Chitosan hydrogels for sustained drug delivery

Soline Peers, Alexandra Montembault, Catherine Ladavière

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Corresponding Author: Dr. Catherine LADAVIERE, Ph.D.

Corresponding Author's Institution: CNRS

First Author: Soline PEERS

Order of Authors: Soline PEERS; Alexandra MONTEMBAULT; Catherine LADAVIERE, Ph.D.

Abstract: Sustainable and controlled delivery of drugs is at the centre of a huge amount of undertaken researches. The ability of hydrogels, high water content materials, to achieve a local and delayed-delivery has already been demonstrated for a wide variety of therapeutic agents and various polymer natures. In particular, chitosan, a polysaccharide, stands out as a first choice polymer for hydrogels elaboration in biomedical, cosmetic, and health related applications, thanks to its interesting properties (as harmlessness, biodegradability, antimicrobial capacity and mucoadhesivity). Moreover, chitosan also allows drugs to go easier through biological barriers. The main objective of this review is to report the various uses of chitosan hydrogels as drug delivery devices to control and/or delay the release of drugs loaded into these polymeric matrices. A wide spectrum of corresponding biomedical applications of these systems can be encountered in the literature, whatever the physicochemical nature of drugs (hydrophilic, hydrophobic, macromolecular), as detailed in this review.

Suggested Reviewers: Florence Agnely Pr
Pharmaceutical, Paris Sud University
Florence.Agnely@u-psud.fr
For her expertise in the development of materials for controlled release

Didier Le Cerf Pr
Chemistry, Rouen University
Didier.LeCerf@univ-rouen.fr
For his work on polysaccharide-based hydrogels.

Yves Grohens Pr
LIMATB, Centre de recherche
yves.grohens@univ-ubs.fr
For his knowledge on natural polymers.

Graphical Abstract

TOC

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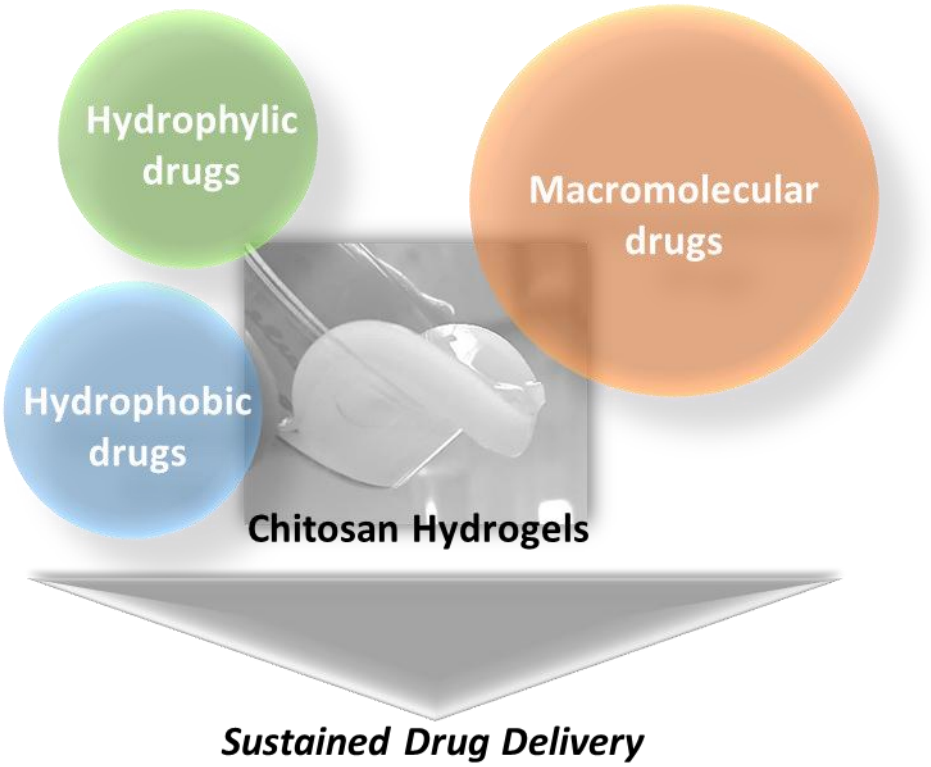

