinking, and pH of the medium. Another hydrogel was synthesized by crosslinking an AAm–chitosan mixture (8:2, *v*/*v*) with MBA for controlled delivery of amoxicillin[20]. The hydrogel matrix released 56.47% and 77.096% of amoxicillin after 24 h and 74 h, respectively.

3.2. *Modification through ionic crosslinking*

The swelling behavior of the crosslinked chitosan hydrogel is influenced by the ionization of functional groups along the polymer chains and the ionization of crosslinking agent. For controlled delivery of glipizide[6], pH-sensitive hydrogel beads of chitosan and TPP were prepared by ionic gelation. Both the swelling ratio and drug release were directly related to the pH of the dissolution medium.

Mucoadhesive hydrogel microparticles composed of poly (methacrylic acid-vinyl pyrrolidone)–chitosan (PMVC) and *N*-vinyl pyrrolidone (NVP) were prepared[21] using the ionic gelation method. Incorporation of NVP improved the release of insulin from the hydrogel at acidic pH. NVP enhanced the mucoadhesion behavior of hydrogel particles when studied in rat intestine. Ionic crosslinking of chitosan and PEG to form hydrogel beads using sodium TPP as crosslinking agent has been reported[22]. A maximum loading efficiency of 90% was obtained with 10% (*w*/*v*) TPP at pH 6.0 for 30 min. To intestinally deliver a drug without losing drug in the stomach, pH-sensitive chitosan hydrogel microspheres were prepared *via* ionotropic crosslinking with sodium TPP and dextran sulfate[23]. The release of drug from hydrogel microspheres was insignificant in simulated gastric fluid over 3 h, but nearly 100% of the drug was released in simulated intestinal fluid within 6 h.

pH-sensitive methotrexate chitosan-based microgels (<200 nm diameter) were prepared by ionically crosslinking *N*-[(2-hydroxy-3-trimethyl ammonium) propyl] chitosan chloride in the presence of TPP[24]. It was observed that the crosslinked microgels exhibited a significant increase in cell motility of HeLa cells compared to non-crosslinked microgels.

3.3. *Modification through grafting*

Grafting of natural polymers such as chitosan containing two types of reactive groups – amino and hydroxyl – is of considerable interest for modification of the polymer structure. Chemical grafting is initiated by generating one or more free radicals on the chitosan chain and allowing these radicals to react with polymerizable monomers that will constitute the grafted chain.

AAm was grafted and polymerized onto chitosan by Pourjavadi and Mahdavinia[25] and the grafting was initiated by ammonium persulfate (APS) under an inert atmosphere. The graft polymer was hydrolyzed in hot sodium hydroxide solution. The swelling capacity of both hydrolyzed and non-hydrolyzed hydrogels increased with an increase in the AAm concentration up to a certain limit, beyond which the swelling capacity decreased. The non-hydrolyzed graft polymer showed the maximum degree of swelling at pH 3 and hydrolyzed graft polymer showed two maxima at pH 3 and 8. In acidic medium (pH 3), the amino groups of chitosan become protonated and the increased charge density and NH_3^+–NH_3^+ electrostatic repulsion enhanced the osmotic pressure inside the gel particles, resulting in further swelling. However, in basic conditions (pH 8), the hydrolyzed graft polymer showed maximum swelling due to ionization of carboxylic groups and the electrostatic repulsive forces between the charged sites (COO^-). Chen and Tan[26] worked on graft copolymerization of acrylic acid onto the chain of carboxymethyl chitosan and subsequent crosslinking in aqueous medium by the same radical system. The FTIR spectroscopic data confirmed the graft polymerization between acrylic acid and carboxymethyl chitosan. The water absorption of the resultant graft polymer was dependent on the crosslinking agent, initiator, monomer concentration, reaction temperature, reaction time, degree of neutralization of acrylic acid, and water volume of the system.

You et al.[27] synthesized a graft copolymer of chitosan and stearic acid by reacting the amine group of chitosan with the carbonyl group of stearic acid. Nanoparticles prepared from the graft copolymer loaded with paclitaxel effectively delivered the drug into the cytoplasm of cancer cells. Superabsorbent hydrogels were prepared by grafting chitosan with polyaniline by an oxidative method[28]. It has been observed that the crosslinking of the copolymers yielded a composite hydrogel in which the polyaniline was homogeneously embedded.

