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Review

Chitosan-based hydrogels: From preparation to biomedical applications

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ABSTRACT

The advances in the field of biomaterials have led to several studies on alternative biocompatible devices and to their development focusing on their properties, benefits, limitations, and utilization of alternative resources. Due to their advantages like biocompatibility, biodegradability, and low cost, polysaccharides have been widely used in the development of hydrogels. Among the polysaccharides studied on hydrogels preparation, chitosan (pure or combined with natural/synthetic polymers) have been widely investigated for use in biomedical field. In view of potential applications of chitosan-based hydrogels, this review focuses on the most recent progress made with respect to preparation, properties, and their salient accomplishments for drug delivery and tissue engineering.

1. Introduction

In the past few years, the advances in the field of biomaterials has led to several studies on alternative biocompatible materials and to the development of these materials focusing on properties, benefits, limitations, and the use of alternative resources (such as polysaccharides and proteins) for its preparations. Among the most studied biomaterials, hydrogels (HGs) have been standing out owing to their advantages, like biocompatibility, biodegradability, mechanical properties, and responsiveness.

HGs are soft materials composed of three-dimensional networks of hydrophilic polymers, which are able to swell either in water or in biological fluids (Grainger, 2013; Kakkar & Madhan, 2016; Lima-Tenório, Pineda, Ahmad, Fessi, & Elaissari, 2015; Soares et al., 2014; Ullah, Othman, Javed, Ahmad, & Akil, 2015). Depending on the preparation method, hydrogels can be classified into 'physical' gels or 'chemical' gels. In physical ones the polymeric chains are held together by molecular entanglements and/or secondary interactions including ionic crosslinks, hydrogen bonds and hydrophobic interactions. In contrast, in chemical gels the polymeric chains are hold together by irreversible covalent bonds.

The swelling ability of hydrogels in biological conditions, allows the diffusion of nutrients, making them very similar to natural tissues, allowing its biomedical applications. If the HGs have stimuli-responsive properties, they are also called as smart material, and the release of the drug may be controlled by an external stimulus, such as pH, light, magnetic field, temperature, and so forth. In addition, those materials have a wide range of applications, including controlled drug release, contact lenses, scaffolds, cell growth, agriculture, and regenerative medicine.

The interest in the development of polysaccharide-based hydrogels, as smart biomaterials, has strongly grown in the last decade. The polysaccharides possess intriguing properties for development of biomaterials, such as biocompatibility, biodegradability, non-toxicity, and low-cost (Lima-Tenório and Tenório-Neto et al., 2015). Starch, carboxymethylcellulose, alginate, carrageenan, and chitosan are some examples of polysaccharides commonly utilized to prepare hydrogels for biomaterial-related applications (Lima-Tenório and Tenório-Neto et al., 2015). Among them, chitosan, which is a natural cationic and hydrophilic polymer, has been the object of several studies by researchers in the areas of biotechnology (Arteche Pujana, Pérez-Álvarez, Cesteros Iturbe, & Katime, 2013; Soares et al., 2014; Xu et al., 2012).

Chitosan, (1–4)-2-amino-2-deoxy-β-D-glucan (Arteche Pujana et al., 2013), is a polysaccharide obtained from alkaline hydrolysis of chitin, one of the most abundant natural amino polysaccharide extracted from the exoskeleton of crustaceans and insect, from fungal cell walls, etc. (Soares et al., 2014; Xu et al., 2012). There are plentiful of amine groups (-NH_2) and hydroxyl groups (-OH) along the chitosan chain, which can be used as cross-linkable functional groups to react with cross-linking agents for in-situ chemical cross-linking (Arteche Pujana et al., 2013; Xiao, You, Fan, & Zhang, 2016). Moreover, the amine groups can be easily converted to ammonium groups, below pH 6.3,

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making chitosan an ideal candidate for use in the preparation of pH-responsive HGs. Besides non-toxicity and biocompatibility, chitosan can be degraded in vivo by several enzymes, mainly by lysozyme (a non-specific protease present in all mammalian tissues) (Ren, Yi, Wang, & Ma, 2005; Szymańska & Winnicka, 2015). Furthermore, the products from degradation are non-toxic oligosaccharides which can be then excreted or incorporated to glycosoaminoglycans and glycoproteins. These properties make chitosan suitable for clinical use. In addition, chitosan may enhance drug penetration by opening the tight junctions between epithelial cells (Jin, Zhang, Li, Liang, & Jia, 2016; Mohammed, Syeda, Wasan, & Wasan, 2017). These properties make chitosan an ideal candidate for use in the preparation of new materials for biomedical applications.

Present review aims to report and to update the state of the art regarding chitosan-based hydrogels in biomedical field. In this direction, special attention is dedicated to their preparation, properties, and application in both drug delivery and tissue engineering.

2. Formation and properties of chitosan-based hydrogels

Chitosan-based HGs can be prepared either directly from native chitosan (combined by itself or with anionic small molecules) or combined with other polymers. It is well reported that chitosan is soluble in acid medium owing to the presence of amine groups (N-free) from D-glucosamine units. At this pH, N-free units are positively charged limiting the interchain interactions (Sereni et al., 2017). Moreover, the ability to change the apparent charge density under certain experimental conditions (e.g. pH, Ionic strength, temperature) has been exploited to produce hydrogels.

Chitosan (CS) can be self-crosslinked either by increasing the pH or by dissolving in a nonsolvent (Peppas, Hilt, Khademhosseini, & Langer, 2006). This kind of material has biocompatibility (intrinsic of chitosan) and can be considered safe for clinical applications, since, no organic solvent or toxic crosslinker is needed (Kiene, Porta, Topacogullari, Detampel, & Huwyler, 2018). For example, as demonstrated by Montenbault et al. physically-crosslinked chitosan hydrogels were obtained after solvent evaporation of a mixture water/1,2-propanediol (Montembault, Viton, & Domard, 2005). More recently, Xu, Y. and co-authors prepared chitosan hydrogels by adjusting the pH and by freezing the solution. Their hydrogels showed a high cytocompatibility for L929 fibroblasts cells and pH-responsive properties (Xu, Han, & Lin, 2017).

Despite the aforementioned advantages, CS-based hydrogels crosslinked by itself possess a dense scale-mesh like network which only allow for passive diffusion nutrients and metabolic wastes being not suitable for biomedical applications (Chen et al., 2017). Furthermore, it has a weak mechanical properties and uncontrolled dissolution (Kiene et al., 2018). On the other hand, this drawback can be avoided by combining chitosan with either natural or synthetic polymers for tuning the HG properties.

Several approaches have been reported to combine chitosan with synthetic/natural polymers. Each one is chosen in order to obtain desired properties. In general, they include: i) chemical reaction of chitosan with a crosslinker, ii) chemical modification of CS chains to obtain a macromonomer (crosslinker agent), iii) hydrophobic association, iv) electrostatic interactions, and v) hydrogen bonding (Sacco et al., 2014, 2016; Yoo, Seong, & Park, 2016). Furthermore, the amine groups can interact between the polymer chains (by hydrogen bonding) to produce chitosan hydrogels (Baghaie, Khorasani, Zarrabi, & Moshtaghian, 2017; Mahdavinia, Soleymani, Etemadi, Sabzi, & Atlasi, 2018). The advantage of combining CS with other polymers is to obtain a hybrid material with new properties. For example, due to their non-covalent nature of the chitosan-based HGs physically crosslinked, these systems are inherently responsive to external stimulus, such as, pH and temperature. Furthermore, the gel formation may be obtained in mild conditions without a crosslinker allowing, for example, the entrapment of proteins during the synthesis (Yuan et al., 2018). Moreover, the physical interactions are reversible providing to the HGs self-healing properties. Thus, in the last years, several combinations of physically crosslinked chitosan-based hydrogels have been investigated, especially, in biomedical field.

On the other hand, the HG formed by chemical crosslinking are stable with time, preserving the gel properties (Lima-Tenório and Tenório-Neto et al., 2015). In this sense, Tsuda and coworkers reported the synthesis of flexible HGs based on modified chitosan using the UV-light approach. This material decreased bone formation ratio in mice skulls and fibula defects (Tsuda et al., 2009). Mirzaei and coworkers studied the effect of different amounts of glutaraldehyde as crosslinker agent on the preparing chitosan hydrogels. They analyzed the interactions polymer-polymer and polymer-drugs and observed that an increase in the crosslinking agent concentration resulted in a considerable decrease of swelling. Furthermore, the crosslinker concentration have changed the enzymatic activity under gastric conditions (Mirzaei, Ramazani, Shafiee, & Danaei, 2013).

