Sensors International 3 (2022) 100168

Contents lists available at ScienceDirect

Sensors International

journal homepage: www.keaipublishing.com/en/journals/sensors-international

Chitosan based injectable hydrogels for smart drug delivery applications

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ARTICLE INFO

Keywords:
Chitosan
Injectable hydrogels
Crosslinking
Biomaterials

ABSTRACT

Hydrogels have a number of characteristics that make them an ideal candidate for biomedical applications. Chitosan is a well-known natural polymer, and it is favoured due to its good biocompatibility, low toxicity, and biodegradability characteristics. Though chitosan is an ideal drug-delivery polymer, however due to its poor solubility and to increase the mechanical strength, stimuli-responsive injectable hydrogels are prepared. Different cross-linking mechanisms are followed for the preparation of injectable hydrogels. Among them, the most common ones are physical and chemical cross-linking methods. Chitosan based injectable hydrogels can be used for the delivery of genes, proteins, antigens and drugs, and have been effectively applied in experimental therapy of several diseases including Parkinson's Disease, cancer and in different forms of degeneration.

1. Introduction

Hydrogels are three-dimensional networks based on cross-linked polymers, small molecules and colloids, where a number of hydrophilic groups or domains are present such as –OH, –CONH–, –$CONH_2$–, and –SO_3H [1–4]. Occurrence of these groups and domains are responsible of imbibing high amounts of water and biological fluids and help retain a significant fraction of water within its structure. Such structures however do not dissolve or aggregate in aqueous environment due to the formation of chemical or physical bonds between the polymer chains [1,4–6]. Hydrogels also have numerous characteristics that make them an excellent candidate for biomedical applications including drug delivery, wound healing, tissue regeneration, and cell therapy. While the cross-linked structure of hydrogels can swell up to thousand times of its dry volume, fully swollen hydrogels in water and physiological fluids show good biocompatibility, low toxicity and good biodegradability. The polymers used in hydrogel preparations also have bio-adhesive and mucoadhesive characteristics that can enhance tissue permeability and drug residence time [1,7–9].

The water-absorbable swollen hydrogels offer a biomimetic environment and show some common physical characteristics such as low interfacial tension with biological fluids. The soft and elastic characteristics minimize irritations after implantation, while the low interfacial tension minimizes cell adhesion and protein adsorption. These features are particularly attractive for encapsulation of biopharmaceuticals, where there is a rapid increase in the number of drugs approvals [10]. Also, due to their biomimetic properties, hydrogels serve two purposes: they act as supporting material for tissue regeneration and help to deliver drug payload by adsorption or encapsulation method. Hydrogels can either be synthesized by one step or multiple step procedure. The hydrogels vary in size from nanometres to centimetres in its width and can readily fit into any shape to which they are confined [1,2].

Controlled release or on-demand release is one major issue that the hydrogel-based drug delivery must focus on. “Smart” hydrogels are those that can respond to small changes in environmental stimuli including temperature, pH, enzymes, light, ultrasound and magnetic fields. A small change in external environment causes swelling and de-swelling of the stimuli-responsive hydrogel, leading to the release of the entrapped drugs at pre-determined rate [11]. They can further be classified according to their physical behaviours, ionic charges or method of preparation [6]. The porous structure can load all kinds of drugs, and by adjusting the internal and outer environment, the smart hydrogels can be used to control drug delivery [12].

In comparison to other gels, three-dimensional (3D) cross-linked

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Production and hosting by Elsevier

https://doi.org/10.1016/j.sintl.2022.100168
Received 15 December 2021; Received in revised form 25 February 2022; Accepted 1 March 2022
Available online 2 March 2022

injectable hydrogels are receiving more attention as scaffolds for delivering biomolecules. The term "injectable" means that the sol or the pre-gel can be transported to the targeting site by an injection device. Therefore, it can be formed *in vivo* and hence different from *in-situ* forming gels. An injectable hydrogel for biomedical applications follows a number of criteria: (a) formation or degradation of the gel, (b) complete absence of toxic reagents, (c) would not produce toxic product, and (d) it must exhibit necessary mechanical properties after injection [2,13]. Injectable hydrogels can be prepared by both physical as well as chemical cross-linking of polymers. While physically cross-linked hydrogels are considered more safer than chemically cross-linked hydrogels, mechanical properties of chemically cross-linked hydrogels are better compared to the physically cross-linked hydrogels. Dynamic covalent bonding is also another cross-linking method to get safe and high-performance injectable hydrogels. Injection procedure can nevertheless influence the structure and properties of hydrogels [2].

Chitosan is a natural polymer and was discovered by C. Roget in 1859. It is derived by alkaline deacetylation of chitin which is the second most abundant polysaccharide after cellulose and mostly found in crustacean shells. Chitosan has a similar structure and bioactivity as that of glycosaminoglycan, and contains β-(1–4) linked D-glucosamine and N-acetyl-D-glucosamine units arranged randomly [4,13–15]. Over time, several unique features of chitosan including biocompatibility, low immunogenicity and intrinsic bacteriostatic activity have been reported in the literatures. This features have led to several applications of chitosan in biomedical field as bio-substitutes, wound dressings, anti-bacterials and drug carriers [16–19]. Due to its advantageous options, considerable advancements have been achieved in the field of chitosan based injectable hydrogels. This review summarizes the use of chitosan as injectable hydrogels, especially in new generation drug delivery systems.

2. Chitosan as a biomaterial

Among the various naturally occurring biopolymers, chitosan is considered as the most suitable scaffolding material because of its biocompatibility, biodegradability and low toxicity. It is reported that chitosan biodegradation causes innocuous amino sugars that are completely absorbed by the body [20]. The molecular structure of chitosan promises high chemical and thermal stability [21]. Chitosan can be fabricated into different scaffolds for drug delivery by simply modifying its structural and functional moieties. Consequently, chitosan has a broad range of application prospects in translational medicine [22]. Chitosan hydrogel is suitable for delivery of proteins, peptides, antigens, genes and oligonucleotides and can be designed into newer drug delivery systems that release their payloads in response to different environmental stimuli [6,23–25].

Due to the presence of nitrogen group in its molecular structure (Fig. 1), chitosan has a cationic nature and exhibits a tendency to form polyelectrolyte complexes. Chitosan further displays functional properties such as mucoadhesion, transfection and *in situ* gelation, which are attributed to the primary amino acids attached to the polymer chains [26,27]. Cationic nature of the chitosan aids in the formation of carboxylate salts such as lactate, acetate, citrate, etc. that make the scaffolds water-soluble [28,29].

Chitosan can also further be divided according to its molecular weight, degree of deacetylation and degree of crystallinity [30,31]. Poor solubility of chitosan is the main limitation of this polymer; though it can be overcome with simple chemical modifications such as alkylation, carboxy-methylation, or acylation [6,12,32]. A newer improved method for synthesis of highly soluble of chitosan (half N-acetyled chitosan) was designed using a series of chitosan of low molecular weights [33]. Solubilization of chitosan occurs through protonation of the amino group ($-NH_2$) of the D-glucosamine units, wherein the polysaccharide is transformed into a polyelectrolyte in an acidic media. As the degree of protonation increases, so does the solubility of chitosan. It is also well-known that both the molecular weight and degree of deacetylation have a decisive effect on the solubility features [34].