A graft copolymer of sodium acrylate (AAs) and CMCs by free radical polymerization using APS was prepared under a nitrogen atmosphere and another graft copolymer of chitosan

and polyethylene glycol through the Schiff base formation was synthesized[29]. pH-sensitive hydrogel microspheres were prepared by crosslinking carboxymethyl chitosan grafted with acrylate (CMCs-g-AAs) and sodium alginate followed by coating with chitosan grafted with poly (ethylene glycol) (Cs-g-PEG) (Scheme 3).

In addition to the above chemical initiating system, various radiation initiating systems have been tried for the synthesis of chitosan-based graft copolymers. Since the radiation technique is clean, safe and effective, it is a very convenient tool for the modification of natural polymers.

Cai et al.[30] have prepared a graft copolymer of *N*-isopropylacrylamide onto chitosan using ^{60}Co γ-radiation. Grafting percentage and grafting efficiency were improved by increasing the monomer concentration and radiation dose. The resultant grafting hydrogels showed good heat and pH sensitivity with good swelling property. El-Sherbiny and Smyth[31] reported photo-induced grafting of PEG onto to CMC_S to confer pH responsive drug delivery systems (Scheme 4). The grafting percentage and grafting efficiency increased with increasing concentration of the initiator within the range of 5.2–20.8 mM. The optimum concentration was 10.4 mM.

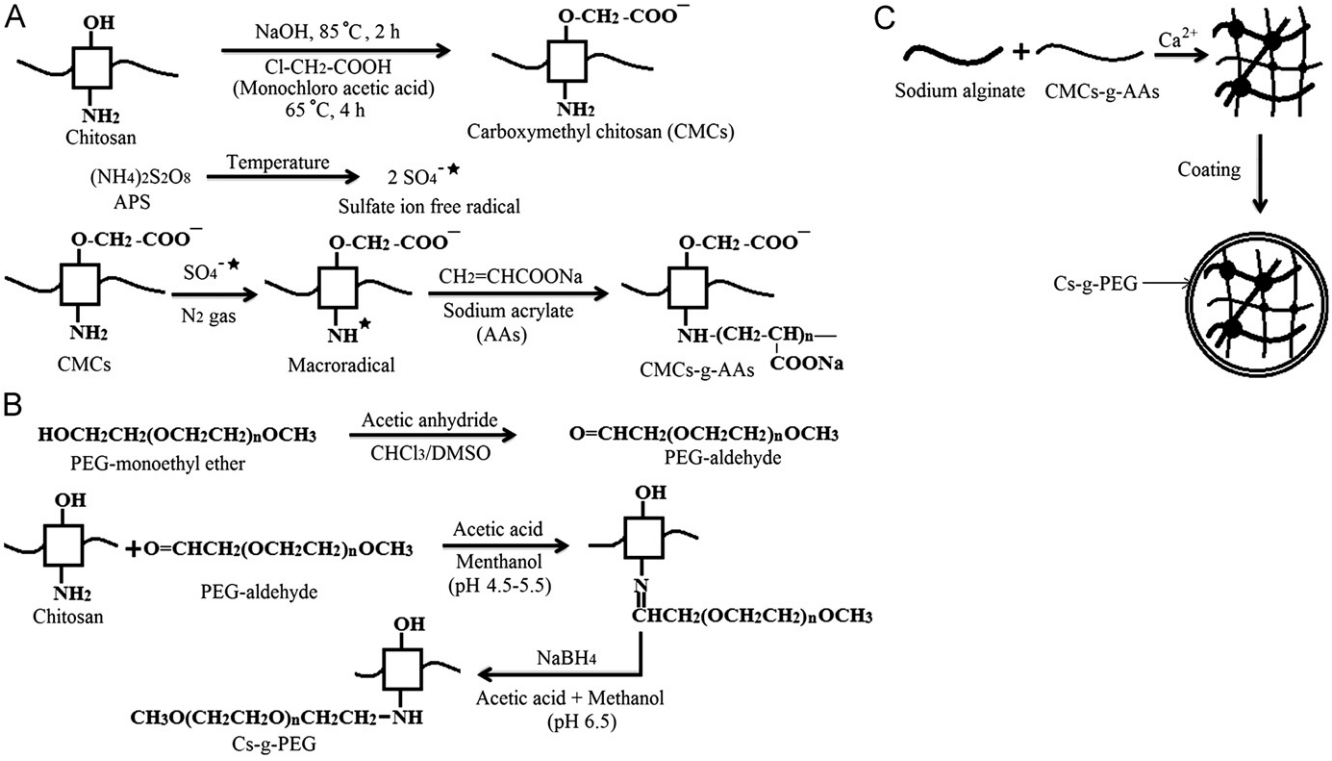
3.4. *Modification through polyelectrolyte complexes (PEC)*