More recently, Zhang and co-authors reported a series of chitosan-based self-healing hydrogels, using a benzaldehyde terminated poly (ethylene glycol), to crosslink chitosan or chitosan derivatives by Schiff base (Li, Wang, Wei, & Tao, 2017; Zhang, Tao, Li, & Wei, 2011). Focusing on the tissue engineering, the advantage of using Schiff-base system is complete avoidance of extraneous toxic crosslinking agents and other triggers that can cause an unwanted tissue response besides being easy prepare due to their simple methodology. However, the Schiff-base (imine) linkages may be hydrolyzed under acidic conditions which is not suitable for oral drug delivery (Li et al., 2017).

Hydrogels formed by two (or more) different monomeric units, with at least one of them hydrophilic have also been reported (Ullah et al., 2015). The polymeric network may be arranged in blocks, alternating or random configuration. Moreover, by adjusting the monomer composition, the properties of copolymers can be tuned, and thus, copolymers present advantages not usually seen in homopolymers. For example, Yang and co-workers have reported the synthesis of a pH-responsive HG film based on a chitosan/poly (acrylic acid) (CS/PAAc) copolymer. The hydrogel swollen in both acidic and basic conditions depending on the composition (Yang et al., 2005). Another example can be found in a work reported by Cao and co-workers. They reported a double-network HG based on oligo(trimethylene carbonate) (TPT)-b-poly(ethylene glycol)-b-oligo(trimethylene carbonate) diacrylate and methacrylate chitosan (CS-MA) where the concentration of CS-MA influenced the swelling behavior and the mechanical properties of the HG (Cao, Yang, Fan, Liu, & Liao, 2015).

Inorganic particles have also been used to confer dual-responsiveness properties to CS-hydrogels. Magnetic- and pH-responsive beads based on chitosan and laponite were reported by Mahdavinia et al. (2018). The beads have shown to be pH-sensitive owing to the $-NH_2$ groups from CS. They investigated adsorption capacity of BSA as function of pH and Ionic strength. The maximum adsorption capacity of hydrogels beads was obtained between isoelectric point of BSA and points zero charge of hydrogel beads. Other examples of chitosan combined to other compounds to form hydrogels with specific properties are summarized in Table 1.

3. Preparation of chitosan-based hydrogels

It is well known that many properties, such as, self-healing, biodegradability, swelling degree, mechanical resistance, and so forth of hydrogels are intrinsically related to the crosslinking methods. Thus, for biomedical applications, the choice of the preparation methods of CS-HG has an important role. For example, to produce biodegradable HGs, it is highly recommended the introduction of labile/unstable bonds which may be cleaved in physiological conditions (Gulrez, Al-Assaf, & Phillips, 2011).

Several crosslinking methods have been developed to form the

Table 1
Summary of chitosan-based composite hydrogels and their properties.

Type of hydrogel	Polymer	Properties	Reference
Chitosan	Chitosan	pH-responsive; highly biocompatible;	Montembault et al. (2005) and Xu et al. (2017)
Chitosan + crosslinker agent	Chitosan + BTDA	The water absorbency of hydrogel increased with decreasing pH (between pH 4.0–8.0). Applied for dye-removal (98% at pH 8.0).	Karimi et al. (2018)
	Maleimide-modified chitosan	Self-crosslink; The gelation kinetics controlled by both curing temperature and pH; the pore size could be controlled by the concentration of chitosan and the freezing temperature.	Chen et al. (2017)
	Carboxymethyl-chistosan + 4,4′-(oxybis (methylene)) bis (2-(2-(2-(oxiran-2- ylmethoxy) ethoxy)- 1,3-dioxolane)	Mild conditions to obtain the HG; properties were tuned by adjust the monomer content. Excellent compatibility and degradabity.	Hu et al. (2017)
Chitosan + natural polymers	Chitosan + Hyaluronic acid	Temperature-responsive (gel at 37 °C) due electrostatic interactions between Chi and HA. Drug release mechanisms controlled by diffusion	Zhang and Jin et al. (2018)
	Chitosan + Carrageenan	Rubbery hydrogels; pH- and salt-sensitive; excellent mechanical properties (compressive strain near 60%)	Liang et al. (2018)
	Chitosan + gelatin	Enzymatically crosslinked; a high average pore size (above 100 μm); high swelling ratio.	Choi et al. (2018)
Modified-chitosan + natural polymers	Carboxymethyl-chitosan + aldehyde-xanthan	Self-healing, cytocompatibility, self-crosslinking, anti-enzymatic hydrolysis	Huang et al. (2018)
	Collagen peptide-chitosan + oxidized konjac glucomannan	Blood compatibility, good coagulation performance, and good cell compatibility. The hydrogel dressings have the capacity to absorb and retain the wound fluid.	Liu and Wen et al. (2018)
Chitosan + synthetic polymers	Chitosan + p(MAA-co-NIPAM)	dual-responsive swelling (pH and temperature).	Rasib et al. (2018)
	Chitosan +3-Sulfopropyl methacrylate potassium salt (N, N1-methylenebisacrylamide as crosslinker)	Superabsorbent; pH-responsive; used to release BSA.	Salama (2018)
Modified-chitosan + synthetic polymers	Chitosan-maleic anhydride + thiol-terminated poly (vinyl alcohol) (TPVA)	Rheological properties tailored by TPVA, rapid gelation behavior (under UV), cytocompatibility.	Zhou et al. (2011)
	GMA-Chitosan + PEGDA	Improved mechanical properties obtained by encapsulation of bone ash; pH-responsive; higher swelling ratio at pH 1.2; cytocompatibility	Aycan and Alemdar (2017)
	Chitosan-g-PNIPAm + methacrylic anhydride	NIR- and temperature-responsive; High swelling ratio;	Wang and Li et al. (2017)
Chitosan + others	Chitosan + PVA + magnetic laponite	Magnetic-responsive beads, high adsorption capacity of BSA between pI of BSA and of hydrogel beads.	Mahdavinia et al. (2018)
	HPP-modified glycol chitosan + $Ru^{2+}(bpy)_3Cl_2$	Injectable hydrogel; photocrosslinkable; The hydrogel possesses good tissue adhesiveness and hemostatic ability.	Lu et al. (2018)
	Chitosan + halloysite nanotubes	Improvement on the HG strength after heating treatment; The swelling capacity and pore size decreased after halloysite nanotubes. Biocompatible.	Huang et al. (2017)

hydrogel matrix structures. They can be divided in two major groups: i) physically and ii) chemically crosslinked (Hamidi, Azadi, & Rafiei, 2008).

3.1. Physical crosslinking

As mentioned earlier, the main advantage of this approach consists on not using the crosslinking agents which, sometimes, are toxic decreasing the biocompatibility. In addition, the hydrogels physically crosslinked are self-healing. They can be obtained either by association with small anionic molecules, by polyanions, by hydrogen bonding, or by hydrophobic associations (Fig. 1).

One method of producing CS-HG without chemical crosslinkers is the so-called freeze-thawing. This method consists on mixing the polymers in an aqueous solution followed by freezing at low temperatures and thawing at room temperature for continuous cycles promoting the physical interactions between the polymer chains. Using freeze-thawing approach the chitosan can be combined with several polymers which can form hydrogen bonding (i.e. starch, alginate, PVA, and so on) (Baghaie et al., 2017).

For example, Iwatsubo, Kishi, Miura, Ohzono, and Yamaguchi (2015) synthesized artificial bones using poly(vinyl alcohol) (PVA) and poly(acrylic acid) (PAA) by repeated freezing and thawing (PVA/PAA-ft-network) and a chitosan/PAA network, sustained by hydrogen bonds between the two polymers. The chitosan/PAA network mimicked the formation of bone matrices, and the PVA/PAA-h-network showed mechanical properties similar to those of fish scales. In the meantime, Ding et al. (2016) prepared several physical HGs using chitosan with various degrees of deacetylation (DDs). The final material showed excellent mechanical properties and cell compatibility, and in an in vitro study, the mouse bone mesenchymal stem cell (mBMSC) culture revealed good adhesion and proliferation, besides induction of the differentiation of mBMSCs into epidermal cells.

Chitosan can be self-crosslinked observing when the initial polymer concentration is over a critical concentration (C*) of chain entanglement and when the balance between hydrophilic and hydrophobic interactions is reached. These values may be achieved after decreasing the apparent charge density, by solvent evaporation, or changing the dielectric constant of the medium (Racine, Texier, & Auzély-Velty, 2018). Using this concept, Boucard et al. (2007) developed a physical HG based on chitosan and cross-linked it by hydrogen bonding to be used in tissue engineering for recovery of damaged tissues. They synthesized a two-layer HG: the first layer to ensure good mechanical properties, made of a rigid protective gel, and the second layer is soft and flexible and allows the material to assume the wound geometry and has good mechanical properties besides gas exchange. The final material did not show considerable toxicity and was effective at tissue regeneration. A schema showing the approach for obtaining the bi-layer chitosan hydrogels as well as the light microscopy the gel layers is shown in Fig. 2.

