Photocrosslinkable Chitosan Hydrogels and Their Biomedical Applications

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ABSTRACT: Chitosan is a bioactive macromolecule with a wide variety of applications due to its excellent biological properties such as biodegradability, biocompatibility, and antibacterial activity, and so forth. This highlight focuses on the preparation of photoactive chitosans, the formation of photocrosslinkable chitosan hydrogels, and the related photopolymerization mechanisms. Moreover, the great potential applications of photocrosslinkable chitosan hydrogels for human tissues are also discussed. © 2018 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2019**, *57*, 1862–1871

KEYWORDS: biomedical application; chitosan; hydrogel; photopolymerization; tissue

INTRODUCTION Chitosan (CS), a linear polysaccharide made up of β-(1,4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units, is derived from chitin. It is the second most abundant natural polysaccharide extracted from the exoskeleton of crustaceans and insect, and from fungal cell walls, and so forth .[1,2] CS has excellent biological properties such as biodegradability, biocompatibility, and antibacterial activity.[3] Furthermore, CS can achieve hemostasis and promote normal tissue regeneration, favoring wound healing and scar prevention.[4] For these reasons, CS has been considered to be one of the most promising biomacromolecules for various biomedical applications such as wound dressings, drug delivery systems, and tissue engineering,[5–7] where it has been widely used in various forms such as membrane,[8] sponge,[9] fiber,[10] micro/nanoparticles,[11] and hydrogel.[12,13]

Among these forms, hydrogel has shown great potential in biological fields due to its hydrated environment similar to native tissues capable of promoting tissue repair and regeneration, its network structure allowing the exchange of nutrients and metabolic wastes, as well as its tunable mechanical property.[14–16] Several approaches, such as ionic crosslinking,[17] hydrogen bonding interaction,[18] and covalent crosslinking[19] have been developed commonly to fabricate hydrogel.

Among these approaches, covalent crosslinking by photopolymerization has recently become popular for the hydrogel fabrication because of fast curing rates at physiological temperatures, a low degree of invasiveness and temporal or spatial control for easy manipulation.[20]

M. Pei and J. Mao contributed equally to this work.

Photocrosslinked CS hydrogel has been reported by many researchers.[21,22] In general, CS is commonly modified into photoactive CS derivative, and the derivative aqueous solution is subsequently transited into hydrogel by photopolymerization under UV irradiation.

In this highlight, we focus on the synthesis of photoactive CS derivatives and thereby the fabrication of photocrosslinkable CS hydrogels. This highlight also provides an overview of the present status and future directions of photocrosslinkable CS hydrogels as wound dressings, drug delivery carriers, tissue engineering scaffolds, and other applications in pharmaceutical, biomedical, and biotechnological fields.

SYNTHESIS OF PHOTOACTIVE CS MACROMERS

The structures of CS and chitin are shown in Figure 1. It can be seen that there is no photoactive group in the molecular chain of CS, and thus it cannot be photopolymerized. However, in view of the structural characteristics of CS, there are a large number of active amino and hydroxyl groups, giving it a chance to introduce the photoactive groups by chemical reactions between —NH_2/—OH and substituting groups. So far, the literature have reported appropriately three approaches to prepare photoactive CS macromers, that is, vinylated CS, azido-functionalized CS, and (methyl)acrylated CS.

Vinylated CS Macromers

Styrenated CS (1 in Fig. 2) with low molecular weight was synthesized by condensation at an amino group of CS with

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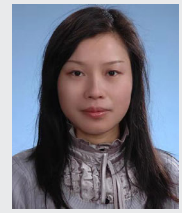

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4-vinylbenzoic acid.[23] Due to its low molecular weight, styrenated CS could completely dissolve in water to form the hydrogel under UV irradiation. However this hydrogel exhibited low mechanical strength, necessary to be further improved in gel strength by mixing with diacrylated poly(ethylene glycol) for the application as photocured scaffolds in tissue-engineered devices.

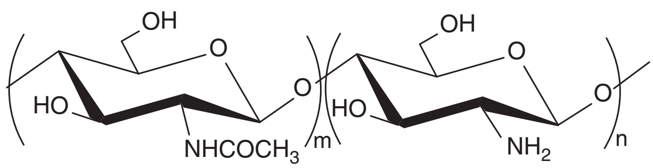

FIGURE 1 The chemical structure of chitin or chitosan.