Chitosan is able to form polyelectrolyte complexes (PEC) with various natural and synthetic anionic polyelectrolytes through strong electrostatic interaction. The complex formation and physical properties of the PEC depend on factors, such as the ionization degree of the cationic and anionic counter parts, pH, temperature, ionic strength, time of interaction and concentration of the polymeric solutions[32].

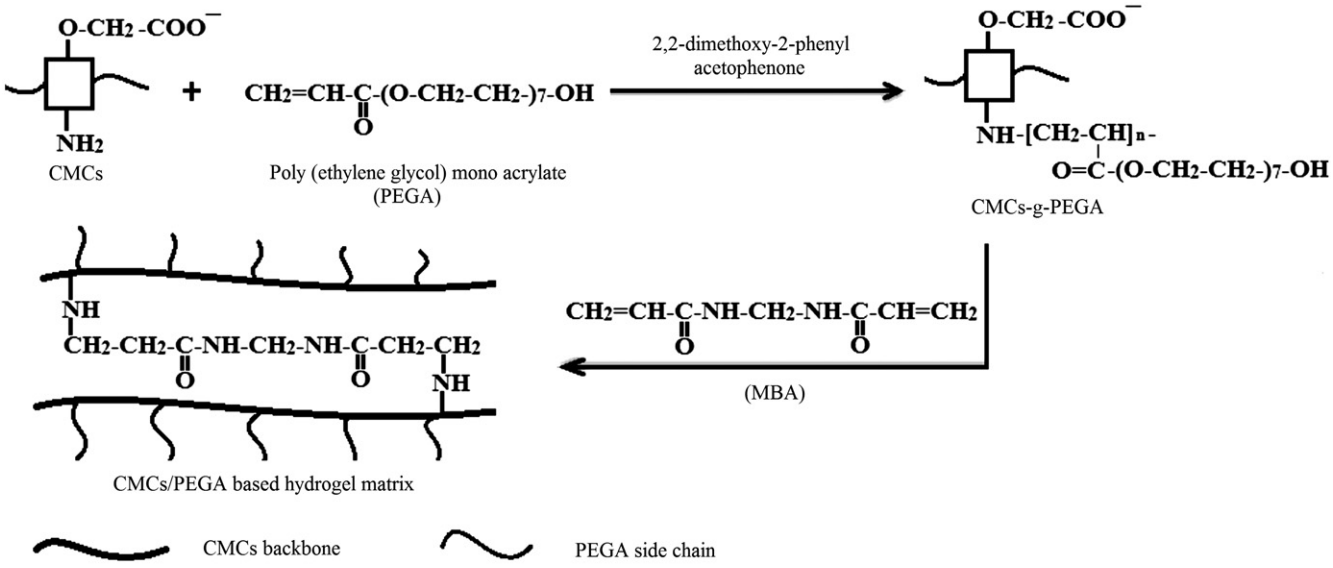
To modify the surface properties and to improve the entrapment efficiency and release of drug, the calcium alginate microparticles were coated with polycations such as chitosan, poly-L-lysine and DEAE-dextran[33]. Coated microparticles showed increased entrapment efficiency and slower release of model protein drug. A polyion complex membrane was prepared with chitosan and sodium alginate for the separation of water and organic mixture[34]. The FTIR spectroscopy, differential scanning calorimetry and thermogravimetric analysis indicated the formation of chitosan and sodium alginate polyion complexes with ionic bonds between protonated amine groups of chitosan and carboxylated groups of alginate. Both ionic crosslinking and physical structure of hydrogel influenced the state of water in ionic hydrogel membranes.