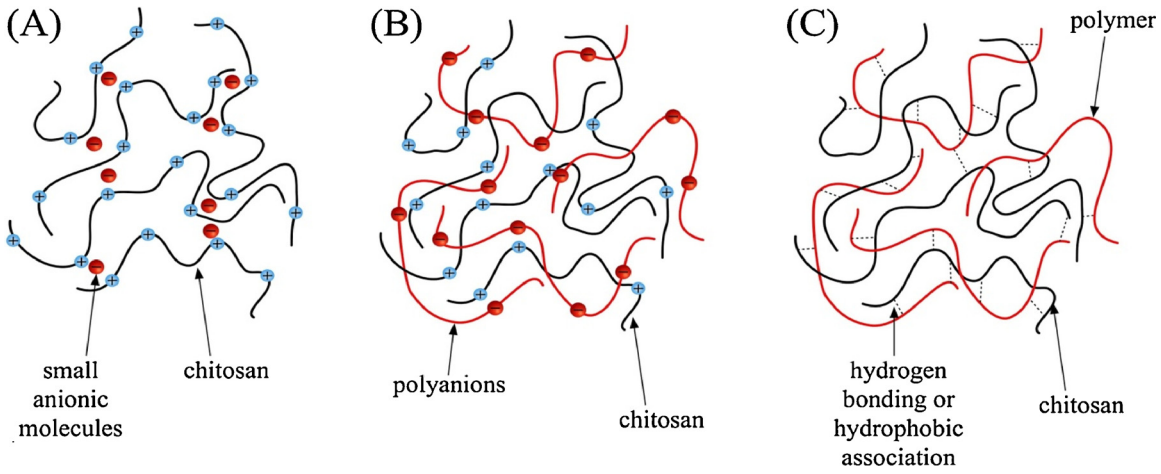

Fig. 1. Schematic representation of physical crosslinking.

Another technique used to produce physical HGs based on chitosan are the association by electrostatic interactions. In such case, the crosslinking can be achieved by small anionic molecules, polyelectrolytes, and metallic anions. Phosphate-bearing molecules, polyacrylic acid, sodium alginate, heparin, and polyglutamic acid are the commonly anions for crosslinking chitosan (Mohamed, Elella, & Sabaa, 2017). For example, Xiao et al. (2016) promoted the physical reticulation from water-soluble chitosan with sodium alginate and soaked the gel in ferrous chloride to form a magnetic HG, with a basic oxidation process. The obtained HG was effective in adsorption of compounds with different charges, in addition to its good thermal stability. Zhang and co-workers have reported a composite hydrogel of chitosan, heparin and poly (gamma-glutamic acid) for wound healing. To produce the hydrogels they obtained a homogeneous solution with chitosan, poly(gamma-glutamic acid) and heparin, then the solution was crosslinked by adding acetic acid (Zhang and Ma et al., 2018).

As demonstrated by Mengatto and colleagues, β-Glycerophosphate disodium salt (GP) can be employed to easily prepare the temperature-responsive chitosan hydrogels (Mengatto, Pesoa, Velázquez, & Luna, 2016). It was supposed the hydrogels could be formed by combination of both electrostatic interactions and hydrophobic associations. In such work, the best conditions for gelation were investigated by a factorial design. They found that concentration of chitosan, GP and temperature were significant factors.

The electrostatic interactions can be also combined to other physical associations to obtain new hydrogel properties. For example, Abioye, Issah, and Kola-Mustapha (2015) used electrostatic interactions as well as hydrogen bonding and hydrophobic interactions to develop a novel ternary chitosan-ibuprofen-gellan nanogel for a drug delivery system for ibuprofen. Chitosan improved the skin penetration, permeability, and the release of ibuprofen owing to extension of ibuprofen–chitosan ionic interactions and concentration.

Although the preparation of HG, by electrostatic route, is relatively easy and do not require the use of crosslinking agents, the challenges of this approach is to control the ions diffusion for obtaining a homogeneous material. To overcome this drawback thermosensitive hydrogels, which are able to perform simple sol-gel transition as function of temperature, have been attracting much attention in biomedical and pharmaceutical fields. It has been reported elsewhere chitosan displays thermo-sensitive behavior activity when combined with β-glycerophosphate (Cui and Liang et al., 2014; Liu et al., 2014; Wang, Chen, Li, Huang, & Liang, 2008).

In addition, chemical modification on the CS structure may induce gelation as function of temperature. For example, chitosan has been modified with 1,2-butene oxide and succinic anhydride (NSHBC). This compound was able to form gel as function of temperature ranging from 17 °C to 32 °C depending on the degree of chemical modification in chitosan (Bai et al., 2018). Using hexanoic anhydride, Z. Li and colleagues reported the chemical modification of glycol chitosan obtaining N-hexanoyl glycol chitosan which are able to gelling at 37 °C (Fig. 3) (Li et al., 2018).

Supramolecular structures are able to accommodate organic/inorganic guest molecules. Such molecules can be of low molecular weight as well as polymers. K. M. Huh and co-workers demonstrated that the poly (ethylene glycol) can be complexed, by inclusion, with a-CD forming a supramolecular assembly. Using such knowledge they modified chitosan with mono-carboxylated PEG which was combined with α-cyclodextrin (a-CD) to produce thermo-responsive supramolecular hydrogels (Huh et al., 2004). In such work, the PEG side-chains are able to form polymer inclusion complexes with a-CD molecules,

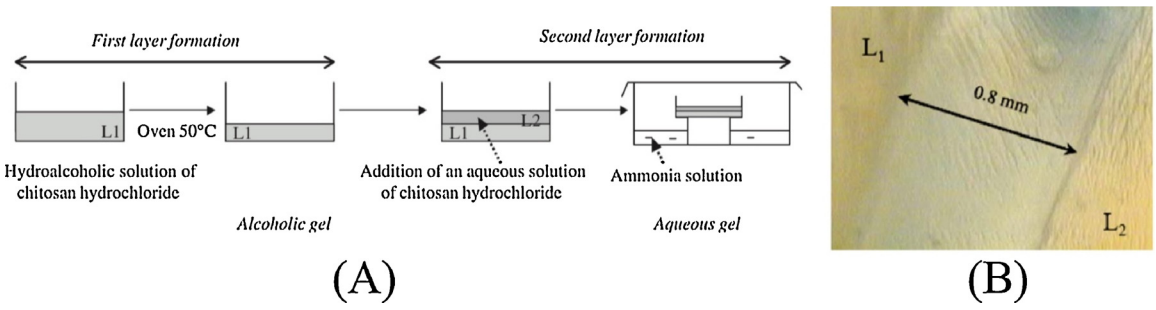

Fig. 2. (A) Experimental set-up processed for the formation of a bi-layer physical hydrogel of chitosan. The first layer (L1) was obtained by evaporation of a hydroalcoholic solution of chitosan. The second (L2) was elaborated by putting in contact ammonia gas with a chitosan solution. This last step also ensured the cohesion between L1 and L2. (B) Light microscopy of the bi-layer physical hydrogel of chitosan. The glue joint is clearly identified between L1 and L2. Reprinted and edited with permissions from reference (Boucard et al., 2007) © Elsevier.

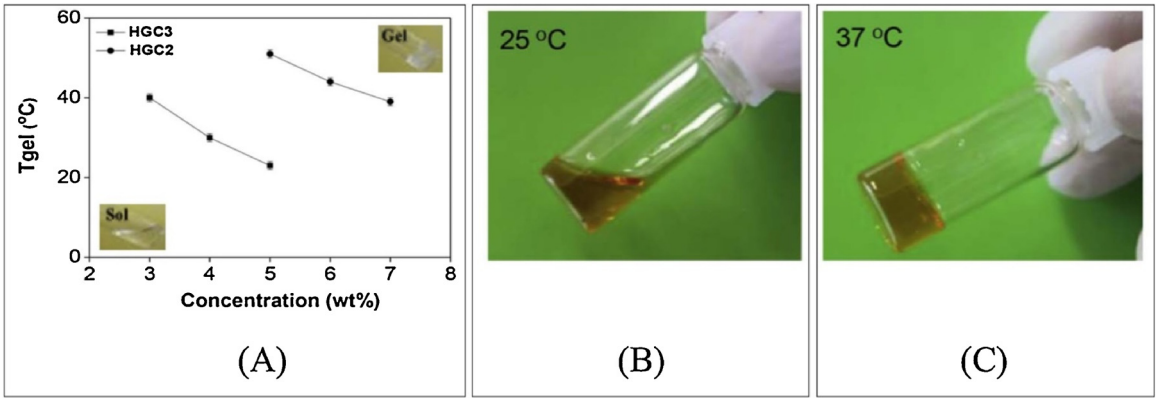

Fig. 3. (A) Thermo-sensitive sol-gel transition diagram of HGC2 and HGC3 solutions for different concentrations, (B) Photographs of in situ gel formation of 4 wt% HGC3 solution in PBS with temperature increased from 25 °C to 37 °C. Reprinted and edited with permissions from reference (Li et al., 2018) © Elsevier.

creating hydrophobic micro-domains with channel-type crystalline structure. The physical properties of the HG could be controlled by several factors, such as, temperature, pH, the PEG content, and the mixing ratio of host and guest molecules.