The processing of chitosan based scaffolds can be facilitated either by physical stimulus or through chemical reactions [35]. Under acidic conditions the primary aliphatic amines get protonated. The free amino groups of chitosan interact with hydrogen ions at lower pH and increase the mechanical properties through interactions with other polymers [12,36]. Chitosan and its derivatives can be processed into different forms like hydrogels, sponges, nanoparticles and so on [12,37].

3. Synthesis-property relationship of injectable hydrogel systems

Numerous types of mechanisms have been applied for the development of chitosan-based injectable hydrogels. Among these, physical cross-linking and chemical cross-linking are the two major and widely followed approaches. Physically cross-linked injectable hydrogels involve synthetic and natural polymers that are stimuli responsiveness as well as temperature, pH or light responsiveness. Conversely, chemically cross-linked injectable hydrogels involve reactions for cross-linking like Schiff based or Michael addition as well as newly developed click chemistry or photo cross-linked injectable hydrogels. Ionic cross-linked or supramolecular interactions are also used separately or in addition to other polymers [13,38]. With the progresses in material sciences, polymer chemistry and supramolecular chemistry, the designs of hydrogel networks have advanced from static and covalent networks to smart, stimuli-responsive, biodegradable and dynamic networks. These

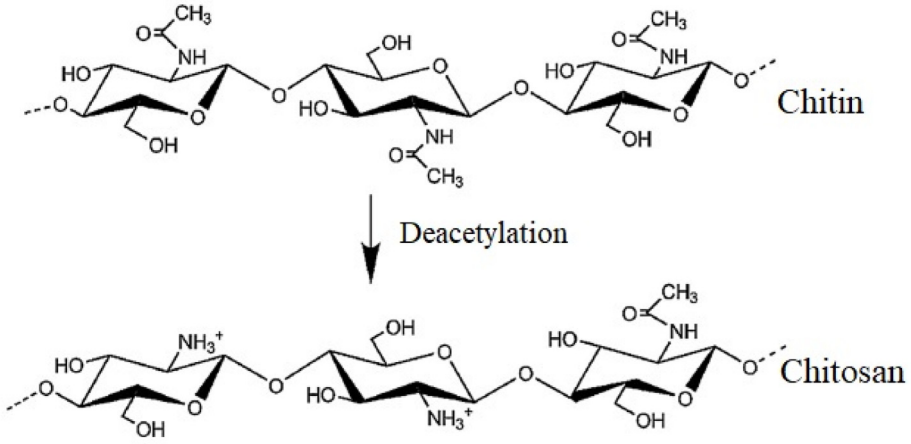

Fig. 1. Conversion of chitin into chitosan.

structures have the ability to mimic extracellular matrices which further enable cellular remodeling, adhesion, strain-stiffening and viscoelasticity through the inclusion of reversible bonds, cleavable linkages, supra-molecular assemblies, or flexible biopolymer backbones [39]. The amino acid groups of chitosan have been extensively studied for designing of both physically and chemically cross-linked hydrogels. However, it should be noted that preparation of hydrogels without any cross-linkers is always preferred due to their low cost and high safety with respect to drug delivery applications (see Table 1) [34,40].

3.1. Physically cross-linked injectable hydrogels

Physical cross-linking involves weak and temporary interactions such as molecular entanglements, hydrogen bonds, ionic and hydrophobic forces which can be reversed through environmental changes like temperature pH, and ionic strength. Physically cross-linked injectable hydrogels are produced by various methods including freeze-thaw cycles, stereo-complex formations, ionic interactions, and hydrogen bonding.

Polymers having hydrophobic domains can be cross-linked in aqueous medium through reverse thermal gelation, otherwise known as 'sol-gel' chemistry. The hydrophobic group is joined to the hydrophilic polymer through post-polymerization grafting to create a polymer-amphiphile. These amphiphiles, though soluble at low temperatures, aggregate at higher temperature to minimize the hydrophobic surface area and contact the bulk water to form the hydrogel. One injectable hydrogel comprising of chitosan-β-glycerophosphate was designed through this approach. This system can deliver macromolecules over prolonged period of time [41].

Polyelectrolyte complexation is another method that does not require any chemical cross-linkers. Polyelectrolyte complexes are formed due to electrostatic interactions between two oppositely charged polyelectrolytes in aqueous solution. The interactions existing between cationic amino groups of chitosan and anionic moieties of other electrolytes (such as pectin, alginates, keratin, etc.) are the keys to the formation of the hydrogel. These systems are however difficult to fabricate in large volumes. Amirian et al. described the fabrication of an injectable hydrogel based on oxidised chitosan and amidated pectin. Though the biopolymers were modified, the hydrogel was completely biocompatible and demonstrated potential for wound dressing applications [42]. Sometimes, nanofillers can also be incorporated to enhance the mechanical strength and functionalities of the hydrogels. It was reported by Ghorbani et al. that inclusion of cellulose nanocrystals in chitosan/pectin hydrogel matrix could reinforce the system by several folds [43].

While chitosan alone may not provide the necessary mechanical strength, researchers have retorted to combine polyvinyl alcohol (PVA) into the blend. This strategy has given way to the application of freeze-thaw process which produce biocompatible hydrogels without the need of any chemical cross-linker. Chitosan/PVA hydrogels have been extensively studied for encapsulation of various bioactives and nanomaterials [44,45]. Formation of these systems are often optimized through modulation of parameters such as thawing temperature, time and the number of freeze-thaw cycles. Hence, the complete control over the mechanical as well as functional aspects of the hydrogel can be realized. Sometimes, chitosan/PVA hydrogels were synthesized using a combination of irradiation and freeze-thaw techniques. In comparison to the hydrogels prepared by irradiation alone, they possessed high swelling capacity and better thermo-mechanical stability. Presence of chitosan also ensured pH sensitiveness and antibacterial effects [34].

3.2. Chemically cross-linked injectable hydrogels

Hydrogels can also be chemically formed through covalent cross-linking of macromers which make the bonds irreversible. These hydrogels are more stable in physiological environment and their mechanical properties may be tuned simply by changing the ratios of reactants during synthesis [46]. Synthesis of injectable chitosan hydrogels can be based on a number of chemical reactions such as photon or thermal polymerization, or polymer-polymer cross-linking reactions to form a 3D network [47]. To incorporate wide variety of biocompatible hydrophilic materials like polyethylene glycol into an injectable hydrogel, the material should be of low viscosity and should immediately convert into gel after injection to avoid loss of precious cargo [13].