Polymer–magnetite hybrid nanoparticles were prepared using polyion complex of carboxymethyl cellulose and chitosan as follows[35]: the carboxymethyl cellulose and chitosan were blended to form polyion complexes (CC particles); the CC particles were chemically crosslinked with genipin to provide gel particles (CCG particles) with improved strength and stability; finally, magnetite was synthesized within CCG particles by the coprecipitation method to obtain polymer–magnetite hybrid nanoparticles (CCGM). The synthetic route for the formation of CCGM particles are shown in Scheme 5.

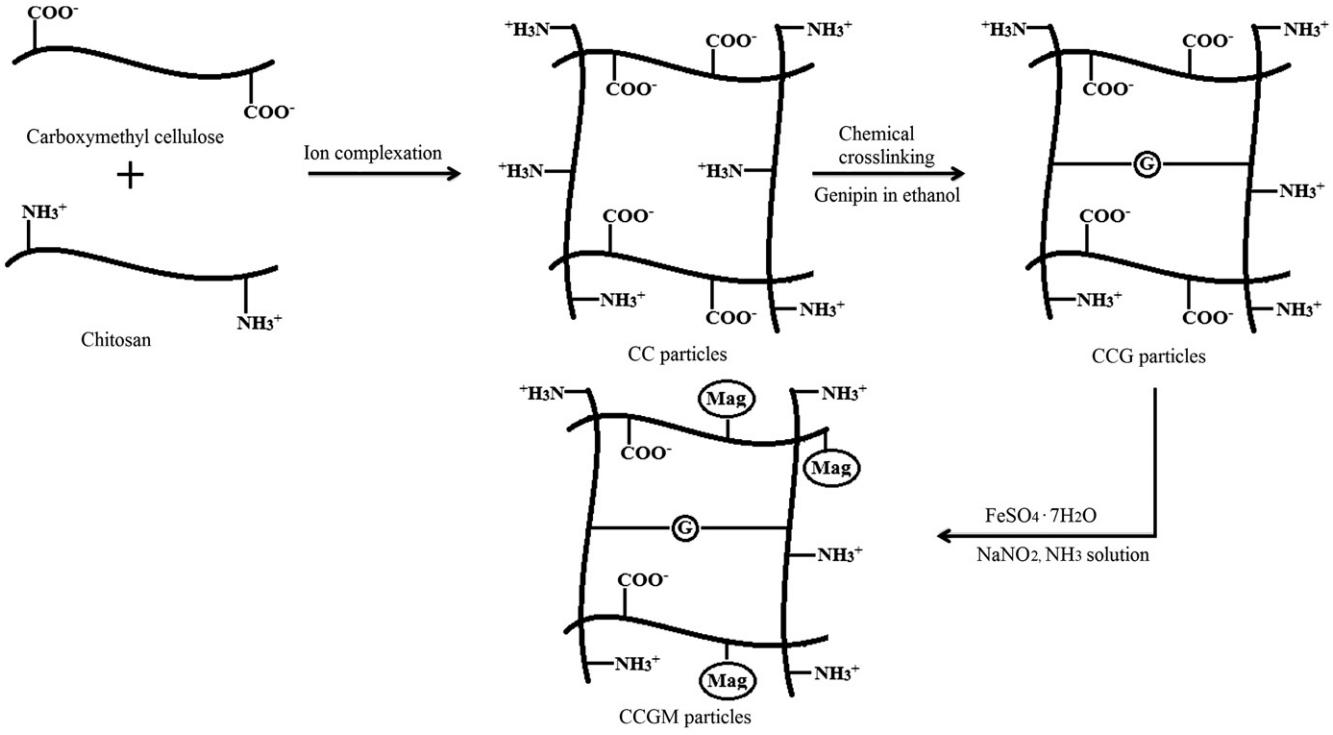
Chitosan has also been used for the preparation of various polyelectrolyte complex products with natural and synthetic polyions such as xanthan, hyaluronic acid, alginate, collagen,



Scheme 3 Proposed pathway for the synthesis of CMCs-g-AAs copolymer (A), synthesis of Cs-g-PEG copolymer (B), and development of alginate/CMCs-g-AAs hydrogel microspheres coated with Cs-g-PEG (C)[44].