Chitosan Hydrogels for Sustained Drug Delivery

S. Peers, A. Montembault*, C. Ladavière*

Université de Lyon, Université Claude Bernard Lyon 1, CNRS, Ingénierie des Matériaux Polymères UMR 5223, F-69622, Villeurbanne, France

* Corresponding authors: alexandra.clayer-montembault@univ-lyon1.fr (A. Montembault), catherine.ladaviere@univ-lyon1.fr (C. Ladavière)

Abstract

Sustainable and controlled delivery of drugs is at the centre of a huge amount of undertaken researches. The ability of hydrogels, high water content materials, to achieve a local and delayed-delivery has already been demonstrated for a wide variety of therapeutic agents and various polymer natures. In particular, chitosan, a polysaccharide, stands out as a first choice polymer for hydrogels elaboration in biomedical, cosmetic, and health related applications, thanks to its interesting properties (as harmlessness, biodegradability, antimicrobial capacity and mucoadhesivity). Moreover, chitosan also allows drugs to go easier through biological barriers. The main objective of this review is to report the various uses of chitosan hydrogels as drug delivery devices to control and/or delay the release of drugs loaded into these polymeric matrices. A wide spectrum of corresponding biomedical applications of these systems can be encountered in the literature, whatever the physicochemical nature of drugs (hydrophilic, hydrophobic, macromolecular), as detailed in this review.

Keywords

Chitosan; hydrogels; drug delivery systems

Introduction

Chitosan is a polysaccharide industrially obtained by N-deacetylation of chitin, the second most common natural polysaccharide in biomass after cellulose [1]. Chitin is a structural material present in the exoskeletons of arthropods (*i.e.*, insects, arachnids, shrimps, crabs) and in the endoskeletons of cephalopods (*i.e.*, squids, cuttlefishes). The highest proportions of chitin are found in crustaceans (up to 30%) and can also be found in some algae and mushrooms [2], [3]. Chitin is mainly localized in skin fibres of crustaceans, and mixed with mineral salts (*e.g.*, calcium carbonate), proteins, pigments, and lipids. Chitosan is then obtained by chitin N-deacetylation process in alkaline or acidic conditions. It is a linear copolymer composed of two sub-units, D-glucosamine and N-acetyl-D-glucosamine units linked by a β (1⟶4) glycosidic bond (**Figure 1**). Its major characteristics are its acetylation degree (*DA*) that is the molar fraction of acetylated units existing in the copolymer and its molar mass (for example, its weight-average molar mass M_w) [4], [5].

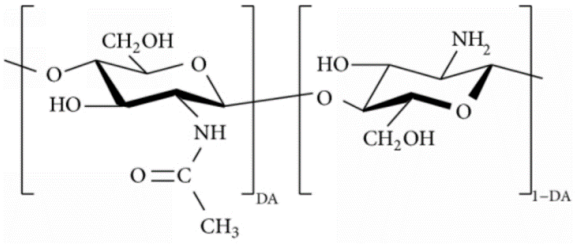

Figure 1. Chitosan chemical formula

Chitosan is a polymer showing interesting biological properties [6] such as harmlessness [7], biodegradability [8], [9], antimicrobial capacity [10]–[12] and mucoadhesivity [13], [14], that made it an excellent candidate for biomedical or cosmetic applications [15]–[17]. Chitosan also presents structural characteristics that are close to the ones found in extracellular matrix, so that it is suitable for growth, organization, and migration of cells during the tissue formation [6], and leads to an easier drug carrying through biological barriers [18]. Finally, note that chitosan is soluble in diluted acid aqueous media and can be found in many shapes such as macro- or nano-particles [19]–[21], sponges [22], films [23], [24], or, what is of interest here, physical and chemical hydrogels.

A gel is composed of a large amount of solvent trapped between the meshes of a three-dimensional network. This quantity depends on the hydrophilic function density that can be

found on the chains [13]. When the solvent involved is water, one talks about hydrogels [25], [26]. Almdal *et al.* [27] described a gel as a soft, solid or solid-like material that "can maintain its form under the stress of its own weight". It is also a resilient material with elastic properties.