The cross-linking reaction involves free amino groups and aldehyde, where acrylates or thiols are used as cross-linker [48]. Cross-linkers such as glutaraldehyde are cytotoxic even at low doses and may release from the hydrogels upon storage [34]. Efficient cross-linking is important to minimize the toxicity. Glycosaminoglycans like hyaluronic acid and chondroitin sulphate also help to cross-link with chitosan *in situ* [49].

The hyaluronic acid is one native tissue-mimetic polymer that can be cross-linked with chitosan *via* Schiff base reaction. The formation of imine linkage between chitosan and oxidised chondroitin sulphate helps in self-healing without involving any external stimulus. Additionally, ratio of chitosan and hyaluronic acid has strong effect on mechanical properties as well as on gelation time and pore size [50]. Though different forms of chitosan such as glycol chitosan and carboxyethyl chitosan have also been experimented, they require a small molecule cross-linkers like adipic acid di-hydrazide to form self-healing gels [51,52]. These kinds of hydrogels can be injected by shear stress/strain forces and rapidly recover to the original gel structure.

Click chemistry is another class of reaction which allows formation of hydrogels at a high speed and high selectivity. It is mainly based on four kind of reactions that includes cycloaddition, nucleophilic ring opening,

Table 1
Advantages and disadvantages of differently cross-linked systems.

Type of hydrogel	Example of cross-linkers	Advantages	Disadvantages
Chitosan-polyelectrolyte complex	Alginate, pectin, keratin, dextran sulphate	No exogenous cross-linkers required, Low toxicity, High pH sensitivity, Protects cargo from gastric environment.	Less control over mechanical properties, Time consuming and requires extensive optimization.
Chemically cross-linked hydrogels	Glutaraldehyde, genipin, EDC/NHS,	Mild reaction conditions, High conversion efficiency, High initiation rate.	Complete removal of cross-linkers is not guaranteed through washing, Some of the cross-linkers are neurotoxic, Not recommended for biomedical use.
Ionic hydrogels	Sodium hydroxide, potassium hydroxide, tripoly phosphate	Preparation is easy, Product has low toxicity, Appealing for pharmaceutical applications.	Less control over mechanical properties.
Photocross-linked hydrogels		Good thermal stability, High water solubility, Low toxicity.	Co-initiator is required.

non-alkyl carbonyl and carbon-carbon addition reactions. These reactions need initiators or catalysts which drive the reactions irreversibly towards one single product [13,53]. Chitosan is initially modified at the amino ends with different initiators, namely mercaptoacetic acid, furan, or maleimide and subsequently cross-linked to form a well-defined biopolymer network [54,55]. Diels-Alder reaction has been used for the preparation of one such cross-linked chitosan hydrogel to preserve the pH-sensitivity and biocompatible nature of chitosan. The microstructures and mechanical properties of the hydrogels were also tuned to serve specific needs [54].

Michael addition is another *in situ* reaction used for the synthesis of injectable hydrogels. It involves addition reactions of an electrophilic conjugated system with a nucleophilic carbon ion. This reaction is mostly attractive due to its high selectivity under mild condition [2].

Photo cross-linking is another approach that involves the exposure of UV (ultraviolet) or visible light for a short duration of time. Combination of both UV cross-linkable components and stimuli responsive polymer can also be used for the synthesis of injectable hydrogels [13]. As the degree of cross-linking depends on the irradiation time and the distance of the irradiation source, increased exposure of UV leads to formation of hydrogel of high mechanical strength. Photo cross-linked hydrogels are prepared by free radical polymerization of monomers and polymers containing certain functional groups (such as methacrylate, acrylate, etc.) in presence of photoinitiators upon exposure to light. Irgacure 2959 is one of the most common photo cross-linkers used to synthesize chitosan hydrogels and is reportedly to produce less cytotoxicity in comparison to others from the Irgacure series [56].

3.3. Ionic cross-linking or supramolecular interaction

Ionic cross-linking is another method of preparation for chitosan based injectable hydrogels. The formation of injectable hydrogels involve divalent ions such as calcium and zinc ions, as well as use of guluronic acid from alginate moiety cross-linked in an ionic manner [57]. Rapid synthesis of carboxymethyl chitosan using cooper, zinc and silver ions have been successful wherein detailed analyses revealed ordered complexation of the metal ions with carboxylic, amino and hydroxyl groups of the polymer chains [58]. The formation of chitosan based injectable hydrogels by involvement of calcium ions has contributed the towards repair of spinal cord and various concentrations of calcium ions may help in managing the mechanical properties [13].

The supramolecular hydrogel formation occurs through the interaction between cyclodextrin and various polymers, where cyclodextrin form supramolecular complexes due to the hydrogen bonds and hydrophilic interactions with the polymer chains [13]. Nanomaterials such as graphene oxide have also been used as a two dimensional cross-linker to fabricate chitosan based supramolecular hydrogels [59].

3.4. 3D printed hydrogels

It is often challenging to design a drug delivery device that combine proper geometry, order pore structure, degradation profile and can withstand mechanical stress during physiological loading. Expansion of 3D printing technology into biomedical engineering has enabled researchers to control and optimize these parameters and generate biocompatible scaffolds for drug delivery applications. 3D printed hydrogels are considered as one advanced approach to upgrade the mechanical strength. It helps to improve defective bones as well as in osteogenesis. Although additive manufacturing of hydrogels through 3D printing is attractive, the properties required of a printable bioink are quite demanding; they should be processable, compatible and must possess tissue-like features. In contrast to conventional hydrogels, injectable hydrogels should be able form their 'hydrogel' structure after their *in vivo* administration [39]. Biopolymers such as carboxymethyl cellulose, polylactic acid, agarose, methacrylates, polylysine, hyaluronic acid, and gelatin have often been applied as bioink materials by various cross-linking mechanisms including click chemistry, ionic bondings and hydrogen interactions [60–62].

Dong and colleagues develop a scaffold unification of 3D printed PCL constructs and bone marrow mesenchymal stem cells (BMMSCs) that were loaded into chitosan hydrogels. Compared to other individual scaffolds, it has more potential efficacy in case of cell seeding, mechanical strength and osteogenic capacity *in vivo* [63]. Xiangling Ye et al. in another study used 3D printing for fabricating poly(3--hydroxybutyrate-*co*-3-hydroxyvalerate)-calcium sulphate hemihydrate loaded with chitosan hydrogels. It helped inducing new bone formation and enhances cell adhesion and proliferations well as osteogenesis of rBMSCs (rat bone marrow stromal cells) [64].

4. Chitosan in smart drug delivery

Chitosan and its derivatives naturally exhibit pH sensitivity, good biocompatibility, enzymatic biodegradability and polycationic nature. Conjugation of various polymers with chitosan and protonation or deprotonation of amino group enhances the pH-responsiveness of chitosan based injectable hydrogels. Polymer chains in lower pH can easily expand to improve the water solubility by protonated the amino group that induces electrostatic repulsion, whereas higher pH reduces the water solubility of chitosan by deprotonated the amino group causing impairment of the globular structure. Hence, for this case, swelling property and water solubility depend on the pK_a value and external pH conditions [65,66].