Scheme 4 Proposed pathway for the preparation of CMCs-g-PEG copolymer and its hydrogel matrix[46].



Scheme 5 Proposed synthetic route for the formation of CCGM particles[28].

gum kondagogu, pectin, carrageenen, gelatin, dextransulfate, Y-glutamic acid, carboxymethylcellulose, Eudragit S100 and polyalkyleneoxide-maleic acid copolymer.

4. Applications

4.1. Drug delivery application

Since chitosan is positively charged, it can interact with negatively charged polymers, macromolecules and polyions. Chitosan-based hydrogel systems can be used for oral, transdermal, nasal, rectal and ocular drug delivery.

4.1.1. Oral drug delivery

Oral delivery is widely accepted for drug administration. Chitosan-based hydrogels have been investigated in a number of therapeutic oral delivery systems either as controlled release matrices or functional biomaterials. Chitosan-based hydrogel has been used for the delivery of drugs to specific sites of the body such as oral cavity, stomach, small intestine and colon. The site-specific delivery of the drug to the oral cavity can be

used to treat a number of diseases of the mouth, such as stomatitis, periodontal disease, fungal and viral infections, and oral cavity cancers, thereby avoiding the first pass metabolism effect. Chlorhexidine buccal tablets were prepared using drug-loaded chitosan microparticles by a spray-drying technique[36]. The antimicrobial activity of the drug against *Candida albicans* was improved. Moreover, an *in vivo* study showed that the formulation exhibited a prolonged release of the drug in the buccal cavity. Antibacterial activity of chitosan-based thermosensitive hydrogel was effective against the periodontal pathogens-*Porphyromonas gingivalis*, *Prevotella internedia* and *Actenobacillus actinomycetem* comitans[37]. Experimental results demonstrated that the thermosensitive hydrogel acts as the vehicle as well as an activator for the antibacterial process.

Chitosan based hydrogel systems can be designed to deliver drugs locally to the stomach or the upper part of GIT to improve bioavailability. Chang et al.[38] developed amoxicillin-loaded pH-sensitive hydrogels composed of chitosan and poly(γ-glutamic acid) for the treatment of *Helicobacter pylori* (*H. pylori*) infection in the peptic ulcer disease. A confocal laser scanning microscopic study indicated that the hydrogels could infiltrate the cell–cell junctions and interact with *H. pylori* infection sites in the intercellular spaces. Hydrogel of chitosan and polyacrylic acids containing amoxicillin and clarithromycin were also clinically evaluated for *H. pylori* eradication[39]. Clinical trial experiments indicated that the polyionic hydrogel could serve as suitable candidates for amoxicillin and clarithromycin site-specific delivery in the stomach. Modified chitosan hydrogels loaded with metronidazole, tetracycline and theophylline were developed for stomach-specific delivery. Modified chitosan hydrogels can bypass the acidic environment of the stomach and release the loaded drug into the intestine.

A novel pH-sensitive composite hydrogel of chitosan-graft-poly (acrylic acid), attapulgite, and sodium alginate was developed for controlled release of diclofenac sodium (DS)[40]. The cumulative release rate of DS from the composite hydrogel beads was insignificant at pH 2.1 and 100% at pH 6.8 within 24 h. However, most of the loaded DS was released within 2 h at pH 7.4. A novel pH-sensitive hydrogel bead composed of *N*-succinyl chitosan and alginate was developed by Dai et al.[41] using nifedine. The *in vitro* release of nifedipine from the hydrogel bead was 11.6% at pH 1.5 while 76% at pH 7.4. Superporous hydrogel based on poly(acrylic acid-*co*-AAm) and *N*,*O*-CMCs was prepared for the oral delivery of insulin[42]. *In vivo* results showed that the oral administration of insulin-loaded hydrogel yielded notable insulin absorption and a hypoglycemic effect. Moreover, the biocompatibility of the hydrogel was confirmed by an oral acute and sub-acute toxicity study in mice. Similarly, superporous hydrogels were prepared and evaluated for their potential in effective insulin absorption *via* the oral route[43].