Azido-Functionalized CS Macromers

A common approach to obtain a photoactive CS macromer is to modify CS with azido groups (Fig. 3). As a photoactive group usually used in the photochemical immobilization method in the modified polymer material, the azido group can undergo an irreversible photolysis under light irradiation to convert into highly active nitrene groups, which can react with the amino group to form the crosslinking structure (—N=N—) and thus change the solution into the hydrogel. Jameela et al.[24] obtained photocrosslinkable derivative of CS by coupling 1-chloro-2-hydroxy-3-azidopropane onto CS (2 in Fig. 3), and used it as a drug delivery matrix. Amoozgar et al.[25] also prepared photocrosslinkable CS by partial conjugation of 4-azidobenzoic acid to CS (3 in Fig. 3). This CS aqueous solution could form a hydrogel under UV light irradiation. The detailed mechanism is that, UV irradiation could induce photolytic conversion of aryl azide to reactive nitrene, which underwent ring expansion and reacted with the amines of photocrosslinkable CS to form an inter-/intra- molecular CS network. However, this UV-crosslinkable CS unexpectedly caused a granulomatous reaction when applied in the peritoneal cavity of rabbits, suggesting its insufficient biocompatibility.[26]

(Meth)Acrylated CS Macromers

Up to now, the most commonly used method to prepare photocrosslinkable CS is by grafting methacryloyl groups to CS molecular chain. For instance, Tsai et al.[27] prepared acryloyl CS (4 in Fig. 4) by the reaction between *N*-phthaloylated CS and acryloyl chloride in the organic solvent homogeneously. Elizalde-Pena et al.[28] also synthesized methacryloyl chiotsan (5 in Fig. 4) by ring-opening reaction between CS and glycidyl methacrylate (GMA). However, this methacrylated CS was not soluble at physiologic pH, while significant water solubility of methacrylated oligochitosan was obtained only for tetramers. In addition, considering the issue about biocompatibility of UV-curable CS derivatives, a series of methacryloyl chiotsan (6 in Fig. 4) with different structure were prepared by reductive *N*-alkylation via Schiff's bases using photosensitive aldehydes in less-toxic agents.[29] Interestingly, some of methacryloyl CS (i.e., 6a in Fig. 4) did not show inflammatory effect on surrounded tissue in subcutaneous implantation experiments, indicating its good biocompatibility.[30]

However, these photopolymerizable CS derivatives are only soluble at acidic pH and almost insoluble in the water environment at the neutral pH condition, while solubility at physiological pH values is required for the synthesis of useful biocompatible hydrogels for biological applications, especially for tissue engineering. Thus, a useful photopolymerizable CS precursor would be soluble in aqueous solution at physiological pH.

Water-Soluble Photoactive CS Macromers

Some water-soluble photoactive CS derivatives have previously been reported in literature. Considering hydrogels formation at physiological condition is crucial requirement for biomedical applications, CS is often modified by hydrophilic units such as carboxymethyl, glycol, hydroxypropyl, and lactose moiety, so as to change its intrinsic acid-soluble property, and then grafted with photocrosslinkable groups along its chemical chains.

Water-soluble photoactive CS with azide and lactose moieties in its molecular chain (7 in Fig. 5) was synthesized through a condensation reaction with amino groups of CS.[31,32] The introduction of lactose resulted in a water-soluble CS at neutral pH-values. About 2.5% and 2.0% of the amino groups in the CS chains reacted with azide benzoic acid and lactobionic acid, respectively.[33] This kind of photoactive CS aqueous solution can rapidly be converted into a hydrogel within 60 s under UV irradiation through crosslinking of the azide and amino groups of its molecular chains.

Poon et al.[34] introduced highly hydrophilic carboxymethyl into CS molecular chain and then prepared water-soluble methacrylated *O*-carboxymethylchitosan (8 in Fig. 5) by further reaction with GMA. The photocrosslinked anionic hydrogels based on this kind of CS derivative had a controlled *in vitro* biodegradation, and could support attachment and proliferation of Smooth Muscle Cells, indicating their cytocompatibility.

Kufelt et al.[35] applied succinic anhydride chemistry to convert CS in a water-soluble species by the formation of *N*-succinylchitosan, and subsequently use GMA to conjugate with succinyl groups forming photosensitive CS (9 in Fig. 5). This kind

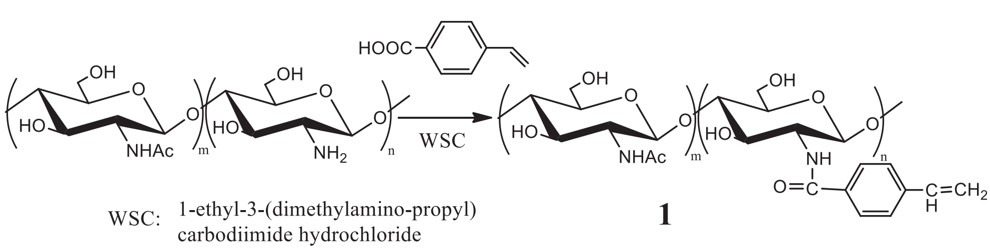

FIGURE 2 Synthesis of styrenated chitosan.