Moreover, in neutral and alkaline solutions, chitosan shows poor solubility and mechanical performance. Many physical and chemical strategies have been developed to improve the solubility of chitosan without impairing any other important characteristics [35,66]. Blending of various polymers with chitosan can improve the pH responsiveness characteristics. For example, application of sodium bicarbonate as gelling agent into chitosan-hydroxyapatite allows quick gelation within 4 min exhibiting good viability, proliferation and dispersion as a potential cell carrier [67]. Development of another approach by Chen Zhao et al. through combining chitosan and amorphous calcium phosphate to develop a composite hydrogel system in which an acidifier of glucono δ-lactone is used to achieve a pH-responsive injectable hydrogel with good biocompatibility as well as effective cell-adhesion and proliferation [35,68].

Temperature is one of the many factors responsible for formation of injectable hydrogels. Incorporation of thermo-responsive polymers are generally responsible for preparation of chitosan-based smart hydrogels [69]. A desirable chitosan-based thermo-responsive injectable shows sol-gel transition as the temperature changes, i.e., liquid at room temperature and phase transition begins at physiological temperature. Ideally chitosan based thermo-responsive injectable hydrogels provide applications in tissue-engineering such as enhanced bone tissue formation as well as cellular activity due to its attractive characteristics of mould-ability, good biocompatibility and biodegradability [35,66,70]. The first thermo-responsive injectable hydrogel was prepared by Chenite et al. where β-glycerophosphate and chitosan were used to form the matrix and that enhances drug efficacy and prolongs drug retention time. These injectable hydrogels have sol-gel transition temperature at 37 °C and can be used to deliver growth factors, small drugs, nucleic acid and cells [71]. Poly (N-isopropylacrylamide) (PNIPAM) is also a thermo-responsive polymer which gives phase transition behaviour at around 30 °C [72].

Light responsive hydrogels have also received some attention in the field of drug delivery, due to their advantages of non-invasive, remote-controlled, and instant delivery. Such hydrogels have a pivotal role in photothermal therapy wherein photo-absorbing agents convert light energy to heat at the target site. While conventional light-responsive hydrogels with visible or UV light irradiation could suffer from limitations in penetration depth, near-infrared (NIR) responsive hydrogels can be an alternate strategy to control the release (Fig. 2). NIR responsive

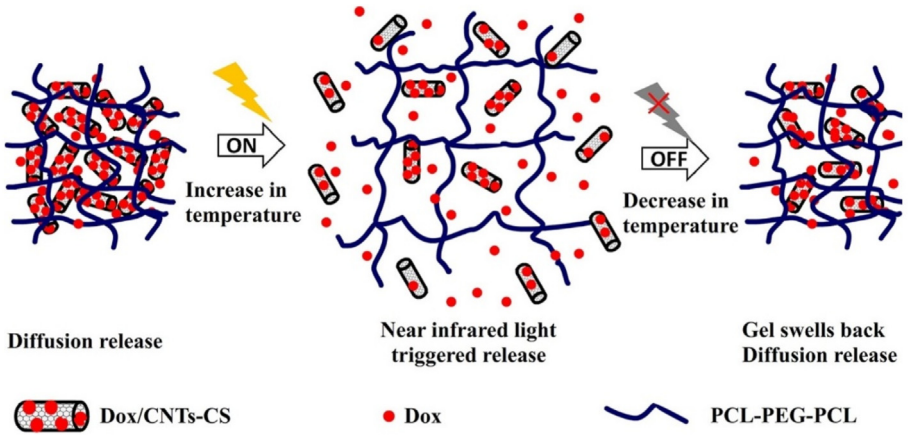

Fig. 2. Mechanism of NIR triggered release of doxorubicin loaded light responsive hydrogel [73].

agents are incorporated within the hydrogel matrix so that the integrity of the hydrogel is compromised due to sol-gel transition upon stimulated by NIR. Once the radiation is off, the drug release is restricted with the structural recovery of the hydrogel from the thermal effect [73,74].

Dual responsive hydrogel with chitosan is also a potential development for drug delivery. Phenylboronic acid with chitosan introduced sensitivity to glucose, while the pH sensitivity property is due to the presence of oxidised dextran [72]. Another polymer with chitosan that has been used to obtain sol-gel transition at physiological pH and temperature is glycerol phosphate [75].

5. Recent trends in chitosan injectable hydrogels

Bacterial infections are one of the biggest challenges faced by the healthcare community and formation of bacterial biofilms have significantly contributed to the development of bacterial resistance to antibiotic therapy [76]. Self-healing chitosan hydrogels designed through supramolecular interactions have demonstrated antibacterial property against a wide range of organisms. Incorporation of reduced graphene oxide had lent a photothermal capacity which specifically caused membrane rupture, protein denaturation and bacterial destruction upon photo-activation (Fig. 3) [76,77]. Injectable chitosan hydrogels containing drugs such as amoxicillin and ibuprofen could release their cargo upon stimulated by electric fields or pH change. They were also regarded as ideal candidates for smart drug delivery in antibacterial therapy [78].

In chemotherapy, the adverse effects of injectable hydrogels were reduced by controlling the rate of drug release as well as drug loading, degradation, and swelling behaviour of the hydrogels. These injectable hydrogels have also been used in control release of regenerative medicine and in immunotherapy. For the delivery of site-specific therapeutic agents, injectable hydrogels are expected to play a greater role as compared to conventional hydrogels. Additionally, injectable hydrogels have also been used as carriers or scaffolds for delivery of cells or bioactive moieties such as enzymes, proteins, etc. Recent studies on injectable hydrogels indicate high drug entrapment and can be used for the treatment of different types of cancer as well as effective treatment on diabetes and microbial infection [79,80]. For cervical carcinoma treatment, Wei and colleagues developed an injectable hydrogel that could deliver doxorubicin and combretastatin giving synergistic anticancer effect [81].

A significant portion of the global population, especially the elderly people, suffer from various kinds of degenerative ailments, both at neural and peripheral levels. Hydrogels have been an attractive platform for encapsulation and delivery of variety of cytokines and stem cells for tissue regeneration and cytoarchitecture preservation [82]. Yizhou et al. designed an injectable hydrogel with dopamine and gelatin quaternized into chitosan base to treat Parkinson's disease [83]. Other degenerative conditions such as disc herniation, macular degeneration and intervertebral disc damage can also be alleviated through the use of chitosan based thermo-sensitive hydrogels. These injectable formulations can restore the biomechanical function and thus lessen lower back pain [84–86].