Chitosan with pending hydrophobic side-groups (e.g. hydrophobized polysaccharides, palmitoyl, alkyl chains or even cholesterol) have also been used to form physical hydrogels by hydrophobic interactions (De Jong, Van Eerdenbrugh, Nostrum van, Kettenes-Van Den Bosch, & Hennink, 2001). However, such structures are sometimes fragile or show lower water absorption. To overcome these drawbacks, Noble et al. prepared a non-covalently cross-linked chitosan hydrogel from a amphiphilic palmitoyl glycol chitosan (Noble, Gray, Sadiq, & Uchegbu, 1999). The hydrogel was obtained by freeze-drying which gave a white spongy-like material. They observed that the HG swollen up to 20 times (in relation of the initial weight of dry gel) at pH 9.0.

3.2. Chemical crosslinking

To provide a good mechanical strength and preserving the HG properties over the time, the chemical crosslinking methods are used. In addition, unlike the physical crosslinking, the chemically one allows the formation of hydrogels with uniform properties. However, for biomedical applications, enormous attention must be paid on the cleavaging of the cross linker (which can be done either by chemical or by enzymatic ways) in order to avoid the release of toxic compounds.

In general, the chemical crosslinking methods which are used can be divided in free-radical polymerization, condensation reactions, and addition reactions (Fig. 4).

The factors which affect such approach are the concentration of crosslinking agent and the crosslinking time (Wong, Ashton, & Dodou, 2015). For radical polymerization, chitosan may be modified to have polymerizable groups. This approach may be induced by UV, visible light, or increasing the temperature. The photopolymerization is started by free radicals produced by photoinitiators upon UV or visible light irradiation, promoting attacks on double bonds of monomers and propagating the radical attack, creating a cross-linked polymer network (Hu et al., 2012). These kinds of HG are being applied to tissue engineering and drug delivery systems (Zhou, Ma, Shi, Yang, & Nie, 2011), because the aqueous macromer solution allows for noninvasive delivery and a fast polymerization considering the radical initialization and the physiological conditions in situ (Ma et al., 2010). For example, photosensitive groups can be attached to C-2 amino of hydroxyethyl chitosan (HECTS) to obtain a photocrosslinkable monomer as have been demonstrated by Qiao et al. (2017). The HG shown great biocompatibility and biodegradability in vivo and intraocular being applied as a sustained drug release system to improve success ratio of glaucoma surgery.

Zhou et al. (2011) synthesized, by the Michael reaction, the (methacryloyloxy)ethyl carboxyethyl chitosan (MAOECECS), a photo-crosslinkable precursor, and blended it with a photoinitiator in an aqueous environment, with UV irradiation, thereby creating the HGs for subsequent characterization. The material was slowly biodegraded in the

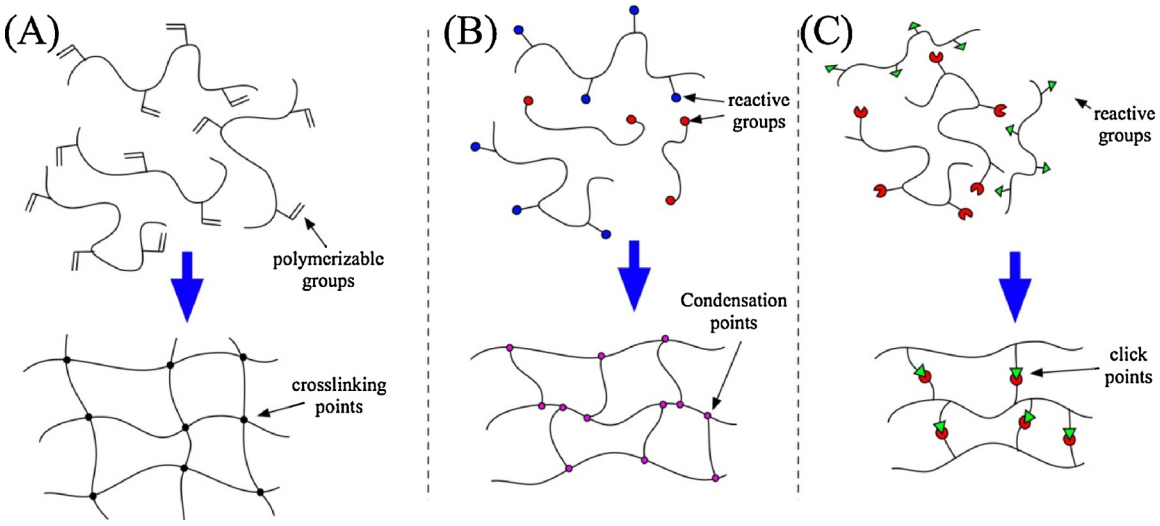

Fig. 4. Schematic representation of chemical crosslinking: (A) free-radical, (B) condensation, and (C) addition reactions.

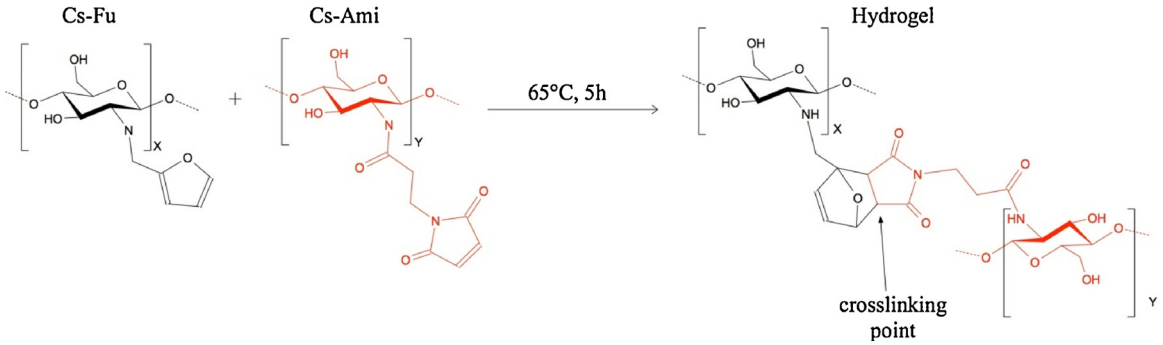

Fig. 5. Diels-Alder reaction between Cs-Fu and Cs-Ami to produce Chitosan-hydrogels (Guaresti et al., 2017).

presence of lysozyme, with the decrease of the cross-linking density and was noncytotoxic. In contrast, Qi, Xu, Wang, Nie, and Ma (2013) also developed a biocompatible and nontoxic HG, based on chitosan, photo-cross-linkable because of methyl acryloyl glycine (MAG), CS-MAG. The resultant material showed lower crystallization degree than chitosan did and decreased thermal stability. On the other hand, the HGs had favorable water absorption and a porous network influenced by the initiator concentration: higher concentrations led to a homogeneous pore size distribution.

Hu et al. (2012), using methacrylated glycol chitosan (MeGC) and three blue light initiators – camphorquinone (CQ), fluorescein (FR), and riboflavin (RF) – evaluated the influence of the initiator on the final injectable HG. They were able to conclude that HGs initiated with RF were more favorable in terms of cell viability and mechanical properties. They also evaluated the influence of time of irradiation, concluding that longer irradiation leads to HGs with greater mechanical strength, but the cytocompatibility decreased. The stability and degradation were dependent on its mechanical properties. Ma et al. (2010) developed an injectable composite HG based on methacryloyloxy ethyl carboxyethyl chitosan (EGAMA-CS), polyethylene glycol dimethacrylate (PEGDA), and N,N-dimethylacrylamide (DMMA) by photopolymerization. The materials showed excellent mechanical behavior, good thermal stability, and no considerable cytotoxicity and were able to promote cell adhesion and proliferation. Both materials have potential applications to tissue engineering.