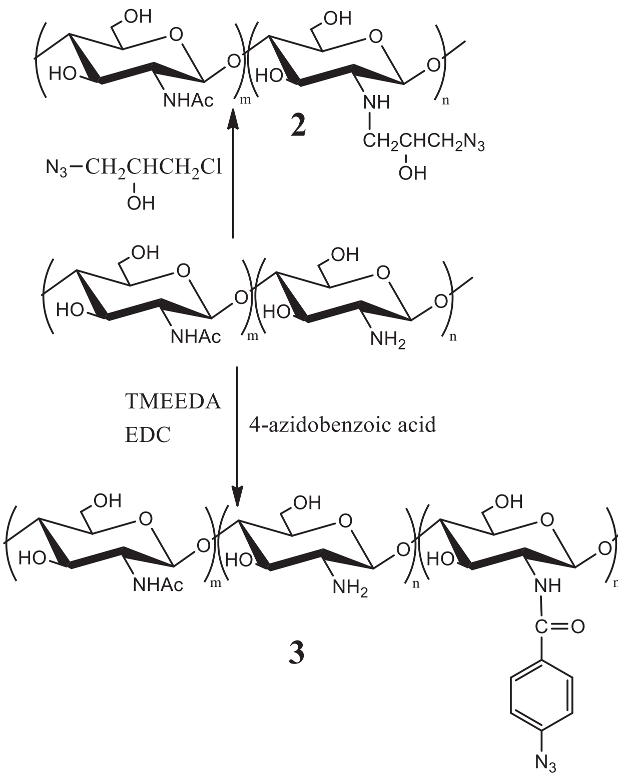

FIGURE 3 Synthesis of azido-functionalized chitosan.

of photosensitive CS is biocompatible and can be applied for the biofabrication of reproducible 3D scaffolds using rapid prototyping techniques. Hu et al.[36] prepared water-soluble CS derivative with nitrocinnamate as pendant groups (10 in Fig. 5) by the conjugation of carboxymethylated CS with *N*-hydroxyl succinimide-activated nitrocinnamate in the presence of *N*,*N*-dicyclohexylcarbodiimide. Such a modified polysaccharide could exhibit self-photogelation ability in the absence of potentially toxic photoinitiator or catalyst and be suitable for hydrogel biomaterials.

Of course, one strategy to obtain the water-soluble photoactive CS macromers is to use a water-soluble CS as a raw material. For example, Amsden et al.[37] adopted a water-soluble glycol CS to prepare a photopolymerizable CS prepolymer (11 in Fig. 6) through the grafting of methacrylation using GMA in aqueous media at pH 9. This *N*-methacrylated glycol CS was found to be noncytotoxic up to a concentration of 1 mg/mL, and could be crosslinked in solution using UV light and Irgacure 2959 photoinitiator under various conditions to yield gels with low sol content and high equilibrium water content.

Aforementioned water-soluble photoactive CS derivatives are successfully synthesized through various reactions. However, these synthesis protocols normally involve multistep chemical modification, which could lead to relatively low yields of final products. Besides, azo-derivatized CS hydrogels was found to have high storage moduli or pore sizes that are not ideal for neurite out growth.[38] Furthermore, conversion of azido groups to high reaction-active nitrene groups during UV photopolymerization may result in denaturation of protein drug capsulated in the hydrogels.[37] Meanwhile, the hydrogel derived of vinyl CS may be not formed well and mechanically weak,[34,39] sometimes need additional form time, which is possibly due to low-molecular-weight of CS precursor or insufficient crosslinking of hydrogel networks.

To solve these problems, a simple one-step modification of CS, water-soluble photocrosslinkable (methacryloyloxy) ethyl carboxyethyl CS (12 in Fig. 7), was prepared through Michael-addition reaction between CS and ethylene glycol acrylate methacrylate.[40] This kind of photoactive CS derivative could be water-soluble when the degree of substitution of hydrophobic groups (ester group) grafted onto CS molecular chains was regulated in the range of 0.10 to 0.35.[40] And this CS derivative was found to be noncytotoxic up to a concentration of 1.0 mg/mL. However, it is frustrating that this CS derivative could not be water-soluble any more when the molecular weight of CS exceeded 200 kD.

Another simple one-step modification of CS, that is, water-soluble photocrosslinkable CS derivatives including maleic chitosan (MCS, 14 in Fig. 7) and methacrylamide chitosan (13 in Fig. 7), were also proposed.[41,42] However, formic acid or acetic acid was used as a solvent in this reaction condition, which could result in degradation of CS molecular chain to make final hydrogels relatively weaker. Additionally, the trace toxic acid solvent in hydrogel products is harmful when it is applied to human tissue. To address this issue, we adopted high-molecular-weight CS as a raw material to prepare water-soluble MCS under mild and heterogeneous reaction conditions by using dimethyl sulfoxine as a solvent.[43] This kind of CS derivatives are compatible to mouse fibroblasts and could be potential as tissue engineering scaffolds.

PHOTOCROSSLINKING PROCESSES BASED ON PHOTOACTIVE CS MACROMERS

Aqueous solutions of photoactive CS macromers can be rapidly converted into hydrogels at a few seconds or minutes under photo-irradiation through inter/intra- molecular crosslinking in the presence/absence of a photoinitiator. The typical photopolymerization mechanisms of photoactive CS macromers are chain growth, chain-step growth, and cycloaddition.