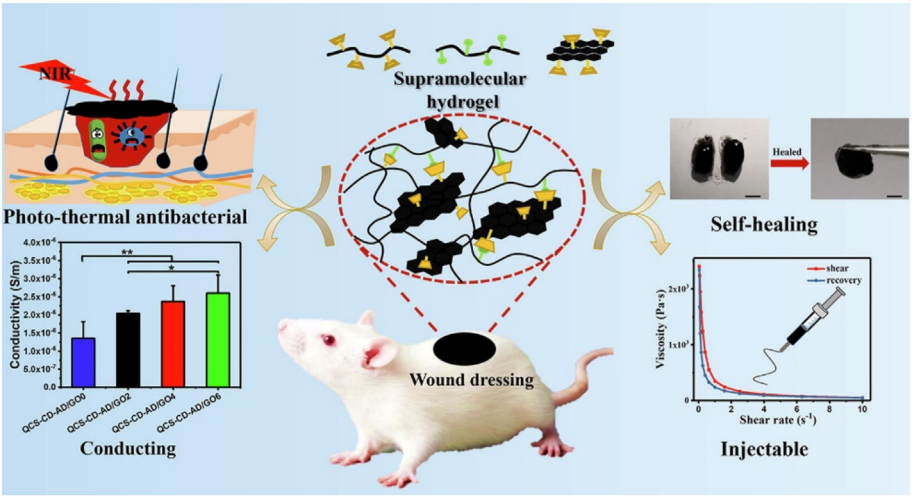

Fig. 3. Chitosan supramolecular hydrogel with photo-thermal antibacterial activity [77].

6. Conclusion and future perspectives

Recently, chitosan based injectable hydrogels have emerged as a successful platform for delivery of drugs. Chitosan is a natural polymer and because of its good bioavailability and low toxicity, it is acceptable as a material for biomedical application. It has been categorized by the United States Food and Drug Administration as 'safe'. Hence chitosan is widely explored to design new generation biomaterials in various physical forms, like nanoparticles, films, sponges and hydrogels. Chitosan based hydrogels can be used for delivery of drugs, proteins, genes, antigens, etc., but due to low solubility of chitosan in water, number of harsh preparation procedures are followed. Chemically cross-linked injectable hydrogels are limited to certain reactions hence they have few applications compared with physical cross-linking method. However, chemically cross-linked injectable hydrogels have advantages as they take less time compared to physical cross-linking injectable hydrogels. Sometimes, natural polymers are used in ionic or supramolecular injectable hydrogels that helps to maintain a bridge between physically and chemically cross-linking injectable hydrogels. These strategies may be further exploited to design pH- or thermo-sensitive hydrogels which can facilitate delivery of wide range of drugs at a specific pH or temperature.

It is also essential to note that the encapsulation as well as the release of the drugs from the hydrogel matrix depends on the degree of acetylation of chitosan, nature of the entrapped molecules, and its functionalization. Incomplete understanding of critical information such nature of the drug-polymer interactions and drug loading plays a major part in the therapeutic inefficiency of the hydrogel. Hence, a detailed idea on the specific application of the hydrogel should be conceived prior to the selection of the drug and synthesis strategy.

Declaration of competing interest

The authors would like to declare that there are no conflicts of interest in publication of this article.