There are two types of chitosan hydrogels: chemical and physical hydrogels [29]. Chemical hydrogels are crosslinked with covalent bonds thanks to free ammoniums attached along the chitosan chain. Different ways can be used to achieve this chemical crosslinking and are reported in the literature. Among them, crosslinking agents as for example formaldehyde [28], glutaraldehyde [13], [29]–[32] or genipin, or other compounds with several reactive chemical functions [33]. These molecules can be nevertheless toxic or their biocompatibility not well-known, which is undesirable in drug delivery applications.

Another way consists in hydrogel crosslinking by establishing covalent bonds with other polymers. For example, Tan *et al.* [34] made a covalent crosslinking between hyaluronic acid (HA) and chitosan by reacting ammoniums coming from CS and aldehydes coming from HA ("Schiff base reaction"). UV irradiation can also be used to achieve chemical crosslinking. Several researchers [35]–[39] worked on UV irradiation of azide functions ($-N_3$) to create highly reactive nitrene (R-N). Gelation of the hydrogel is operated after reaction between nitrenes and ammoniums coming from CS, forming an azo bonding (R-N=N-R''). Besides, some interpenetrating networks (IPN) or semi- interpenetrating networks (semi-IPN) were developed and consists in a second polymer mesh interlinked with the chitosan hydrogel's one previously crosslinked [40].

Depending on crosslinking density, chemical hydrogels can be less sensitive to degradation and have often better mechanical properties than physical hydrogels. The latters are called « reversible » hydrogels. Their formation is based on low energy bonding as Van der Waals or hydrogen bonds. Polyelectrolyte complexes can also be obtained thanks to electrostatic interactions between polymers. In this case, interactions take place between protonated ammonium of CS and anionic charges from the other polymer [41]. On the other hand, ionic interactions can establish between the gel and incorporated anionic molecules or drugs [42], [43], [13]. Physical chitosan hydrogels are often used in numerous biomedical applications. Nevertheless, their degradation can be faster than chemical hydrogels due to their lower mechanical properties.

Among these physical or chemical chitosan hydrogels, responsive ones can be achieved thanks to a gelation occurring « *in situ* », on the site of interest, with formulations that are often injectable [44]–[47]. To this end, three methods can be employed [48]: i) the use of crosslinkers under effective control of crosslinking kinetics, ii) crosslinking operated by the action of enzymes (tyrosinase [49], [50]), or iii) physical crosslinking of CS chains with the modification of an environmental parameter (temperature, pH). For example, a pH-sensitive release of drug can be very interesting in cancer treatments because the pH is locally decreased around the tumour. Drugs are only released in this acidic environment without damaging surrounding tissues [51]. When the gelation occurs with a temperature modification, corresponding « thermosensitive » hydrogels are obtained by neutralization of chitosan acidic solution by an anionic base, such as polyol (*e.g.*:, glycerophosphate (GP)) [52]. The use of polyol base enables hydrogel formation at body temperature, *i. e.* 37°C, thanks to interactions within the hydrogel matrix [53] : i) a decrease of electrostatic repulsion between CS chains as a result of CS ammoniums neutralization (electrostatic interactions between cationic ammoniums from CS and anionic phosphates from polyol), ii) hydrophobic interactions which take place between polymer chains with the temperature increase, making the gelation possible [48].

The main objective of this review is to report the studies about all these chitosan hydrogels, specifically used as drug delivery devices to control the release of drugs loaded into the gel matrix. It also reports their numerous biomedical applications encountered in the literature, whatever the physicochemical nature of drugs (hydrophilic, hydrophobic, macromolecular drugs).

1. Sustained drug delivery and burst release

Nowadays, several studies demonstrate the great interest of chitosan hydrogels for the sustained delivery of different drugs in biomedical area. Indeed, their porous hydrophilic three-dimensional structure allows the incorporation of drugs while creating a diffusion barrier, and hindering their release. The release of drugs from hydrogels follows three mechanisms reported by Azevedo *et al.* [7] i) chemically controlled: polymer chain breakage *via* hydrolytic or enzymatic degradation, releasing incorporated drugs, ii) swelling-controlled: when the molecule diffusion is faster than the hydrogel swelling, the later conditioning the release, iii) diffusion-controlled**:** when hydrogel swelling faster than diffusion phenomenon.

This latter mechanism is the most suitable to hydrogels due to their high swelling capacity in a water environment. Drug diffusion is governed by the pore size in the hydrogel matrix. The higher swelling rate, the bigger the pores, and the faster the small size drug diffusion through the hydrogel. On the contrary, the release of macromolecules, having a higher hydrodynamic radius, is generally slower [30].