Chain Growth Mechanism

For UV photopolymerization, a common photoinitiator is Darocur 2959, which is selected due to its solubility in water and a reduced cytotoxic effect for different cell types.[44] When Darocur 2959 is exposed to UV light, free radicals (benzoyl and ketyl) are generated and further attach the alkene groups of photoactive macromers to form intermolecular covalent bonds, leading to formation of hydrogel network[42] that is chain growth mechanism of photopolymerization. Based on this mechanism, many photocrosslinked CS hydrogel systems were prepared. Saraiva et al.[42] synthesized photocrosslinkable

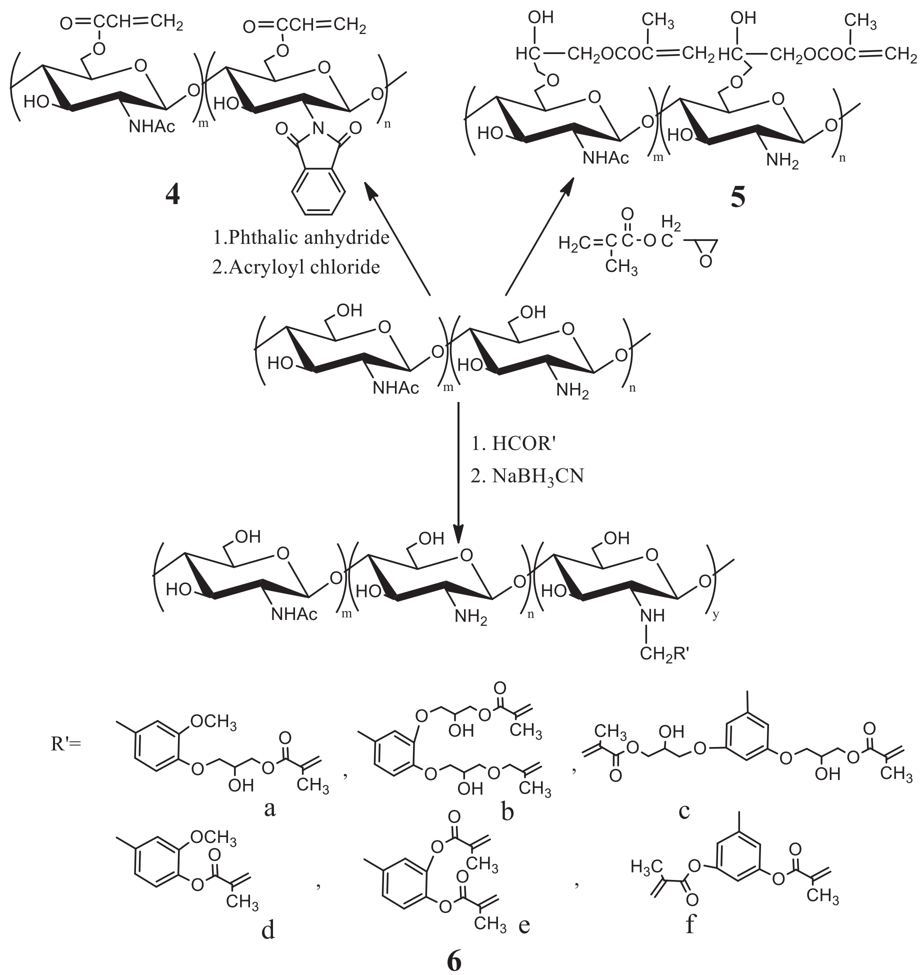

FIGURE 4 (Meth) acryloyl chitosan.

hydrogels composed by methacrylamide CS and methacrylamide gelatin, using Darocur 2959 as a photoinitiator. A series of photoactive CS based hydrogel systems were also prepared such as photopolymerized (methacryloyloxy) ethyl carboxyethyl CS hydrogel,[45] photopolymerized injectable maleilated CS/poly(ethylene glycol) diacrylate hydrogels,[38] and photopolymerized maleilated CS/methacrylated poly (vinyl alcohol) hydrogels.[43] All these hydrogel systems illustrated good cytocompatibility and could support the cell attachment and proliferation, indicating their potential as tissue engineering scaffolds.

Step Growth Mechanism

In step growth mechanism, first, photoactivated photoinitiator abstracts a proton from the thiol group to produce a thiyl radical. Second, thiyl radical reacts with an alkene group of photoactive macromers to form a carbon-centered radical, which reacts further to form hydrogel network. Thiol-ene photopolymerization is involved in step growth polymerization. Thiol-ene polymerization has many advantages over free-radical polymerization: less sensitive to oxygen inhibition and formation of more homogeneous networks due to crosslinks between complementary reactive groups.[46] Thiol-acrylate photopolymerization is another step growth polymerization. In thiol-acrylate systems, acrylic vinyl monomer can significantly react with thiyl radical to undergo step growth polymerization, and meanwhile it can be homopolymerizated under UV irradiation.[47] Based on this mechanism, methacrylamide CS/dithiothreitol hydrogel[48] and maleilated CS/thiol-terminated poly(vinyl alcohol) hydrogel[49] are formed via photopolymerization technique, respectively.