References

[1] N. Bhattarai, J. Gunn, M. Zhang, Chitosan-based hydrogels for controlled, localized drug delivery, Adv. Drug Deliv. Rev. 62 (2010) 83–99.
[2] Y. Sun, D. Nan, H. Jin, X. Qu, Recent advances of injectable hydrogels for drug delivery and tissue engineering applications, Polym. Test. 81 (2020) 106283.
[3] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv. Drug Deliv. Rev. 64 (2012) 223–236.
[4] M. Hamidi, A. Azadi, P. Rafiei, Hydrogel nanoparticles in drug delivery, Adv. Drug Deliv. Rev. 60 (2008) 1638–1649.
[5] B.D. Ratner, A.S. Hoffman, Synthetic Hydrogels for Biomedical Applications, 1976.
[6] U. Ahmad, M. Sohail, M. Ahmad, M.U. Minhas, S. Khan, Z. Hussain, M. Kousar, S. Mohsin, M. Abbasi, S.A. Shah, Chitosan based thermosensitive injectable hydrogels for controlled delivery of loxoprofen: development, characterization and in-vivo evaluation, Int. J. Biol. Macromol. 129 (2019) 233–245.
[7] N.A. Peppas, J.J. Sahlin, Hydrogels as mucoadhesive and bioadhesive materials: a review, Biomaterials 17 (1996) 1553–1561.
[8] N.A. Peppas, P. Bures, W.S. Leobandung, H. Ichikawa, Hydrogels in pharmaceutical formulations, Eur. J. Pharm. Biopharm. 50 (2000) 27–46.
[9] M. Kurakula, G.K. Rao, V. Kiran, M.S. Hasnain, A.K. Nayak, Alginate-based hydrogel systems for drug releasing in wound healing, in: Alginates Drug Deliv, Elsevier, 2020, pp. 323–358, https://doi.org/10.1016/B978-0-12-817640-5.00013-3.
[10] C.A. Dreiss, Hydrogel design strategies for drug delivery, Curr. Opin. Colloid Interface Sci. 48 (2020) 1–17, https://doi.org/10.1016/j.cocis.2020.02.001.
[11] C. He, S.W. Kim, D.S. Lee, In situ gelling stimuli-sensitive block copolymer hydrogels for drug delivery, J. Contr. Release 127 (2008) 189–207, https://doi.org/10.1016/j.jconrel.2008.01.005.
[12] M. Mu, X. Li, A. Tong, G. Guo, Multi-functional chitosan-based smart hydrogels mediated biomedical application, Expet Opin. Drug Deliv. 16 (2019) 239–250.
[13] K. Wang, G. Buschle-Diller, R.D.K. Misra, Chitosan-based injectable hydrogels for biomedical applications, Mater. Technol. 30 (2015) B198–B205.
[14] I. Younes, M. Rinaudo, Chitin and chitosan preparation from marine sources. Structure, properties and applications, Mar. Drugs 13 (2015) 1133–1174.
[15] A.K. Nayak, M.S. Hasnain, Plant Polysaccharides in Drug Delivery Applications, 2019, pp. 19–23, https://doi.org/10.1007/978-981-10-6784-6_2.
[16] H. Ding, C.-J. Zhao, X. Cui, Y.-F. Gu, W.-T. Jia, M.N. Rahaman, Y. Wang, W.-H. Huang, C.-Q. Zhang, A novel injectable borate bioactive glass cement as an antibiotic delivery vehicle for treating osteomyelitis, PLoS One 9 (2014), e85472.
[17] F. Ding, H. Deng, Y. Du, X. Shi, Q. Wang, Emerging chitin and chitosan nanofibrous materials for biomedical applications, Nanoscale 6 (2014) 9477–9493, https://doi.org/10.1039/C4NR02814G.
[18] M.S. Hasnain, A.K. Nayak, Chitosan as responsive polymer for drug delivery applications, in: Stimuli Responsive Polym. Nanocarriers Drug Deliv. Appl., vol. 1, Elsevier, 2018, pp. 581–605, https://doi.org/10.1016/B978-0-08-101997-9.00025-4.
[19] S. Jana, A. Saha, A.K. Nayak, K.K. Sen, S.K. Basu, Aceclofenac-loaded chitosan-tamarind seed polysaccharide interpenetrating polymeric network microparticles, Colloids Surf. B Biointerfaces 105 (2013) 303–309, https://doi.org/10.1016/j.colsurfb.2013.01.013.
[20] M. Barman, S. Mahmood, R. Augustine, A. Hasan, S. Thomas, K. Ghosal, Natural halloysite nanotubes/chitosan based bio-nanocomposite for delivering norfloxacin, an anti-microbial agent in sustained release manner, Int. J. Biol. Macromol. 162 (2020) 1849–1861, https://doi.org/10.1016/j.ijbiomac.2020.08.060.
[21] A. Karakeçili, B. Topuz, S. Korpayev, M. Erdek, Metal-organic frameworks for on-demand pH controlled delivery of vancomycin from chitosan scaffolds, Mater. Sci. Eng. C 105 (2019) 110098.
[22] B. Abinaya, T.P. Prasith, B. Ashwin, S. Viji Chandran, N. Selvamurugan, Chitosan in surface modification for bone tissue engineering applications, Biotechnol. J. 14 (2019) 1900171.
[23] R.C.F. Cheung, T.B. Ng, J.H. Wong, W.Y. Chan, Chitosan: an update on potential biomedical and pharmaceutical applications, Mar. Drugs 13 (2015) 5156–5186.
[24] S. Jagga, M.S. Hasnain, A.K. Nayak, Chitosan-based scaffolds in tissue engineering and regenerative medicine, in: Chitosan Biomed. Appl., Elsevier, 2022, pp. 329–354, https://doi.org/10.1016/B978-0-12-821058-1.00014-9.
[25] A. Kundu, M.S. Hasnain, A.K. Nayak, Chitosan-based hydrogels in drug delivery, in: Chitosan Drug Deliv, Elsevier, 2022, pp. 361–387, https://doi.org/10.1016/B978-0-12-819336-5.00008-X.
[26] A. Oryan, S. Sahvieh, Effectiveness of chitosan scaffold in skin, bone and cartilage healing, Int. J. Biol. Macromol. 104 (2017) 1003–1011, https://doi.org/10.1016/j.ijbiomac.2017.06.124.
[27] A. Ali, S. Ahmed, A review on chitosan and its nanocomposites in drug delivery, Int. J. Biol. Macromol. 109 (2018) 273–286, https://doi.org/10.1016/j.ijbiomac.2017.12.078.
[28] M.N.V.R. Kumar, R. Muzzarelli, C. Muzzarelli, H. Sashiwa, A.J. Domb, Chitosan chemistry and pharmaceutical perspectives, Chem. Rev. 104 (2004) 6017–6084.
[29] M.T. Ansari, S. Murteza, M.N. Ahsan, M.S. Hasnain, A.K. Nayak, Chitosan as a responsive biopolymer in drug delivery, in: Chitosan Drug Deliv, Elsevier, 2022, pp. 389–410, https://doi.org/10.1016/B978-0-12-819336-5.00002-9.
[30] J.-K.F. Suh, H.W.T. Matthew, Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review, Biomaterials 21 (2000) 2589–2598.
[31] J.L. Drury, D.J. Mooney, Hydrogels for tissue engineering: scaffold design variables and applications, Biomaterials 24 (2003) 4337–4351.
[32] M. Prabaharan, Chitosan derivatives as promising materials for controlled drug delivery, J. Biomater. Appl. 23 (2008) 5–36.
[33] N. Kubota, N. Tatsumoto, T. Sano, K. Toya, A simple preparation of half N-acetylated chitosan highly soluble in water and aqueous organic solvents, Carbohydr. Res. 324 (2000) 268–274, https://doi.org/10.1016/S0008-6215(99)00263-3.
[34] H. Hamedi, S. Moradi, S.M. Hudson, A.E. Tonelli, Chitosan based hydrogels and their applications for drug delivery in wound dressings: a review, Carbohydr. Polym. 199 (2018) 445–460, https://doi.org/10.1016/j.carbpol.2018.06.114.
[35] K. Lavanya, S.V. Chandran, K. Balagangadharan, N. Selvamurugan, Temperature- and pH-responsive chitosan-based injectable hydrogels for bone tissue engineering, Mater. Sci. Eng. C 111 (2020) 110862.
[36] B. Ding, H. Gao, J. Song, Y. Li, L. Zhang, X. Cao, M. Xu, J. Cai, Tough and cell-compatible chitosan physical hydrogels for mouse bone mesenchymal stem cells in vitro, ACS Appl. Mater. Interfaces 8 (2016) 19739–19746.
[37] Z.A. Khan, S. Jamil, A. Akhtar, M.M. Bashir, M. Yar, Chitosan based hybrid materials used for wound healing applications-A short review, Int. J. Polym. Mater. Polym. Biomater. 69 (7) (2019) 419–436, https://doi.org/10.1080/00914037.2019.1575828.
[38] J.-A. Yang, J. Yeom, B.W. Hwang, A.S. Hoffman, S.K. Hahn, In situ-forming injectable hydrogels for regenerative medicine, Prog. Polym. Sci. 39 (2014) 1973–1986.
[39] A.A. Aldana, S. Houben, L. Moroni, M.B. Baker, L.M. Pitet, Trends in double networks as bioprintable and injectable hydrogel scaffolds for tissue regeneration, ACS Biomater. Sci. Eng. 7 (2021) 4077–4101, https://doi.org/10.1021/acsbiomaterials.0c01749.
[40] K. Ghosal, A. Das, S.K. Das, S. Mahmood, M.A.M. Ramadan, S. Thomas, Synthesis and characterization of interpenetrating polymeric networks based bio-composite alginate film: a well-designed drug delivery platform, Int. J. Biol. Macromol. 130 (2019) 645–654, https://doi.org/10.1016/j.ijbiomac.2019.02.117.
[41] T.R. Hoare, D.S. Kohane, Hydrogels in drug delivery: progress and challenges, Polymer (Guildf) 49 (2008) 1993–2007, https://doi.org/10.1016/j.polymer.2008.01.027.
[42] J. Amirian, Y. Zeng, M.I. Shekh, G. Sharma, F.J. Stadler, J. Song, B. Du, Y. Zhu, In-situ crosslinked hydrogel based on amidated pectin/oxidized chitosan as potential wound dressing for skin repairing, Carbohydr. Polym. 251 (2021) 117005, https://doi.org/10.1016/j.carbpol.2020.117005.
[43] M. Ghorbani, L. Roshangar, J. Soleimani Rad, Development of reinforced chitosan/pectin scaffold by using the cellulose nanocrystals as nanofillers: an injectable hydrogel for tissue engineering, Eur. Polym. J. 130 (2020) 109697, https://doi.org/10.1016/j.eurpolymj.2020.109697.