One of the major obstacle encountered in drug delivery is the « burst effect » (or « burst release »), that is to say the sudden, fast and uncontrolled drug release immediately after administration. « Burst release » phenomenon is explained by adsorbed drugs at the surface, which often release faster than drugs incorporated inside the matrix. Various strategies can be employed to reduce this phenomenon and consequently to improve the efficiency of the pharmaceutical treatment. Among these strategies, the drug incorporation into hydrogels has revealed interesting results for sustained drug delivery application as demonstrated in the studies reported in this review.

Indeed, the elaboration of CS hydrogels for the delivery of i) hydrophilic, ii) hydrophobic, or iii) macromolecular drugs was extensively investigated in the literature. These studies are described in the following parts according to the hydrophilic or hydrophobic properties of drugs, as well as their molar mass (above 1,000 $g.mol^{-1}$ for macromolecules). It is worthy of note that the drugs are referred all along this review to their international non-proprietary name (INN). The INN of a pharmaceutical active agent is a non-commercial name from the World Health Organization, unequivocally used in various languages.

2. Chitosan hydrogels for the controlled release of hydrophilic drugs

Chitosan chemical and physical hydrogels represent interesting candidates for the delivery of hydrophilic drugs thanks to their high water content (>80%), as shown in several following studies.

Chemical hydrogels

A chemical hydrogel composed of CS and polyvinyl alcohol (PVA), crosslinked with triethyl orthoformate and impregnated with doxazocin (alpha-adrenergic blocking agent used in this study as an anticancer drug) was elaborated by Jamal *et al.* [54] for the treatment of cervical cancer. Drug was incorporated by incubation of hydrogel (so, after gelation) overnight at room temperature in a doxazocin solution. As mentioned by the authors, the more

concentrated crosslinking agent, the higher the hydrogel density, and the smaller the pores (according to SEM observations). The authors also demonstrated that the most effective formulation for the angiogenesis decrease is the one with 8 wt. % crosslinking agent and a doxazocin concentration about 1 mg/mL. Furthermore, this formulation has showed the best anti-proliferative property for cancer cells (Hela cells).

In situ gelling CS chemical hydrogels were also broadly studied because they could be administered by oral route. This route was employed by Xu *et al.* [55] for the controlled release of lidocaine (which is a local anaesthetic/antiarrhythmic drug, and was incorporated before gelation) from chemical CS and catechol hydrogels, crosslinked with genipin. In this study, the catechol was used for its mucoadhesive properties (and not as a crosslinking agent). Although catechol reduced final rheological properties of the hydrogel, it increased the hydrogel density and mucoadhesion. The retention of drug was thus more effective, and the penetration of lyzozymes (proteins implied in antibacterial defence), was reduced. Indeed, 66% of lidocaine was released *in vitro* from the hydrogel CS/catechol after 5h contrary to 85% from the "simple" CS hydrogel at the same time. According to the authors, the smaller the pores, the more effective the drug retention. Note that a delayed release of lidocaine has already been observed by Kristl *et al.* [56] in 1993 who have incorporated the drug into CS hydrogels (without catechol).

In situ gelling hydrogels have also been used for the localized injection of anticancer drugs. Chang *et al.* [51] have worked on the administration of 5-fluorouracile (5-FU) from CS chemical hydrogels, incorporated before gelation. The hydrogel showed auto-regenerative properties thanks to the PVA addition [57]. Moreover, this PVA addition provided i) the local reorganization of polymer chains and the decrease of surface energy due to hydrogen bonds between CS chains and water molecules, ii) the establishment of novel interactions between PVA and CS chains after local dissolution of CS chains (in the acidic tumour environment). These authors also mentioned that the 5-FU release was promoted in the acidic tumour environment, reducing the side effects and protecting the healthy surrounding tissues.