Cycloaddition Mechanism

Macromolecules with cinnamate group incorporated can always undergo cis–trans isomerization and photodimerization reactions,[50] in which cinnamate moieties can be covalently bonded to adjacent cinnamate moieties to form the cyclobutane ring (crosslinking points), resulting in formation of hydrogel network structure. Hu et al.[36] synthesized a carboxymethylated CS derivative containing nitrocinnamate moiety and subsequently used its aqueous solution to prepare the photocrosslinked hydrogel upon exposure to UV irradiation by the photocycloaddition reaction.

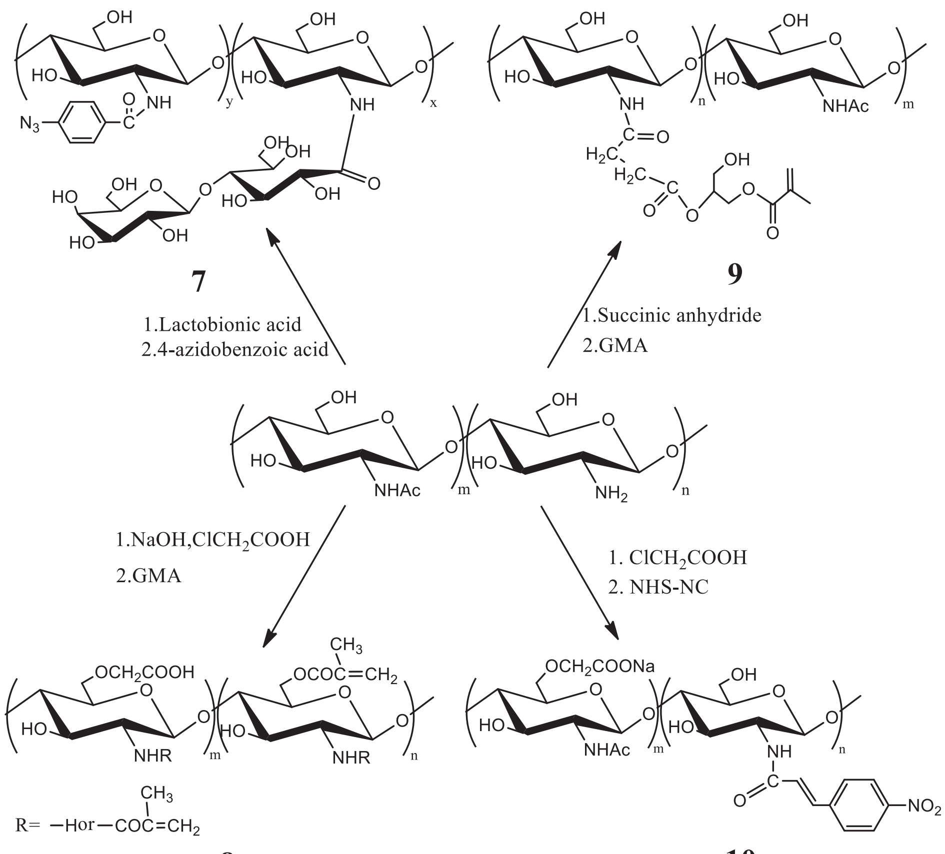

FIGURE 5 Synthesis of water-soluble photoactive chitosan (I).

BIOMEDICAL APPLICATIONS OF PHOTOCROSSLINKED CS-BASED HYDROGELS

Due to their excellent biological properties such as biocompatibility, biodegradability, and wound healing as well as promotion of tissue regeneration, photocrosslinked CS-based hydrogels have been extensively utilized for various biomedical applications such as wound dressings, drug delivery, and tissue engineering.

Tissue Engineering Scaffolds

Photocrosslinked CS-based hydrogels as tissue engineering scaffolds can provide appropriate physicochemical and mechanical properties to enhance cell adherence, proliferation, and differentiation, eventually aiming to the regeneration of a new tissue such as heart tissue, neural tissue, and cartilage tissue. Rask et al.[51] synthesized photocrosslinkable azidobenzoic acid modified CS conjugated with the angiopoietin-1-derived peptide, a peptide thought to mediate attachment and survival responses of cardiomyocytes, and they found that the hydrogel based on the CS derivatives could promote survival of neonatal rat heart cells. As neural tissue engineering scaffolds, photocrosslinkable hydrogels can potentially conformally fill irregular neural tissue defects and serve as stem cell delivery systems. Photocrosslinkable hydrogels based on methacrylated CS facilitated enhanced neurite differentiation and extension, demonstrating their potential for neural tissue engineering[52] (Fig. 8). Photocrosslinkable CS hydrogels were also used to encapsulate bone marrow stromal cells to improve osteogenic differentiation, and animal experiment findings showed that these hydrogels could promote osteogenesis for clinical bone repair.[53]

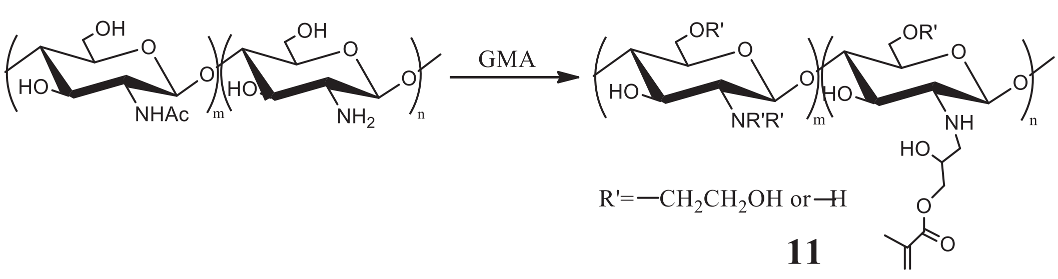

FIGURE 6 Synthesis of water-soluble photoactive chitosan (II).