Another novel temperature- and pH-sensitive hydrogel based on chitosan grafted with poly(acrylic acid), poly(hydroxy propyl methacrylate), poly(vinyl alcohol) and gelatin was prepared for controlled drug delivery of oxytetracycline[44]. It was observed that the release of the drug increased with the increasing time, temperature and pH and reached to the maximum after 48 h at pH 9.

A pH-sensitive hydrogel using CMCs and alginate was also prepared by crosslinking with genipin for site-specific protein drug delivery in the intestine[45]. The release of BSA at pH 1.2 was relatively low, but increased significantly at pH 7.4. It may be due to the formation of hydrogen bonds between CMCs and alginate at pH 1.2 that restricts the swelling. At pH 7.4, the hydrogel swelled more significantly due to large swelling force created by electrostatic repulsion between the ionized acid groups.

Colon specific drug delivery systems are gaining importance for use in the treatment of chronic diseases, such as irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, and also for the systemic delivery of protein and peptide drugs. Xu et al.[46] prepared dual crosslinked gel beads composed of alginate and chitosan for the colonic site-specific delivery of bovine serum albumin (BSA). The release of BSA from all beads was much faster in simulated colonic fluid than in simulated intestinal fluid. Chitosan hydrogel beads were prepared by the crosslinking method followed by enteric coating with Eudragit S100 for targeted delivery of Satranidazole to the colon[47]. The chitosan beads prevented premature drug release in simulated gastric fluid. However, most of the loaded drugs were released in the colon, an environment rich in bacterial enzymes that degrade chitosan. Several chitosan-based formulations are being investigated as carriers for colon-specific delivery of 5-aminosalicyclic acid, prednisolone, metronidazole, 5-fluorouracil and indomethacin.

4.1.2. *Transdermal drug delivery*

Transdermal drug delivery systems in the form of hydrogel membranes can deliver drugs for systemic effects through skin at a predetermined and controlled rate. This system presents the advantage of avoiding the first pass metabolism effect. Drug delivery can be easily interrupted on demand by simply removing the devices. The hydrogel patch composed of chitosan and starch crosslinked with GA has been prepared for controlled release of α-hydroxy acid contained in tamarind fruit pulp extract[48]. The resultant patches showed good bio-adhesive properties and the amount of tartaric acid released was proportional to the square root of time (Higuchi's model[49,50]).

Similarly, a curcuminoid-loaded hydrogel patch composed of chitosan and starch was developed for cosmetic applications[51]. A rapid rate of curcumin release was observed by the vertical diffusion cell method. A transdermal delivery system for the treatment of cutaneous leishmaniasis was developed by incorporating berberine into a chitosan hydrogel[52]. The *in vitro* skin perfusion studies indicated that only trace amounts of berberine permeated through the rat skin due to its low oil–water partition coefficient. Surfactants can enhance percutaneous absorption of berberine.

Novel lidocaine hydrochloride-loaded transdermal chitosan patches were developed using a chitosan membrane for rate control and a chitosan hydrogel as a drug reservoir[53]. *In vitro* drug release was prolonged by chitosan having a 95% degree of deacetylation.

4.1.3. *Ocular drug delivery*

The major problem with conventional eye drops is retention in the eye. The administration of ophthalmic drugs in hydrogels has been shown to increase the contact time of the drugs with cornea, thereby increasing ocular bioavailability. An *in situ* thermosensitive hydrogel composed of chitosan and β-glycerophosphate (GP) was prepared[54]. The hydrogel enhanced the transcorneal permeation to 7-fold over an aqueous solution, improved ocular bioavailability, minimized the need for

frequent administration and decreased the ocular side effects of ofloxacin. Another *in situ* thermosensitive hydrogel composed of chitosan and poly (*N*-isolpropylacrylamide) was prepared for ocular delivery of timolol maleate[55]. The drug release from the hydrogel was also doubled.