The creation of covalently crosslinked HGs may also be mediated by reaction between chitosan and a compound with two or more functional groups, for example, epichlorohydrin (Xiao et al., 2016) or glutaraldehyde (Arteche Pujana et al., 2013; Xiao et al., 2016). However, this kind of reagent intended for cross-linking the chains is considered toxic (Arteche Pujana et al., 2013; Xiao et al., 2016), and it is necessary, for biological applications, to instead use biocompatible agents like genipin (Arteche Pujana et al., 2013). Dimida et al. (2015) synthesized a chitosan HG using genipin as a cross-linking agent, aiming to study the kinetics of the reaction. The HG was physicochemically characterized and analyzed according to detailed images. Study of the kinetic reaction of chitosan and genipin at different thermal conditions and with different crosslinker concentrations in the absence of ethanol or dimethyl sulfoxide for genipin dissolution allowed them to conclude that genipin crosslinking produces stabilization of the chains of chitosan due to its concentration. On the other hand, Maggi, Ciccarelli, Diociaiuti, Casciardi, and Masci (2011) used genipin in polyion complex micelle (PIC) nanoreactors, thus preparing a biocompatible chitosan nanogel without organic solvents. The amount of genipin used in the synthesis was an important parameter for the size control of the nanogels in solution.

Yao, Liao, Chung, Sung, and Chang (2012) combined genipin, a natural reagent, and citrate, a chemical reagent, in the cross-linking process to develop a scaffold for tissue engineering and drug delivery systems. The combination of those two cross-linking agents was necessary due poor mechanical properties of chitosan scaffolds, leading to a material with considerably higher Young's modulus, nanohardness, in addition to the attachment of fibroblast cells to the chitosan films, suggesting that the material holds promise as a scaffold.

Chitosan have amino groups (-NH_2) which may be used to form dynamic covalent bonds with aldehydes. These reactions, so-called Schiff base linkages, are reversible which endows the hydrogel self-healing properties. F. Ding and colleagues developed an acrylamide-modified chitosan and oxidized alginate-based hydrogels with recoverable self-healing and mechanical properties. In such work, both the self-healing ability and the mechanical properties were recovered by adjusting the pH (Ding et al., 2017). Following the same, Li et al. (2014) developed an injectable HG with biodegradability properties, and nontoxic for application to postsurgical peritoneal adhesions using N,O-carboxymethyl chitosan (NOCC) and aldehyde hyaluronic acid (A-HA), by inducing the cross-linking via the Schiff base reaction between the amino groups of NOCC and aldehyde groups of A-HA, leading to a flexible HG. More recently, Guaresti et al. reported a covalently cross–linked chitosan hydrogel. They synthetized firstly furfural-functionalized chitosan (Cs-Fu) and also maleimide–functionalized chitosan (Cs-AMI). Thus, the hydrogel could be obtained by Diels-Alder reaction as demonstrated in Fig. 5. The Diels–Alder cross–linked hydrogel maintained the characteristic pH–sensitivity and the antibacterial activity of original chitosan and therefore being a potential candidate for tissue engineering applications (Guaresti et al., 2017).

3.3. Interpenetrating and semi-interpenetrating polymer networks (IPN and semi-IPN)

Although the advantages of using the chemical crosslinkers (especially, in enhancing the mechanical strength) their use are limited due to the residues of initiators and cross-linkers which may reduce the biocompatibility even after thorough purification (Liu, Zhang, & Li, 2015). To overcome these shortcomings as well as to control the diffusion of solutes in hydrogels, multicomponent networks, such as, semi- or interpenetrating polymer networks (IPN) have been projected (Ngadaonye, Geever, Killion, & Higginbotham, 2013). The IPN is a polymer with two or more networks which are interlaced but not covalently bonded to each other. These networks cannot be separated unless chemical bonds are broken. On the other hand, if one of the components has a linear structure instead a network, it will be called semi-IPN (Priya, Raja, & Raj, 2016). (Fig. 6).

The chitosan-based IPN can be obtained combining CS with either synthetic or natural polymers. Such approach, with altered morphologies, perform better in a synergistic way, to the physico-chemical and drug release properties (Cui, Jia, Guo, Liu, & Zhu, 2014; Zhang et al., 2015). Usually, the commonly methods used for obtaining chitosan-based IPN hydrogels consist on mixing the monomers with chitosan and, afterwards, to polymerize/crosslink the monomers (network 1) followed by crosslinking the CS (network 2) (or vice versa). N-isopropylacrylamide (NIPA), methacrylic acid (MAA), poly(ethylene glycol diacrylate) (PEGDA), Triethylene glycol dimethacrylate

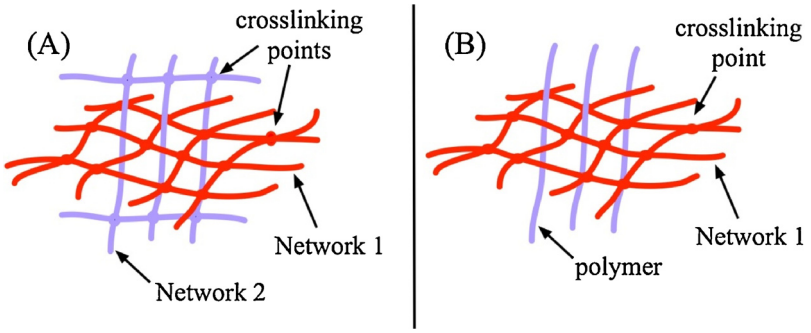

Fig. 6. Schematic representation of (A) IPN and (B) semi-IPN.

(TEGDMA), (hydroxyethyl)methacrylate (HEMA), N-vinylpyrrolidone (NVP) are the most used monomers to produce IPN and semi-IPN chitosan-based hydrogels with synthetic polymers. An example of using such approach was reported in a work published by Chen, Liu, Jin, and Chen (2014). They reported the synthesis of pH- and temperature-sensitive carboxymethyl chitosan/poly (N-isopropilacrylamide-co-methacrylic acid) IPN hydrogels. Firstly, a solution with NIPA, MAA and chemical crosslinker agent (N,N'-methylene-bisacrylamide) were prepared. Then carboxymethyl chitosan (CMCS) were added together with riboflavin (drug model). The polymerization of NIPA and MAA was carried out at room temperature for 12 h. After that, the semi-IPN was immersed in $CaCl_2$ solution to crosslink the CMCS. García, Ruiz-Durántez, and Valderruten (2017) the synthesis of IPN hydrogels based on chitosan and polyHEMA for controlled release of quetiapine. In such work, they prepared solution containing HEMA and CS. Then, CS was firstly crosslinked with glutaraldehyde generating a semi-IPN for further chemical crosslinking of HEMA. A different strategy was demonstrated by Lim et al. where chitosan was crosslinked by ethylenediaminetetraacetic acid (EDTA) (Lim, Kim, & Jun, 2016). In this work, they first obtained a hydrogel using HEMA, EGDMA and NVP. The samples were placed in an aqueous solution of chitosan and EDTA until the complete uptake of chitosan. Then, the sample was immersed in an aqueous solution containing 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N- hydroxysulfoxuccinimide (NHS) to crosslinking chitosan with EDTA.

Natural polymers, such as, gelatin and alginate can be used to produce IPN chitosan-based HG (Wang et al., 2018). For example, Zhang, obtained IPN hydrogels by bienzymatic crosslinking approach. First of all, chitosan was grafted with phloretic acid (CS-PA). Then, CS-PA was solubilized with gelatin. To this solution horseradish (HRP), transglutaminase (TG), and H_2O_2 was added and the gelation process was monitored by rheometer. The results indicated the formation of dual networks: one gelatin network crosslinked by TG and another chitosan-PA network crosslinked by HRP in the presence of a low concentration of H_2O_2 (Zhang et al., 2015). Other examples of IPN hydrogels based on chitosan are summarized in Table 2.

Despite their advantages, some aspects must be observed for the IPN chitosan-based hydrogels. For example, glutaraldehyde is toxic being carcinogenic at particular concentrations. In addition, crosslinking of chitosan amine groups can lead to decrease its bioadhesive properties which is not suitable for biomedical applications.

4. Biomedical applications of chitosan-based hydrogels

4.1. Drug delivery

Drug delivery systems have emerged as an alternative method of diseases treatment, once the active substance is loaded into a carrier or device, then providing the controlled and sustained drug release at a specific site and at a specific rate, avoiding the over dosage and reducing side effects (Lima-Tenório and Pineda et al., 2015; Soares et al., 2016). Among the different controlled-release systems, the hydrogels, because of their particular properties, are being widely investigated for the design of the ideal future controlled release systems (Hamidi et al., 2008).

The hydrogel-based delivery systems are of two major categories: i) conventional (or time-controlled systems), and ii) stimuli-responsive release systems. The difference between them is that the stimuli-responsive HG undergo changes in response to external stimuli, such as pH, temperature, electric field (Grainger, 2013; Samchenko, Ulberg, & Korotych, 2011), ionic strength, magnetic field (Grainger, 2013; Lima-Tenório and Pineda et al., 2015), and so on. Due to their unique properties, such as reversible swelling, absorptive capacity, permeability, mechanical properties, and high sensitivity to external stimuli (Samchenko et al., 2011), these HGs are also called smart materials. By far, chitosan-based hydrogels, especially because of their pH-sensitivity, have proven to be very efficient for the delivery of biologically active molecules (Liu, Gao, Lu, & Zhou, 2016).