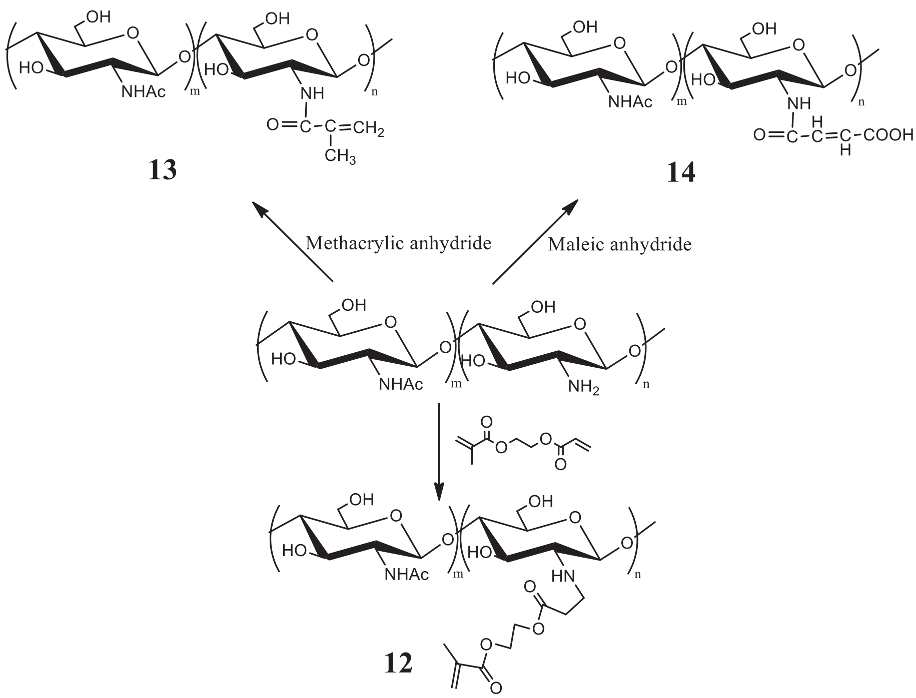

FIGURE 7 Synthesis of water-soluble photoactive chitosan (III).

Wound Dressings

Photocrosslinkable CS hydrogel has been found to induce wound contraction and healing,[31] indicating its potential as a wound dressing. Furthermore, photocrosslinkable CS hydrogel containing fibroblast growth factor-2 can stimulate wound healing in healing-impaired db/db mice, suggesting that it may be a promising wound dressing, especially in the treatment of healing-impaired wounds[54] (Fig. 9). Apart from acute wounds, photocrosslinkable CS based hydrogels are used for chronic wound.[21]