[44] M.T. Khorasani, A. Joorabloo, H. Adeli, Z. Mansoori-Moghadam, A. Moghaddam, Design and optimization of process parameters of polyvinyl (alcohol)/chitosan/nano zinc oxide hydrogels as wound healing materials, Carbohydr. Polym. 207 (2019) 542–554, https://doi.org/10.1016/j.carbpol.2018.12.021.
[45] K. Kalantari, E. Mostafavi, B. Saleh, P. Soltantabar, T.J. Webster, Chitosan/PVA hydrogels incorporated with green synthesized cerium oxide nanoparticles for wound healing applications, Eur. Polym. J. 134 (2020) 109853, https://doi.org/10.1016/j.eurpolymj.2020.109853.
[46] Z. Xu, K.M. Bratlie, Click chemistry and material selection for in situ fabrication of hydrogels in tissue engineering applications, ACS Biomater. Sci. Eng. 4 (2018) 2276–2291, https://doi.org/10.1021/acsbiomaterials.8b00230.
[47] L. Yu, J. Ding, Injectable hydrogels as unique biomedical materials, Chem. Soc. Rev. 37 (2008) 1473–1481.
[48] G. Xu, X. Wang, C. Deng, X. Teng, E.J. Suuronen, Z. Shen, Z. Zhong, Injectable biodegradable hybrid hydrogels based on thiolated collagen and oligo (acryloyl carbonate)–poly (ethylene glycol)–oligo (acryloyl carbonate) copolymer for functional cardiac regeneration, Acta Biomater. 15 (2015) 55–64.
[49] H. Tan, C.R. Chu, K.A. Payne, K.G. Marra, Injectable in situ forming biodegradable chitosan–hyaluronic acid based hydrogels for cartilage tissue engineering, Biomaterials 30 (2009) 2499–2506.
[50] E. Öhnstedt, H. Lofton Tomenius, E. Vågesjö, M. Phillipson, The discovery and development of topical medicines for wound healing, Expet Opin. Drug Discov. 14 (2019) 485–497.
[51] C. Qian, T. Zhang, J. Gravesande, C. Baysah, X. Song, J. Xing, Injectable and self-healing polysaccharide-based hydrogel for pH-responsive drug release, Int. J. Biol. Macromol. 123 (2019) 140–148, https://doi.org/10.1016/j.ijbiomac.2018.11.048.
[52] S.W. Kim, D.Y. Kim, H.H. Roh, H.S. Kim, J.W. Lee, K.Y. Lee, Three-dimensional bioprinting of cell-laden constructs using polysaccharide-based self-healing hydrogels, Biomacromolecules 20 (2019) 1860–1866, https://doi.org/10.1021/acs.biomac.8b01589.
[53] H.S. Abandansari, M.H. Ghanian, F. Varzideh, E. Mahmoudi, S. Rajabi, P. Taheri, M.R. Nabid, H. Baharvand, In situ formation of interpenetrating polymer network using sequential thermal and click crosslinking for enhanced retention of transplanted cells, Biomaterials 170 (2018) 12–25.
[54] O. Guaresti, C. García–Astrain, T. Palomares, A. Alonso–Varona, A. Eceiza, N. Gabilondo, Synthesis and characterization of a biocompatible chitosan–based hydrogel cross–linked via 'click' chemistry for controlled drug release, Int. J. Biol. Macromol. 102 (2017) 1–9, https://doi.org/10.1016/j.ijbiomac.2017.04.003.
[55] N. Yang, Y. Wang, Q. Zhang, L. Chen, Y. Zhao, In situ formation of poly (thiolated chitosan-co-alkylated β-cyclodextrin) hydrogels using click cross-linking for sustained drug release, J. Mater. Sci. 54 (2019) 1677–1691, https://doi.org/10.1007/s10853-018-2910-3.
[56] M. Rajabi, M. McConnell, J. Cabral, M.A. Ali, Chitosan hydrogels in 3D printing for biomedical applications, Carbohydr. Polym. 260 (2021) 117768, https://doi.org/10.1016/j.carbpol.2021.117768.
[57] C.A. McKay, R.D. Pomrenke, J.S. McLane, N.J. Schaub, E.K. DeSimone, L.A. Ligon, R.J. Gilbert, An injectable, calcium responsive composite hydrogel for the treatment of acute spinal cord injury, ACS Appl. Mater. Interfaces 6 (2014) 1424–1438.
[58] F. Wahid, H.-S. Wang, C. Zhong, L.-Q. Chu, Facile fabrication of moldable antibacterial carboxymethyl chitosan supramolecular hydrogels cross-linked by metal ions complexation, Carbohydr. Polym. 165 (2017) 455–461, https://doi.org/10.1016/j.carbpol.2017.02.085.
[59] D. Han, L. Yan, Supramolecular hydrogel of chitosan in the presence of graphene oxide nanosheets as 2D cross-linkers, ACS Sustain. Chem. Eng. 2 (2014) 296–300, https://doi.org/10.1021/sc400352a.
[60] X. Wang, J. Qi, W. Zhang, Y. Pu, R. Yang, P. Wang, S. Liu, X. Tan, B. Chi, 3D-printed antioxidant antibacterial carboxymethyl cellulose/ε-polylysine hydrogel promoted skin wound repair, Int. J. Biol. Macromol. 187 (2021) 91–104, https://doi.org/10.1016/j.ijbiomac.2021.07.115.
[61] M. Whitely, S. Cereceres, P. Dhavalikar, K. Salhadar, T. Wilems, B. Smith, A. Mikos, E. Cosgriff-Hernandez, Improved in situ seeding of 3D printed scaffolds using cell-releasing hydrogels, Biomaterials 185 (2018) 194–204, https://doi.org/10.1016/j.biomaterials.2018.09.027.
[62] I. Noh, N. Kim, H.N. Tran, J. Lee, C. Lee, 3D printable hyaluronic acid-based hydrogel for its potential application as a bioink in tissue engineering, Biomater. Res. 23 (2019) 3, https://doi.org/10.1186/s40824-018-0152-8.
[63] L. Dong, S.-J. Wang, X.-R. Zhao, Y.-F. Zhu, J.-K. Yu, 3D-printed poly (ε-caprolactone) scaffold integrated with cell-laden chitosan hydrogels for bone tissue engineering, Sci. Rep. 7 (2017) 1–9.
[64] X. Ye, L. Li, Z. Lin, W. Yang, M. Duan, L. Chen, Y. Xia, Z. Chen, Y. Lu, Y. Zhang, Integrating 3D-printed PHBV/Calcium sulfate hemihydrate scaffold and chitosan hydrogel for enhanced osteogenic property, Carbohydr. Polym. 202 (2018) 106–114.
[65] M. Rizwan, R. Yahya, A. Hassan, M. Yar, A.D. Azzahari, V. Selvanathan, F. Sonsudin, C.N. Abouloula, pH sensitive hydrogels in drug delivery: brief history, properties, swelling, and release mechanism, material selection and applications, Polymers (Basel) 9 (2017) 137.