To increase its ocular bioavailability, Genta et al.[56] prepared bioadhesive chitosan microspheres for ophthalmic administration of acyclovir. *In vivo* ocular studies on rabbits indicated a high concentration of acyclovir for an extended period of time.

4.1.4. Nasal drug delivery

The nasal mucosal membrane presents a potentially useful site for the delivery of drugs by combining a decreased first pass effect with greater patient acceptability. Chitosan hydrogels improve nasal absorption of drugs since they facilitate the paracellular transport of large molecules across the mucosal surface by opening tight junctions. Nazar et al.[57] synthesized *N*-trimethyl chitosan chloride and formulated it into a hydrogel with PEG and GP for nasal drug delivery. The hydrogel containing *N*-trimethyl chitosan with medium average molecular weight and a low degree of quaternization yielded an aqueous formulation that exhibits a sol–gel transition at 32.5 °C within 7 min. A thermosensitive *in situ* gel system was prepared by mixing chitosan and polyvinyl alcohol for the nasal delivery of insulin[58]. The prepared hydrogel exhibited a sol-gel transition at 37 °C for approximately 12 min. The release of insulin maintained steady blood glucose levels for 6 h.

Alsarra et al.[59] evaluated different polymeric hydrogels, such as polyvinyl pyrrolidone (PVP), chitosan and carbopol, for the nasal delivery of acyclovir. The release rates of acyclovir from PVP gels were higher compared to chitosan or carbopol gels. A histopathological study indicated that the PVP was a safe hydrogel for mucosal delivery.

A novel thermosensitive hydrogel system was prepared by simply mixing quaternized chitosan and PEG with a small amount of α-β-GP for nasal drug delivery[60]. The *in vivo* study showed that the prepared hydrogel decreased the blood glucose concentration (40–50% of initial blood glucose concentration) after 4–5 h of administration. Spray-dried microspheres based on methylpyrrolidinone chitosan were developed for nasal administration of metoclopromide[61]. The *in vitro* and *in vivo* study indicated that the microspheres could be a suitable nasal delivery system for the administration of metoclopramide.

4.1.5. Rectal drug delivery

The rectum has been studied as a favorable site of drug delivery for local and systemic action. This route offers certain advantages with local targeting of drugs to the organs. It may also represent an alternative to intravenous or other injection routes of drug administration. The DS-loaded chitosan microspheres were incorporated into hydrogels containing hydroxypropyl methylcellulose (HPMC) and carbopol 934 for rectal administration[62]. The viscosity of rectal hydrogels influences the drug release and distribution of hydrogels in the distal portion of the large intestine.

4.2. Tissue engineering applications

Tissue and organ loss or damage is a major human health problem. Transplantation of tissue or organs is a standard therapy to treat these patients. However, this therapy is severely limited due to the shortage of donors. Tissue engineering (TE) is one of the available therapies to treat the loss or damaged tissue and organ. It involves *in vitro* seeding and attachment of human cells onto a scaffold, followed by the culturing of the cells to form the new organ or tissue. Chitosan is a promising polymer for tissue engineering for its non-toxicity, biocompatibility and biodegradability. Moreover, chitosan has structural similarity to glucosaminoglycans which are the major component of the extracellular matrix.

Ladet et al.[63] developed a chitosan-based multi-membrane hydrogel (MMH) to investigate the *in vitro* responsiveness of articular chondrocyte-like cells. The cells aggregated, proliferated and maintained their phenotype with the production of a large amount of cartilage-type matrix proteins.

An insulin-loaded, glucose-sensitive hydrogel composed of *N*-succinyl chitosan (SCS) and aldehyde hyaluronic acid covalently conjugated with glucose oxidase (GOD) and catalase was developed for adipose tissue regeneration[64]. The GOD converts glucose to gluconic acid in the presence of glucose. The gluconic acid reduced the pH value of the microenvironment which triggered the swelling of the pH-sensitive hydrogel and consequently facilitated the release of insulin by a diffusion-mediated process.