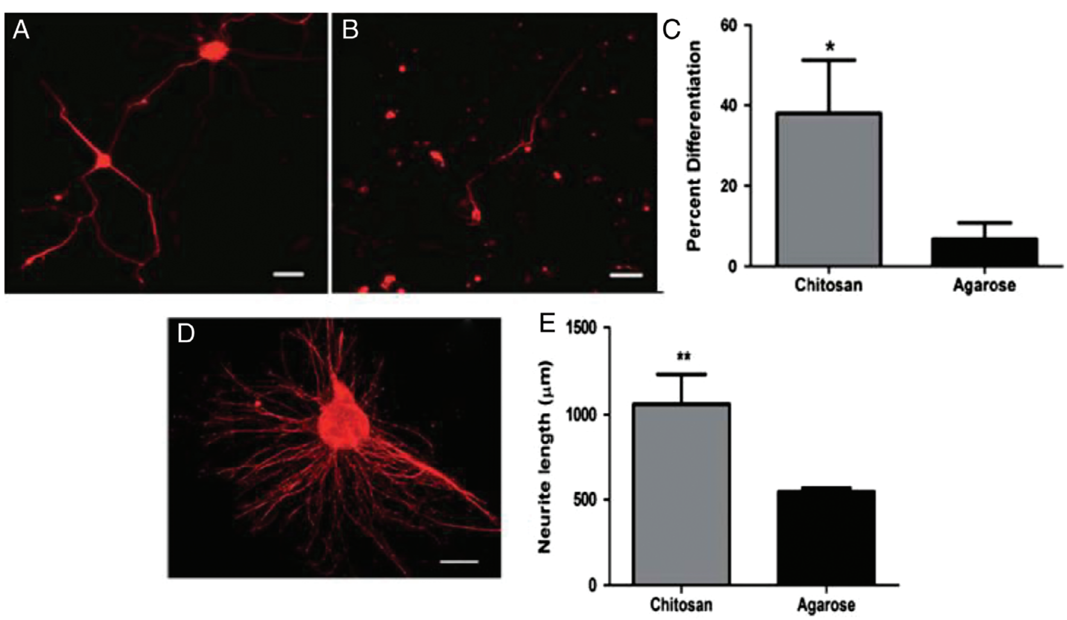

FIGURE 8 Cortical neuron differentiation and DRG neurite extension within hydrogels. (a) Cortical neuron showing extensive neurite outgrowth within chitosan hydrogels. (b) Neurite extension within agarose hydrogels. (c) Quantification of neurite outgrowth within hydrogels: significant higher percentage of neurite extension from chitosan hydrogels compared to agarose hydrogels. (d) Confocal micrograph of 3D neurite extension in chitosan hydrogels. (e) Quantification of neurite extension in hydrogels: significant higher neurite length within chitosan hydrogels as compared to agarose hydrogels.[52]

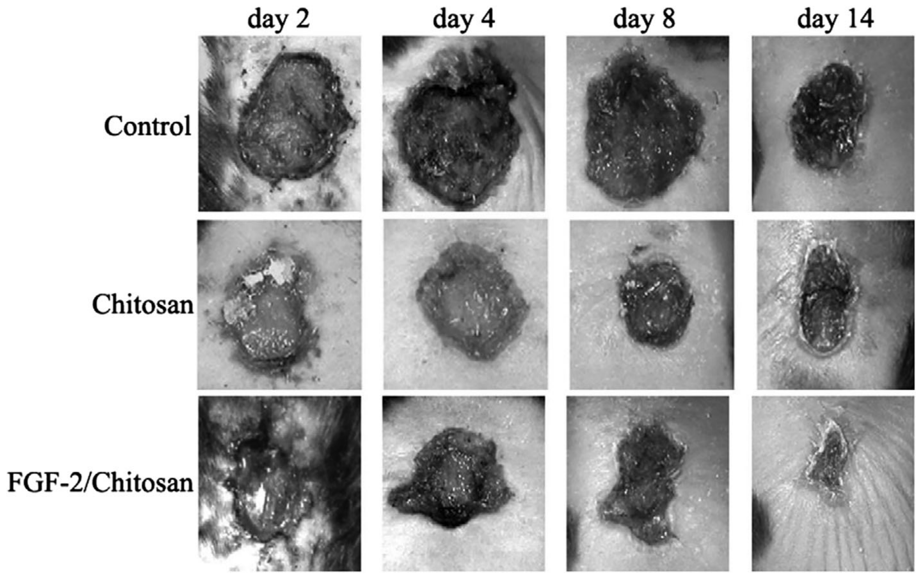

FIGURE 9 Wound closure of FGF-2-incorporated photocrosslinkable chitosan hydrogel-treated db/db mice. The photographic findings showed that wound healing in both photocrosslinkable chitosan hydrogel-treated and FGF-2-incorporated photocrosslinkable chitosan hydrogel-treated db/db mice occurred faster than the control wound.[54]

Drug Delivery Carriers

Hydrogels are usually expected to be drug carriers for the controlled release of drugs by maintaining an effective concentration in the target for longer periods of time. If the drugs are hydrophobic (e.g., paclitaxel) or easy to react with crosslinking agents (e.g., albumin drug reacted with aldehyde crosslinking), hydrogels matrix may be excellent carriers for these drugs. Obara et al.[55] used photocrosslinked CS hydrogels to encapsulate paclitaxel, and studied its effect on subcutaneous tumor growth in mice. The results indicated that paclitaxel-incorporated photocrosslinked CS hydrogel has an inhibitory activity on angiogenesis and tumor growth (Fig. 10). Theophylline was loaded in the photocrosslinkable CS hydrogel to sustain release of the drug for prolonged period.[24] Qiao et al.[22] evaluated a photocrosslinkable hydroxyethyl CS hydrogel as a potential drug release system for glaucoma surgery, and found that heparin loaded hydrogel could effectively maintain filtration bleb and lowing intraocular pressure after glaucoma filtration surgery for prolonged time. And this photocrosslinkable CS hydrogel was a potential drug delivery device for the ocular diseases.