[66] G. Tang, Z. Tan, W. Zeng, X. Wang, C. Shi, Y. Liu, H. He, R. Chen, X. Ye, Recent advances of chitosan-based injectable hydrogels for bone and dental tissue regeneration, Front. Bioeng. Biotechnol. 8 (2020) 1084.
[67] A. Rogina, A. Ressler, I. Matić, G.G. Ferrer, I. Marijanović, M. Ivanković, H. Ivanković, Cellular hydrogels based on pH-responsive chitosan-hydroxyapatite system, Carbohydr. Polym. 166 (2017) 173–182.
[68] C. Zhao, N.T. Qazvini, M. Sadati, Z. Zeng, S. Huang, A.L. De La Lastra, L. Zhang, Y. Feng, W. Liu, B. Huang, A pH-triggered, self-assembled, and bioprintable hybrid hydrogel scaffold for mesenchymal stem cell based bone tissue engineering, ACS Appl. Mater. Interfaces 11 (2019) 8749–8762.
[69] W.M. Argüelles-Monal, J. Lizardi-Mendoza, D. Fernández-Quiroz, M.T. Recillas-Mota, M. Montiel-Herrera, Chitosan derivatives: introducing new functionalities with a controlled molecular architecture for innovative materials, Polymers (Basel) 10 (2018) 342.
[70] J. Qu, X. Zhao, P.X. Ma, B. Guo, pH-responsive self-healing injectable hydrogel based on N-carboxyethyl chitosan for hepatocellular carcinoma therapy, Acta Biomater. 58 (2017) 168–180.
[71] A. Chenite, C. Chaput, D. Wang, C. Combes, M.D. Buschmann, C.D. Hoemann, J.C. Leroux, B.L. Atkinson, F. Binette, A. Selmani, Novel injectable neutral solutions of chitosan form biodegradable gels in situ, Biomaterials 21 (2000) 2155–2161.
[72] X. Xu, Y. Liu, W. Fu, M. Yao, Z. Ding, J. Xuan, D. Li, S. Wang, Y. Xia, M. Cao, Poly (N-isopropylacrylamide)-based thermoresponsive composite hydrogels for biomedical applications, Polymers (Basel) 12 (2020) 580.
[73] X. Dong, C. Wei, J. Liang, T. Liu, D. Kong, F. Lv, Thermosensitive hydrogel loaded with chitosan-carbon nanotubes for near infrared light triggered drug delivery, Colloids Surf. B Biointerfaces 154 (2017) 253–262, https://doi.org/10.1016/j.colsurfb.2017.03.036.
[74] A. GhavamiNejad, M. SamariKhalaj, L.E. Aguilar, C.H. Park, C.S. Kim, pH/NIR light-controlled multidrug release via a mussel-inspired nanocomposite hydrogel for chemo-photothermal cancer therapy, Sci. Rep. 6 (2016) 33594, https://doi.org/10.1038/srep33594.
[75] S. Kim, S.K. Nishimoto, J.D. Bumgardner, W.O. Haggard, M.W. Gaber, Y. Yang, A chitosan/β-glycerophosphate thermo-sensitive gel for the delivery of ellagic acid for the treatment of brain cancer, Biomaterials 31 (2010) 4157–4166.
[76] Y. Chen, Y. Gao, Y. Chen, L. Liu, A. Mo, Q. Peng, Nanomaterials-based photothermal therapy and its potentials in antibacterial treatment, J. Contr. Release 328 (2020) 251–262, https://doi.org/10.1016/j.jconrel.2020.08.055.
[77] B. Zhang, J. He, M. Shi, Y. Liang, B. Guo, Injectable self-healing supramolecular hydrogels with conductivity and photo-thermal antibacterial activity to enhance complete skin regeneration, Chem. Eng. J. 400 (2020) 125994, https://doi.org/10.1016/j.cej.2020.125994.
[78] J. Qu, X. Zhao, P.X. Ma, B. Guo, Injectable antibacterial conductive hydrogels with dual response to an electric field and pH for localized "smart" drug release, Acta Biomater. 72 (2018) 55–69, https://doi.org/10.1016/j.actbio.2018.03.018.
[79] R. Xing, K. Liu, T. Jiao, N. Zhang, K. Ma, R. Zhang, Q. Zou, G. Ma, X. Yan, An injectable self-assembling collagen–gold hybrid hydrogel for combinatorial antitumor photothermal/photodynamic therapy, Adv. Mater. 28 (2016) 3669–3676.
[80] P. Kesharwani, A. Bisht, A. Alexander, V. Dave, S. Sharma, Biomedical applications of hydrogels in drug delivery system: an update, J. Drug Deliv. Sci. Technol. (2021) 102914.
[81] L. Wei, J. Chen, S. Zhao, J. Ding, X. Chen, Thermo-sensitive polypeptide hydrogel for locally sequential delivery of two-pronged antitumor drugs, Acta Biomater. 58 (2017) 44–53.
[82] S. Papa, I. Vismara, A. Mariani, M. Barilani, S. Rimondo, M. De Paola, N. Panini, E. Erba, E. Mauri, F. Rossi, G. Forloni, L. Lazzari, P. Veglianese, Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine in situ preserving cytoarchitecture and promoting functional recovery in spinal cord injury, J. Contr. Release 278 (2018) 49–56, https://doi.org/10.1016/j.jconrel.2018.03.034.
[83] Y. Ren, X. Zhao, X. Liang, P.X. Ma, B. Guo, Injectable hydrogel based on quaternized chitosan, gelatin and dopamine as localized drug delivery system to treat Parkinson's disease, Int. J. Biol. Macromol. 105 (2017) 1079–1087.
[84] Z. Li, H. Shim, M.O. Cho, I.S. Cho, J.H. Lee, S.-W. Kang, B. Kwon, K.M. Huh, Thermo-sensitive injectable glycol chitosan-based hydrogel for treatment of degenerative disc disease, Carbohydr. Polym. 184 (2018) 342–353, https://doi.org/10.1016/j.carbpol.2018.01.006.
[85] S.E. Gullbrand, T.P. Schaer, P. Agarwal, J.R. Bendigo, G.R. Dodge, W. Chen, D.M. Elliott, R.L. Mauck, N.R. Malhotra, L.J. Smith, Translation of an injectable triple-interpenetrating-network hydrogel for intervertebral disc regeneration in a goat model, Acta Biomater. 60 (2017) 201–209, https://doi.org/10.1016/j.actbio.2017.07.025.
[86] K. Wang, M. Zheng, K.L. Lester, Z. Han, Light-induced Nrf2−/− mice as atrophic age-related macular degeneration model and treatment with nanoceria laden injectable hydrogel, Sci. Rep. 9 (2019) 14573, https://doi.org/10.1038/s41598-019-51151-7.