Tran et al.[65] prepared an *in situ* forming rutin-releasing chitosan hydrogel as an injectable dressing for wound healing. An *in vivo* wound-healing study on rat dorsal wounds demonstrated that rutin-conjugated hydrogel exhibited improved wound healing as compared with the hydrogel without rutin.

Yang et al.[66] prepared a series of hydrogels from an aqueous solution of gelatin and carboxymethyl chitosan by radiation-induced crosslinking at ambient temperature for wound healing. The obtained hydrogels promoted cell attachment and rapid growth of fibroblast on the materials.

A novel injectable scaffold was developed by combining collagen-coated poly-lactide microcarriers and a crosslinkable chitosan hydrogel loaded with cartilage tissue of rabbit ear for cartilage regeneration[67]. A novel thermosensitive hydrogel composed of chitosan and pluronic was developed as an injectable cell delivery carrier for cartilage regeneration[68].

Despite the enormous progress of bone tissue engineering, there are still a number of barriers for the treatment of bone injuries. Luca et al.[69] studied the effects of carrier and pH on recombinant human BMP-2 (rhBMP-2) induced non-osseous bone formation. They observed that the injection of rhBMP-2 in a chitosan hydrogel induced bone formation in the muscle of rats.

In another study, an injectable thermosensitive hydrogel composed of chitosan and PVA was developed for bone tissue engineering[70]. It was observed that the rabbit bone marrow mesenchymal stem cells proliferated in the hydrogel within 3 weeks.

Tang et al.[71] prepared injectable hydrogels composed of chitosan and methyl cellulose that were liquid at low temperature (about 4 °C) and gel under physiologic conditions (37 °C) for cartilage tissue regeneration. The obtained hydrogel resulted in good cell viability and proliferation.

A set of novel injectable hydrogels composed of chitosan derivative, polyethylene glycol dimethacrylate and *N*,*N*-dimethylacrylamide was prepared for bone tissue regeneration[72]. The resultant hydrogels were promoted cell attachment and proliferation.

Severe hepatic failure accounts for many deaths each year worldwide. Liver transplantation is limited due to the shortage

of available organ donors. Therefore, liver tissue engineering is a potential approach to provide liver tissue for patients suffering from hepatic failure. Yang et al.[73] developed porous polymer scaffolds composed of alginate and galactosylated chitosan (GC) for liver tissue engineering. The resultant scaffolds were suitable for improving hepatocyte adhesion and maintenance of cell viability.

Most neurons in the central nervous system (CNS) do not proliferate or renew themselves. Therefore, interest has focused upon cell replacement therapies to repair damage in the CNS. Thermally responsive hydrogels composed of chitosan and GP were prepared for neural tissue regeneration[74]. A photo-crosslinkable hydrogel based on chitosan was synthesized by conjugating 4-azidobenzoic acid with chitosan and crosslinked by UV illumination for peripheral nerve anastomosis[75].

New blood vessel formation is a promising alternative to treat patients suffering from restricted or obstructed blood flow caused by coronary and peripheral arterial disease. Mathews et al.[76] investigated the attachment and growth of bovine aortic endothelial cell (BAEC) and smooth muscle cells (BASMC) on PVA hydrogels modified with water-soluble and water-insoluble chitosan. A cell adhesion study indicated that BAEC and BASMC successfully adhered to the PVA–chitosan membranes.

5. Conclusions

This review summarizes the potential applications of modified chitosan hydrogel for biomedical and pharmaceutical uses, particularly with regard to drug delivery, *in vitro* cell culture, and tissue engineering. The most attractive feature of chitosan for these applications is biocompatibility, as well as ease of preparing derivatives with new properties. Modified chitosan hydrogels have great utility in developing controlled-release formulations of almost all types of bioactive molecules. Recently, modified chitosan hydrogels have been extensively explored for tissue engineering applications, *e.g.*, cell transplantation and tissue regeneration. It can be concluded that for tissue engineering and drug delivery chitosan-based hydrogels are expected to become a promising matrix for use in regenerative medicine and drug delivery.

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