The main drawback on conventional chemotherapy treatment is the non-specificity of the drugs. In this sense, injectable hydrogels pH sensitive are of great interest for anti-cancer drug delivery, once intratumoral injection and in-situ forming HGs can enhance the drug bioavailability to the tumor site and reduce systemic toxicity. Qu, Zhao, Ma, and Guo (2017), for example, have developed an in situ forming hydrogel system based on N-carboxyethyl chitosan (CEC) and dibenzaldehyde terminated poly (ethylene glycol) (PEGDA), and demonstrated their potential as delivery vehicle of doxorubicin (DOX) for hepatocellular carcinoma therapy by employing the pH-responsive of Schiff base. The HGs exhibited in vitro pH-dependent gel degradation and DOX release, being suitable for tumor therapy.

Another study focused on the chitosan-based hydrogels for anticancer delivery was reported by Zhang and Jin et al. (2018). In this work, the authors have reported the preparation of injectable temperature-sensitive hydrogels based on chitosan, hyaluronic acid (HA) and sodium glycerophosphate (GP) for pH sensitive drug release and adhesion to cancer cell. The in vitro DOX release at pH 6.86 and pH 4.00 was investigated, and the results showed the hydrogels are pH sensitive. The introduction of hyaluronic acid depressed the initial burst release of doxorubicin: the higher the HA content, the better sustained drug release behavior of the hydrogel, especially at acid media. Moreover, when incubated with human cervical cancer cells (Hela), the hydrogels reveal the remarkable influence of HA on modulating cancer cell adhesion.

In addition to the pH responsiveness of injectable chitosan-based hydrogels for tumor site specific administration of drug, their three-dimensional network is a good heat storage construction, being able to induce high temperature hyperthermia under microwave exposure, concentrating the heat and enhancing the treatment efficiency. Hyperthermia has shown promising results in cancer treatment when applied alone or in combination with radio- or chemotherapy. The treatment success is due to tumor tissues' being compact and disorganized, with a penetrable vascular network, low blood flux, and

Table 2
Summary of IPN hydrogels based on chitosan.

Type	Network 1 | Network 2	Technique	Ref
IPN	Chitosan-co-gelatin (CS-G) | poly(vinylpyrrolidone) (PVP) (CS-G chemically crosslinked with 1,2-epoxy-4-vinylcyclohexane and PVP crosslinked with glutaraldehyde)	Thermal Microwave Ultrasound	Wang et al. (2018)
	poly(HEMA-co-EGDMA) | poly(chitosan methacrylated) hydrogel of poly(HEMA-co-EGDMA) immersed in a chitosan methacrylated solution with initiator before IPN synthesis	Free radical polymerization 2h	Kang et al. (2017)
	Carboxymethyl chitosan/poly(N-isopropilacrylamide-co-methacrylic acid) | carboxymethyl chitosan (CMCS) Polymerization of poly(N-isopropilacrylamide-co-methacrylic acid), followed by HG immersion in $CaCl_2$ solution to crosslink the CMCS	The polymerization of NIPA and MAA was carried out at room temperature for 12 h.	Chen et al. (2014)
Semi-IPN	poly(HEMA-co-TEGDMA) | N-Carboxyethyl chitosan (Chemically crosslinked)	UV-light (photopolymerization)	Zhou et al. (2008)
	Chitosan-formaldehyde (CS-FA) | Polyacrylamide (CS-FA crosslinked by Schiff base)	50 °C, 1h	Mahdavinia et al. (2008)
	Poly (N-isopropylacrylamide) (PNIPA) | Chitosan PNIPA was chemcally crosslinked with N,N'-Methylenebisacrylamide by free radical polymerization	40 °C, 24h	Guo and Gao (2007)
	Chitosan-g-poly (acrylic acid) (CS-PAA) | Polyvinyl alcohol (PVA) CS-PAA was crosslinked with N,N'-Methylenebisacrylamide by free radical polymerization	80 °C, 3h	Liu et al. (2011)
	Poly (methacryloylglycylglycine) (MAGG) | Chitosan MAGG chemically crosslinked with ethyleneglycol dimethacrylate (EGDMA)	Freeze-thawing followed by free radical polymerization (40 °C, 4 h)	Dash et al. (2012)
	Poly (acrylamide) (PAM) | Chitosan PAM crosslinked with N,N'-Methylenebisacrylamide by free radical polymerization	70 °C, 3h	Wei et al. (2013)
	Chitosan | polyacrylonitrile Chitosan was crosslinked with glutaraldehyde	Casting forming film at room temperature	Al-Mubaddel et al. (2017)

better absorbing heat when compared to normal tissues, being consider as a minimally invasive therapy. Among the treatments involving hyperthermia, there's photothermal therapy (PTT), radiofrequency ablation and microwave ablation.

For instance, Wang and Wang et al. (2017) proposed the application of a chitosan-based injectable ionic HG inducing a high temperature rise for microwave tumor ablation. The ionic hydrogel (denoted as s-HY) was prepared by blending telechelic difunctional poly (ethylene glycol) (DF-PEG) saline solution and glycol chitosan saline solution. In addition, DOX was encapsulated within the hydrogel for combination chemotherapy. Regarding in vivo microwave ablation therapy, the surface temperature in the tumor area was up to 50 °C in the group of DOX-loaded s-HY (DOX-HY), being wider high temperature range in tumors compared with the microwave (MW) and control groups (Fig. 7(a)). The tumor growth was also investigated, after being treated for 14 days (Fig. 7(b)). An effective suppression of tumor growth was demonstrated for the mice treated with DOX-HY + MW and HY + MW. A slight decrease in the tumor volume of DOX-HY + MW group compared with HY-MW can be result of microwave ablation and chemotherapy. Fig. 7(c) shows photographs of nude mice after 14 days treatment. As shown, both DOX-HY + MW and HY-MW groups showed much fewer cells than the control group. However, the thermal therapy by DOX-HY + MW had a significant suppression of tumor growth.

Another strategy to get a hydrogel for hyperthermia therapy on tumors is achieved by the incorporation of magnetic nanoparticles, leading to the development of magneto responsiveness HGs. Zhu et al. (2015) developed a thermosensitive HG for intratumoral injection, aiming to improve the therapeutic efficacy and decrease doxorubicin toxicity. They made a chitosan-based HG and β-glycerophosphate salt, followed by incorporation of polyethylenimine (PEI)-modified superparamagnetic graphene oxide (GO/IONP/PEI). DOX was pre-loaded on the HG, creating a drug delivery system called DOX-GO/IONP/PEI-gel. The HG in an aqueous solution showed a sol-gel transition behavior depending on temperature changes and superparamagnetic properties of GO/IONP/PEI. In comparison to free DOX, the HG showed good efficacy at passing through cell membranes, causing more apoptosis and showing high antitumor efficacy toward MCF-7 cells in vitro. The same results were obtained in vivo, without significant toxicity. When the external alternating magnetic field was applied, the antitumor efficacy was higher, indicating that the proposed magneto-responsive HG has potential application fo cancer magnetic hyperthermia therapy.

Xie et al. (2017), in turn, have prepared an injectable chitosan hydrogel crosslinked with telechelic difunctional poly(ethylene glycol) (DF-PEG-DF), loaded of both doxorubicin (DOX) and docetaxel (DTX) for chemotherapy and iron oxide for magnetic hyperthermia induced stimuli responsive drug release. Improved synergistic antitumor activity was observed for the dual-drug-loaded magnetic hydrogel, and the alternative magnetic field-trigger control release of drugs in codelivery system showed a more efficient antitumor effect of cancer chemotherapy.

It is important to highlight that chitosan-based hydrogels are being also studied and developed in therapy of other diseases. Table 3 gives a great overview about other recent reports on chitosan-based hydrogels as drug delivery systems.

4.2. Tissue engineering

Tissue engineering aims at promoting the biological and functional regeneration of damaged or unhealthy tissues by combining cells and bioactive molecules spread into a support material or a scaffold (Pescosolido et al., 2011). The use of HGs in tissue engineering is advantageous because they are capable of three-dimensional organization of cells, providing a desirable mechanical integrity to form new tissues and supporting nutrient diffusion to encapsulated cells (Wang and Qian et al., 2017). In addition, a suitable environment has been explored for the living cells before and/or after the scaffold confection due to their biocompatibility; furthermore, it imitates the extracellular matrix because of its high water content at equilibrium (Pescosolido et al., 2011).