Thermosensitive hydrogels have also been obtained by a photo-crosslinking reaction. To this end, chemical species were introduced in the formulation such as methacryloyl, ethylene glycol acrylate methacrylate, or azidobenzoic acid, which allowed the gelation *via* an UV irradiation. Wang and co-workers [58] have elaborated gels of CS/poly N-isopropylacrylamide (PNIPAM)/photothermal carbon (chemical species leading to hydrogel formation and contraction with the increasing temperature). Thanks to these photosensitive

compounds in the formulation, a localized and controlled release of drugs has been achieved by the gel contraction. Non- invasive near infrared irradiation was operated with a deeper tissue penetration than UV-Visible light. A contraction of CS hydrogel was observed (> 40% of its initial volume) after irradiation due to photothermal carbon. Doxorubicin, incorporated before gelation, was thus released 40 times faster after hydrogel irradiation than a conventional diffusion.

Antibiotics have also been embedded into thermosensitive hydrogels. Ren *et al*. [59] inhibited the burst effect observed with metronidazole injections by its incorporation into a CS/gelatin hydrogel. These authors showed an *in vitro* sustained delivery of metronidazole during 12 days. Its release was demonstrated to be depending on the hydrogel formulation (i.e., CS *versus* gelatin concentration). In addition to this antibiotic incorporation, the authors have also worked on the delivery of a neurotransmitter (incorporated before gelation), dopamine, from thermosensitive CS/gelatin chemical hydrogels for Parkinson's disease. The hydrogel formation has resulted from physical and chemical interactions between hydrogel components (quaternized CS, gelatin, and dopamine). It has been shown that pore size (observed by SEM observations) and swelling rate of the hydrogel were influenced by polymer concentration and component ratios.

Drug release can also be regulated by the modification of an external parameter, as for example environmental pH (leading to a pH-sensitive release). With this aim, chemical hydrogels composed of CS and PVA crosslinked by tetraethyl orthosilicate were developed for the controlled delivery of acetylsalicylic acid [60]. First, the release had been studied at acidic pH (pH = 1.2) during 2h to mimic gastric environment, and then during 7h at pH = 6.8 to mimic intestinal environment. About 85% of acetylsalicylic acid was released after these 9 hours. This release is attributed to the swelling capacity of the hydrogel, related to the environmental pH. Indeed, authors mentioned that "hydrogels showed a low swelling at acidic pH and maximum swelling was exhibited at neutral pH.". At a neutral pH, the bigger the swelling, the higher the drug release. In this study, the release was preferred in the intestinal environment because of the presence of mucosa, that allows drug absorption and enhances drug efficiency.

Risbud *et al.* [61] worked on pH-sensitive release of an antibiotic, amoxicillin (incorporated before gelation) from CS/polyvinylpyrrolidone chemical hydrogels obtained by crosslinking with glutaraldehyde (GA), forming semi-interpenetrating network (semi-IPN). Hydrogels could be then dried by two methods, « freeze-drying », or « air-drying » at 37°C

during 72h, and this post-treatment influenced hydrogels morphology and amoxicillin release [62]. The resulting lyophilisates presented more important swelling (after immersion in solutions with different pH), even more at acidic pH (pH = 1 or 2) when CS ammoniums are protonated. In these conditions, the sudden release of antibiotics can be triggered at a given localization, optimal for stomach release for example. These encouraging results confirmed that these freeze-dried hydrogels could be used for *Helicobacter Pilori* treatment, known for gastric mucosa colonization [63].

Zhang *et al.* [64] elaborated thermosensitive CS/hyaluronic acid (HA) chemical hydrogels for the pH-sensitive release of doxorubicin incorporated before gel formation. In this original study, the gelation was operated at 37°C and the release of drugs entrapped in the matrix was governed by surrounding pH. Doxorubicin release was thus slower at pH 4.00 rather than pH 6.86 (**Figure 2**) with only 30% of doxorubicin release at pH = 4.00 after 120h of study, when more than 50% released at pH = 6.86 during the same time. Furthermore, the higher HA concentration in the hydrogel formulation, the slower the doxorubicin release (at pH = 4.00 and 6.86). At pH=6.86, only 45% of doxorubicin was released within 40h through hydrogel with only 3 wt.% of HA contrary to the formulation without HA that released almost all the initially incorporated doxorubicin. Authors attributed this trend to acid dissociation constants of species ($pKa_{Glycerophosphate}$ = 6.34 and pKa_{HA} = 3.00) that influence hydrogen bonds formation with CS protonated ammoniums. Indeed, the higher the pKa, the weaker the dissociation. HA is thus more dissociated than glycerophosphate, so, according to authors, hydrogen bonds between HA carboxylic groups and CS ammonium functions are promoted, limiting burst release.