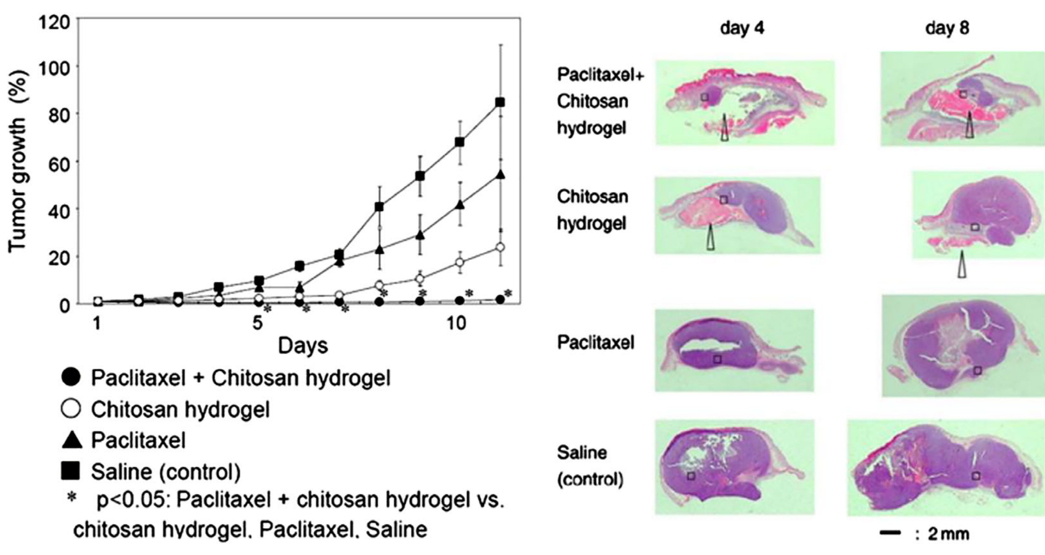

FIGURE 10 Inhibition effect of paclitaxel-incorporated photocrosslinkable chitosan hydrogel on 3LL-tumor growth and tumor vascularization (histological examination) *in vivo.* Significant necrotic tumor tissue was induced in the paclitaxel-incorporated photocrosslinkable chitosan treated tumor after 4 and 8 days (as showed in histological examination).[55]

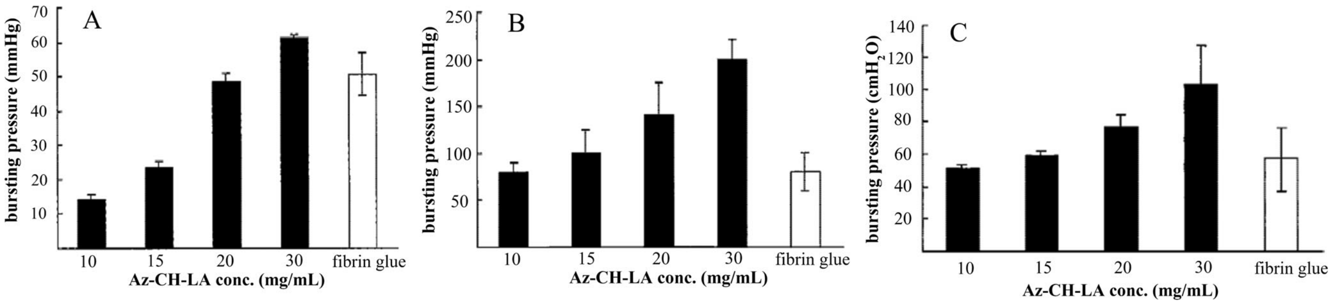

FIGURE 11 Air-sealing strength of photocrosslinkable chitosan hydrogels and fibrin glue at a pinhole on the isolated small intestine (a); at a pinhole on the isolated aorta (b) and at an incision on an isolated trachea (c).[33]

Biological Adhesives

Biological adhesives are adopted for tissue adhesion, hemostasis, and sealing air leakages and body fluids during surgical procedures. Due to its gel-forming property, photocrosslinkable CS is used as potential adhesive materials. Ono et al.[33] used a photocrosslinkable CS with azide and lactose moieties as a biological adhesive for soft tissues. The results showed that the photocrosslinkable CS hydrogel was more effective in sealing air leakages from pinholes on isolated small intestines and aorta and from incisions on isolated trachea compared to the fibrin glue (Fig. 11). Hybrid hydrogels based on photocrosslinkable CS and other polymers were also used as bioadhesives for soft tissues such as peropheral nerves because of their improved mechanical properties and tissue compatibility.[25,27] Attributed to its sealing effect, photocrosslinkable CS hydrogel was developed as hemostatic kit for severe hemorrhage.[56]

CONCLUSIONS AND OUTLOOKS

In this highlight, we focus on the synthesis of photoactive CSs, and the preparation and biomedical applications of photocrosslinkable CS hydrogels. Three main types of photoactive CS macromers including vinylated CS, azido-functionalized CS, and (methyl) acrylated CS were synthesized via chemical modification on the primary amine groups or hydroxyl groups of CS molecular chains. Considering hydrogels formation at physiological condition as the crucial requirement for biomedical applications, water-soluble photoactive CS with relatively high molecular weight is often a good choice. Photocrosslinkable CS hydrogels can be prepared under photo-irradiation via different photopolymerization mechanisms, i.e. chain growth, chain-step growth, and cycloaddition. This highlight also provides the most likely directions of photocrosslinkable CS hydrogels as wound dressings, drug delivery carriers, and tissue engineering scaffolds for tissues such as skin, bone, cartilage, nerve, and other applications.

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CONFLICT OF INTEREST

The authors report no conflict of interest in this work.

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