In the last decade, many reports focused on chitosan-based hydrogels for tissue engineering. The choice of chitosan, among other natural polymers, can be explained by its biodegradable, biocompatible, nontoxic, antimicrobial, biologically adhesive, biological activity and hemostatic effects (Liu and Wang et al., 2018). However, the preparation of chitosan-based HG for wound healing application is not a novelty. For example, Fujita et al. (2004) have developed an injectable chitosan/oxidized heparin hydrogel for the controlled release of fibroblast

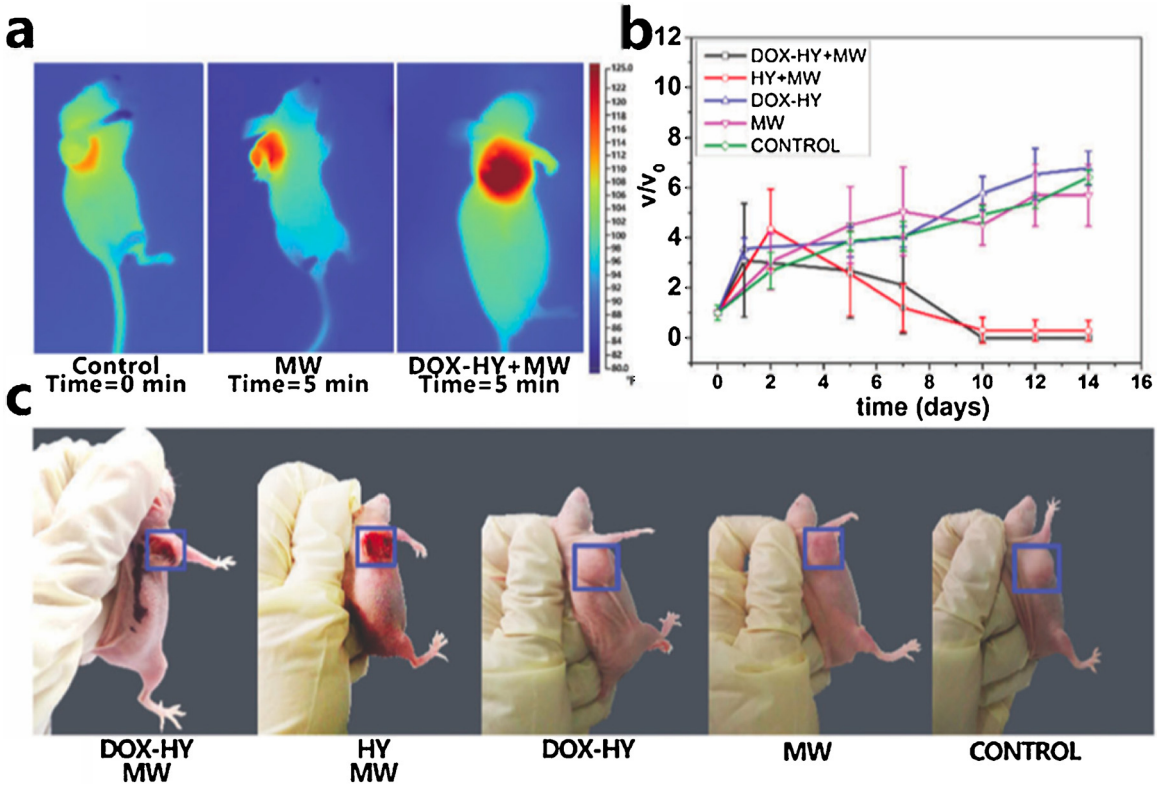

Fig. 7. Evaluation of the ionic hydrogel microwave ablation treatment efficiency in vivo. (a) Infrared thermal photographs of nude mice, DOX-HY + MW group and only MW group, respectively. (b) Relative tumor volume change with SHG-44 tumors under various treatments (n = 5). (c) Photographs of nude mice after 14 days microwave treatment in the DOX-HY + MW, HY + MW, DOX-HY, MW, CONTROL groups, respectively. Reproduced from Ref. (Wang and Wang et al., 2017) with permission from The Royal Society of Chemistry.

Table 3
General overview of chitosan-based hydrogels for drug delivery systems.

HG Formulation	Model Drug	Summary	Reference
Chitosan-grafted-glycidyl methacrylate/poly (ethylene glycol)diacrylate (PEGDA)/bone ash (BA)	Amoxicillin	Optimum amount of BA: 5 v.% for the usage of HG as a drug carrier system; Encapsulation efficiency: 76% (pH 7.4) and 98% (pH 1.2); Cumulative release rates approximately doubled for both HGs (with or without bone ash) at pH 1.2 condition.	Aycan and Alemdar (2017)
Chitosan and Ammonium-quaternary derivative chitosan (O-HTCC)	DOX	Encapsulation efficiency: > 70% (into chitosan nanoparticles) and ≈50% (into O-HTCC); pH 4.5: > 50% of DOX released after first 8 h.	Soares et al. (2016)
Quaternized chitosan/gelatin	Metronidazole and Dopamine	The drug release rate from the hydrogels generally degreased with the DA content decreasing in the samples. Cumulative release of metronidazole lasted more than 12 days for all the hydrogels.	Ren et al. (2017)
Carboxymethyl chitosan (CMC)/poloxamer 407 (F127)	Nepafenac (NP)	Temperature- and pH-responsive HG; NP release affected by temperature and pH; Higher cumulative release at 35 °C and pH 7.4.	Yu et al. (2017)
Chitosan-α,β-glycerophosphate (CS-HG)/ chitosan-based microspheres (CMs)	Methotrexate (MTX)	Both in vitro and in vivo, MTX-loaded CMs-CS-HG demonstrated long-term sustained MTX release. Biological evaluation: CMs-CS-HG showed good hemocompatibility and histocompatibility, and had non-genotoxicity and non-cytotoxicity to Kunming mice.	Dang et al. (2016)
Carboxymethyl chitosan (CMC)/oxidized chondroitin sulfate (OCS)/chitosan-based microspheres (CMs)	Bovine serum albumin (BSA)	Encapsulation efficiency of BSA in CMs was 7.9%. BSA release within the first 12 h: 26%, from CMs; 16%, from hydrogel of CMC and OCS; and 10% from CMs HG. About 30% of BSA was released from the CMs/ gel during 2 weeks, significantly less than those of CMs (80%) and gel (51%).	Fan et al. (2017)
Chitosan/poloxamer 407	Fluconazole (FLU)	Sustained FLU release and increased drug corneal permeability; potential for ophthalmic use for the treatment of fungal keratitis.	Gratieri et al. (2011)
Aldehyde-modified xanthan (Xan-CHO)/ carboxymethyl-modified chitosan (NOCC)	BSA-FITC	HG: self-healing, anti-enzymatic hydrolysis, cytocompatible; Stable release of BSA-FITC within 10 h after injection in liquids; After in vivo degradation for 14 days, the remaining weight was nearly 30% that of the initial weight.	Huang et al. (2018)
Chitosan/tartaric acid (TA)	vitamin B12 and blue dextran (BD)	Vitamin B12: delivered in a few hours from chitosan hydrogel and chitosan film; BD: only a small amount of the model drug was delivered within 11 days.	Ruiz-Caro et al. (2012)
Chitosan/xanthan gum (XG)/cellulose nanocrystals (CNCs)	5-fluorouacil (5-FU)	XG-CS without CNC hydrogels: almost all 100% release was observed within 1 day; XG-CS with CNC hydrogels (from 2 to 10%): the% release of 5-FU decreased.	Madhusudana Rao et al. (2017)

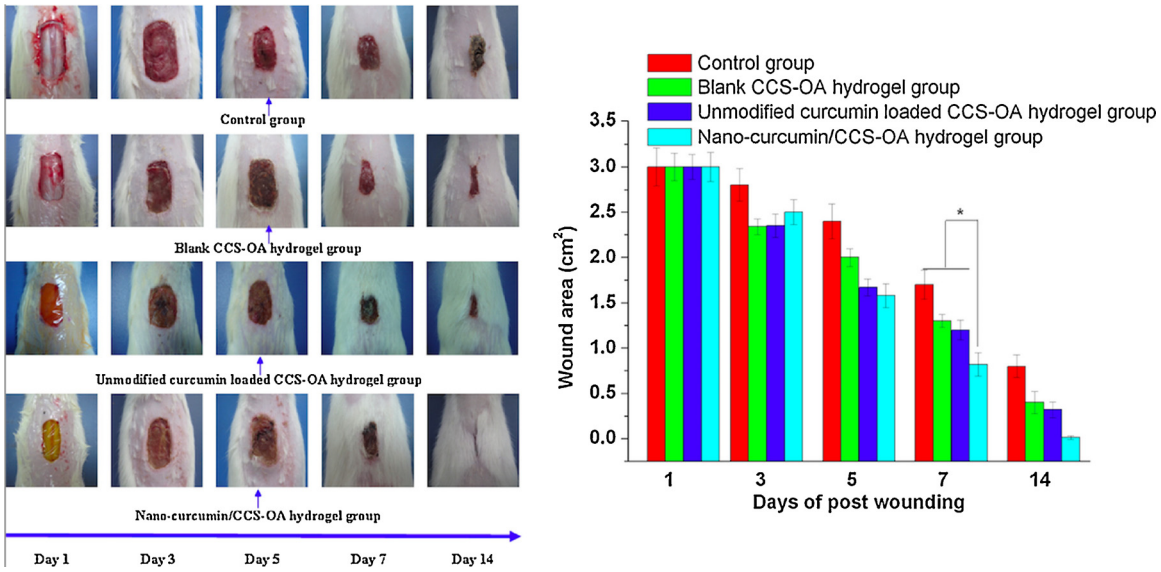

Fig. 8. The photographs of wound treated with saline solution, blank CCS-OA hydrogel, unmodified curcumin loaded CCS-OA hydrogel and nano-curcumin/CCS-OA hydrogel. Each wound on the indicated day is representative of three rats in each group. The data represent the mean ± SD of three rats. The statistical significance of wound area was evaluated on 7th day of post-wounding. *$p < 0.05$, $n = 3$; one-way ANOVA analysis. Reprinted and edited with permissions from reference (Li et al., 2012) © Elsevier.