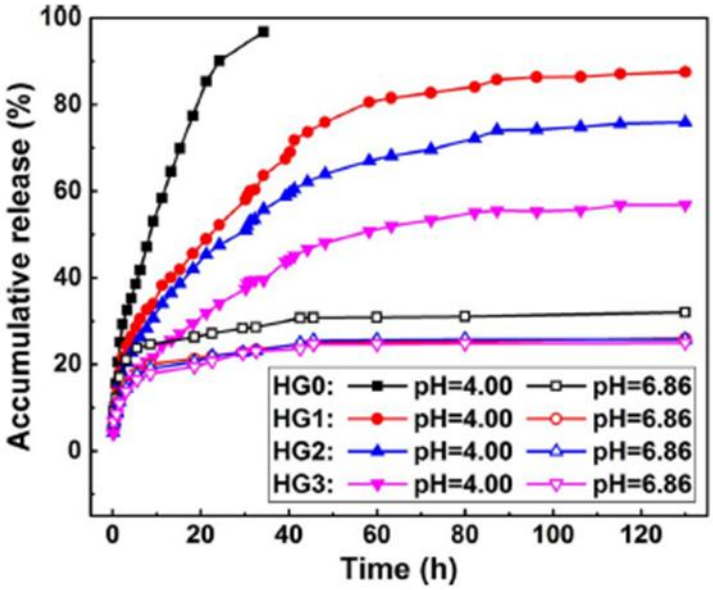

Figure 2. Cumulative release of doxorubicin (% of total incorporated amount) from thermosensitive CS hydrogels (black squares) or from thermosensitive CS/HA hydrogels (3% (w/v), pink triangles; 2% (w/v) blue triangles and 1% (w/v), red circles) at pH=4,00 (filled markers) or at pH = 6,86 (empty markers), from Zhang et al. [64].

The drug release can also be managed by applying an electrical current (leading to a electrical-sensitive release). Ramanathan and co-workers [65] worked on an electrically-modulated release of three drugs, hydrocortisone (neutral), benzoic acid (anionic) and lidocaine (cationic), separately incorporated into CS chemical hydrogels. These authors showed, after normalisation by drug molecular mass, that the release rate followed this order: benzoic acid > hydrocortisone > lidocaine. Hydrocortisone release was governed by electro-osmotic and diffusion forces within the hydrogel, whereas the polarity was implied for the two other drugs. Note that the higher the applied current, the faster the release.

This original property has also been studied by Qu *et al.* [66] for the controlled delivery of amoxicillin from an *in situ* gelling CS/polyaniline chemical hydrogel which were sensitive to various stimuli as for example pH or voltage. In this study, polyaniline was used as one of the polymers for hydrogel elaboration, in order to make them conductive [67], and drug was incorporated before gelation process. These authors demonstrated that the more acidic the pH or the higher the voltage, the faster amoxicillin release. As can be seen in **Figure 3**, the application of an electrical current acted as an « on-off switch » that governed the antibiotic release. Furthemore, in absence of applied current, the amoxicillin release was governed by diffusion caused by a concentration gradient whereas with the applied current, the release was

governed by two mechanisms: i) electric field driving the migration of charged molecules, ii) change in the overall net charge of polyaniline chains (oxidation or reduction).

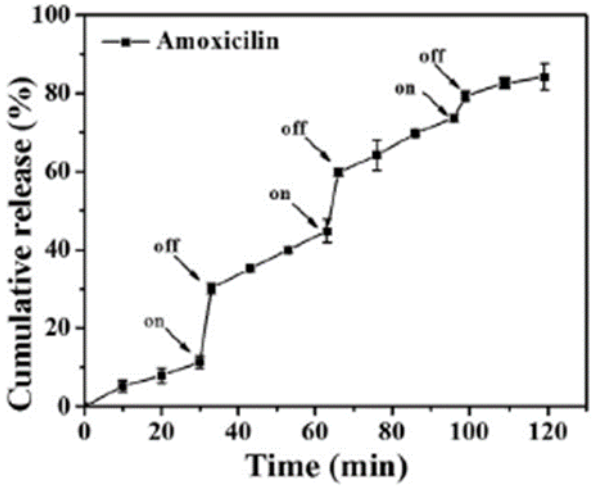

Figure 3. Cumulative release in PBS at pH = 7,4 (% of total incorporated amount) of amoxicillin from CS/polyaniline after application of a 3V electric field (on) during 3 min from Qu et al. [66]

Physical hydrogels

CS physical hydrogels can be employed for the controlled delivery of hydrophilic drugs with antimicrobial and anti-oxidative properties, such as thymol, a drug administered by oral route *via* buccal mucosa. Alvarez Echazu *et al.* [68] studied this hydrogels efficiency against bacteria as for example *staphylococcus aureus* and *streptococcus mutans* during 72 hours. An anti-oxidative property of thymol has been evidenced even at low concentrations (1.25 mg/mL). 100% of initially incorporated thymol (incorporation *via* incubation of hydrogel in a thymol solution during 7h) was released in less than 24 hours by diffusion from the hydrogel to Fusayama Meyer artificial saliva, an artificial environment close to natural saliva.

Chen *et al.* [69] worked on doxorubicin incorporation into gold nanoparticles/CS hybrid physical hydrogels. Indeed, $AuCl_4^-$ ions are able to form electrostatic interactions with CS ammoniums in solution. Doxorubicin incorporation is done by incubation of lyophilized hydrogels into doxorubicin solutions at different concentrations for 2 days. One can regrettably note that no comparison has been done in this work between hydrogels with and without gold nanoparticles. A plateau around 60% of doxorubicin released from hybrid hydrogels was reached after 8 hours at pH = 7.4 and at 37°C (diffusion through the hydrogel)

explained by strong electrostatic interactions between doxorubicin and CS. In order to check this hypothesis, the hydrogel was put into acidic conditions, leading surprisingly to the delivery of additional 30% of doxorubicin. In addition, the use of a low concentration of doxorubicin locally decreases drug cytotoxicity, and doxorubicin side effects.

In situ gelling hydrogels were also used for the delivery of the dipyridamole, an antiplatelet drug [70]. In this study, hydrogel formation at 37°C was provided by $NaHCO_3$ addition in CS solution, and the drug (incorporated before gelation) was progressively released by a diffusion process during one month. The authors noticed that $NaHCO_3$ concentrations had a non-negligible influence on the drug release, and that the higher the concentration, the faster the hydrogel formation, the higher hydrogel density and the slower the release. Electrostatic interactions between H^+ ions (coming from CS solubilisation in aqueous acidic medium) and HCO_3^- allowed to achieve hydrogel formation. This was associated with a CO_2 release, which created additional micropores in the hydrogel matrix.

Ruel-Gariépy *et al.* [71] elaborated a thermosensitive CS/GP physical hydrogel for the delivery of model molecules, drugs or water-soluble proteins. The release of methylene blue (cationic, 320 $g.mol^{-1}$), calcein (anionic, 623 $g.mol^{-1}$), chlorphenamine (cationic antihistaminic, 275 $g.mol^{-1}$), and a fluorescent albumin (anionic, fluorescein isothiocyanate-albumin, FITC-albumin at pH = 7.0, ca 66.10^3 $g.mol^{-1}$) were studied. They showed that the higher the molecular weight, the slower the release (*e.g.*, FITC-albumin *versus* calcein). According to these authors, the charge of the incorporated molecule had no influence at low concentrations, whereas at high concentrations, the release of an anionic molecule was slower thanks to electrostatic interactions with cationic polymer chains. For example, anionic groups of FITC-albumin at pH = 7.0 were linked to ammoniums along the CS chain. They also studied the influence of lysozyme addition, an enzyme degrading CS, on the release of albumin. The lysozyme addition was expected to hydrolyze CS glycosidic bonds, and leads to a consecutive sudden release of anionic species. Nevertheless, despite the lysozyme addition, less than 100% of FITC-albumin were finally released (**Figure 4**). The authors explained this result to the high resistance of this type of hydrogels (*DA* < 25%) to enzymes at the employed concentration in this study (0.25 mg/mL).