Table 4
General overview of chitosan-based hydrogels for tissue engineering applications.

HG Formulation	Cell	Summary	Reference
Chitosan/graphene oxide (GO)	Human embryonic stem cell derived fibroblasts (HEF1) and cardiomyocytes	HG with self-adhesive and self-healing properties, as well as electrical conductivity, were prepared by the incorporation of the mussel-inspired protein polydopamine (PDA); Enhanced cell viability and proliferation.	Jing et al. (2017)
Chitosan/hydroxypropyl cellulose (HPC)/collagen (Col)/elastin (ela)/genipin	Corneal epithelium cells	In vitro cell culture experiments: the chitosan–collagen blend provided the regular stratified growth of the epithelium cells, good surface covering and increased number of the cell layers.	Grolik et al. (2012)
Chitosan/collagen crosslinked with poly(ethylene-glycol) and stabilized carbodiimide	Corneal epithelium cells	The developed composite, covalently bonded to collagen molecules, showed synergistic effect on physical and chemical properties. In vitro and in vivo tests indicated an implantable, elastic, nontoxic material with even better optical clarity when compared to human eye corneas.	Rafat et al. (2008)
Chitosan-graft-glycolic acid (GA)/phloretic acid (PA) enzymatic crosslinked with horseradish peroxidase (HRP) and H_2O_2	Bovine articular chondrocytes	In vitro essays indicated that the injectable HG allowed cells to retain their round shape after two weeks, besides ensuring cell viability	Jin et al. (2009)
N-succinyl-chitosan (S-CS)/aldehyde hyaluronic acid (A-HA	Bovine articular chondrocytes	The S-CS-A-HA HG, crosslinked with Schiff's base, was efficient in promoting cell survival, adhesion, besides of retain spherical shape characteristic of chondrocytic cells, which infers its potential application in cartilage tissue engineering.	Tan et al. (2009)
Chitosan/glycerophosphat salt (GP)	Foetal mouse cortical cells	In order to improve neuron affinity of chitosan/GP, poly-D-lysine (PDL) was immobilized chitosan by azidoaniline photocoupling, leading to an HG with improved cell survival in the optimum concentration of immobilized PDL (0,1%).	Crompton et al. (2007)
Chitosan (CS) blended with collagen (CC), albumin (CA) and gelatin (CG)	Bovine chromaffin cells	Scaffolds based on CC provided a fast attachment of chromaffin cells than CA matrix, surving for at least two weeks under in vitro and in vivo culture conditions	Elçin et al. (1998)
Chitosan/collagen type-II/Chondroitin sulfate (ChS)	Chondrocytes	The photopolymerized HG supported proliferation and deposition of cartilagionous extracellular matrix due to chondrocytes and mesenchymal stern cells encapsulated onto its structure. Once collagen type-II and chondroitin sulfate (ChS) were responsible for increase chondrogenesis, besides of improve cellular condensation, which is interesting in cartilage regeneration.	Choi et al. (2014)
O-carboxymethyl chitosan (O-CMC)/alginate/poly (vinyl alcohol) (PVA)	Adipose derived Stem cells (ASC)	The injectable HG based on O-CMC/alginate with fibrin nanoparticles incorporation was a better than alginate/PVA for ADSCs adhesion, proliferation and differentiation into adipocytes, confirmed by Oil Red O staining technique.	Jaikumar et al. (2015)
Quaternized chitosan (QCS) grafted polyaniline	Adipose-derived mesenchymal stem cells (AMSCs)	The HGs were crosslinked with oxidized dextran. The ones containing grafted polyaniline showed lower citotoxicity and higher antibacterial activity and then the ones based in QCS only.	Zhao et al. (2015)

growth factor (FGF-2). The hydrogel was biodegraded in about 20 days after subcutaneously injected, showing an excellent dressing for the wound healing.

In 2012, Li et al. have proposed a novel injectable hydrogel based in chitosan and alginate derivatives, and loaded with nano-curcumin for the dermal wound repair application. As shown in Fig. 8, after fourteen days of treatment, the wound area in the nano-curcumin was significantly decreased with almost completely wound closure. The authors have also confirmed that nano-curcumin could be released from the proposed hydrogel, significantly enhancing the re-epithelialization of epidermis and collagen deposition in the wound tissue.

More recently, Cheng, Lin, Ling, and Young (2017) proposed a chitosan/gelatin thermosensitive hydrogels for therapeutic angiogenesis. It was observed that by blending gelatin into the HG, it was possible to provide an appropriate microenvironment for adipose-derived stem cells (ASC) survival. In addition, it was possible to allow gradual release of the cells, thus facilitating tissue angiogenesis, demonstrating to be a promising approach for applying ASC-encapsulated chitosan/gelatin hydrogels to accelerate ischemic tissue regeneration.

Wang and Qian et al. (2017) prepared novel scaffolds based on hydroxyethyl chitosan (HECS) and cellulose (CEL), HECS/CEL with bubble like porous structure combining chemical cross-linking, silica leaching, and freeze-drying methods, generating micro- and macropores by a particulate porogen and freeze-drying process. Bioanalysis in vitro showed that the HECS/CEL scaffolds could support the attachment, spreading, viability, and proliferation of osteoblastic MC3T3-E1 cells, being considered as a promising matrix for application to bone tissue engineering.

Polyelectrolyte composite hydrogels have been also investigated for application as cartilage scaffolds. Liang et al. (2018) have constructed a homogeneous composite hydrogels from chitosan and carrageenan, using epichlorohydrin as the crosslinking agent. By the in vitro studies it was observed that the proposed HG promoted the adhesion, viability, and proliferation of ATDC5 cells. Furthermore, the CS/CG composite hydrogels could promote the chondrogenic differentiation of ATDC5 cells in vitro, and the expression of cartilage markers was significantly enhanced with an increase in CG content, indicating that CG played an important role in the chondrogenic differentiation of ATDC5 cells. In this context, CS/CG composite hydrogels was proposed as a promising cell-carrier for cell delivery in cartilage repair.

Table 4 summarizes other reports on chitosan-based hydrogels for tissue engineering applications.

5. Conclusion

With the advances in polymer science, a large number of multifunctional materials has also emerged, with special focus in biomedical applications. Among the several types of biomaterials studied and developed in the past few years, the chitosan-based HGs have attracted much attention because of its advantages like low cost, renewability of the resources, biocompatibility, biodegradability, and versatility.

There is a continuous progress in the development of chitosan-based HGs with potential to be applied in drug delivery and tissue engineering approaches. Some of the progress related to their preparation methods, the advantages and/or disadvantages related to these strategies, and some of the most recent works and results regarding its biomedical application were summarized in this work.

In summary, the chitosan-based HGs can be fabricated only by crosslinking of pure chitosan (homopolymeric), or by chitosan combined with a second polymer (copolymeric), by either chemical or physical crosslinking, and so on. Each of these strategies will lead to several different properties (pore diameters, mechanical properties, swelling performance, and stimuli-sensitivity behavior). In this sense, it is possible to tune the chitosan-based HGs properties, by employing/investigating different strategies and materials.

More successful biomedical application of chitosan-based hydrogels is expected. Thus, much work has to be done for that. A future goal on chitosan-based hydrogels is to get more successful biomedical applications, by developing smart devices capable of both reducing side effects related to drug administration (especially to anticancer drugs, overcoming problems related to the conventional treatment), and delivering cells for potential clinical application in drug delivery and tissue engineering. With the effort of researchers, we expected to enjoy a definite promising prosperity of chitosan-based HG in the near future.

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