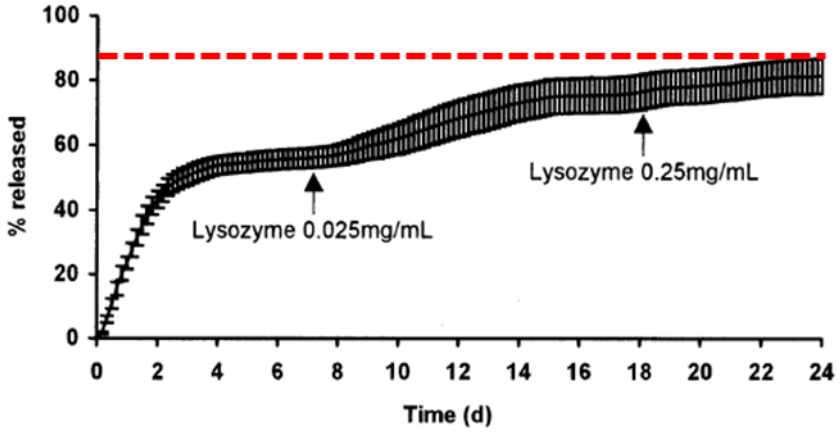

Figure 4. Mean release profile of FITC-albumin incorporated into a CS/GP hydrogel in PBS at pH = 7.4 and 37°C before and after lysozyme additions (at 2 different lysozyme concentrations) [71]

As the formation of thermosensitive hydrogels is achieved at temperatures higher than at ambient temperature, they can also be used for the treatment of irritated mucosa. The formulations can thus be administered before gelation in the form of spray [72] or by intranasal route [73]. To this end, Naik *et al.* [74] elaborated thermosensitive CS/GP physical hydrogels and revealed that 8% of GP were necessary to achieve hydrogel formation at 37°C. The addition of more than 8% of GP resulted in a supertonic solution, incompatible with a nasal administration (due to a solute concentration above the cytoplasm one). They studied the influence of polyethylene glycol (PEG) addition in the formulation, an additive which ensures the hydrogel formation, with a smaller GP amount. Thanks to mucoadhesive properties of CS, electrostatic interactions took place between cationic charges of D-glucosamine units of CS and anionic charges of sulfates and sialic acids of mucin (proteins secreted by mucosa). It has also been shown that CS improved the permeation of nervous system by polar formulations (opening the junctions between epithelial cells without immune response).

Thermosensitive physical hydrogels based on CS derivatives (trimethyl CS and methylpyrrolidone CS) and GP incorporating benzydamine (anti-inflammatory drug) were also elaborated for the treatment of buccal mycosis by Rossi *et al.* [75]. These authors have demonstrated that the hydrogel formation was influenced by the degree of substitution of CS derivative and the CS molar mass. For example, a high substitution degree and a high molar mass prevented the hydrogel formation. They also noticed an antimicrobial activity of CS hydrogels, and as expected, this effect was enhanced by benzydamine addition.

These CS hydrogels with thermosensitive properties were also used for the drug delivery in the inner ear for the treatment of Ménière's disease. Lajud *et al.* [76] used an enzyme, the chitosanase, for a better control of gentamicin (antibiotic) release. A CS hydrogel (20 g/L, 2% w/v) was completely destroyed 30 min after the chitosanase addition at 37°C. Despite a needed second injection to obtain a 100% gentamicin release, this system allows a localized delivery of gentamicin.

As previously mentioned for the CS chemical hydrogel, a pH-sensitive release of doxorubicin has also been obtained by Wu *et al.* [77] with a physical CS hydrogel. Indeed, at acidic pH, 80% of initially incorporated doxorubicin was released in less than 6h (contrary to only 9% at pH higher than 8). **Table 1** summaries the studies described above and others found in the literature about the delivery of hydrophilic drugs from CS hydrogels.

Table 1. Delivery of various hydrophilic drugs from chemical or physical CS hydrogels

Drug	Hydrogel type	Biomedical application	Administration route	Reference
5-fluorouracil	Chemical	Anticancer	Intratumoral	[51]
Benzoic acid	Chemical	Food additive	Unspecified	[65]
Amoxicillin	Chemical	Antibiotics against *Helicobacter Pylori*	Oral	[61], [62]
Acetylsalicylic acid	Chemical, *in situ* gelation	Antalgic, antipyretic, anti-inflammatory, antiplatelet	Oral	[60]
Benzydamine	Physical, *in situ* gelation	Anti-inflammatory for buccal mycosis treatment	Oral	[75]
Methylene blue	Physical, *in situ* gelation	Model molecule	Unspecified	[71]
Calcein	Physical, *in situ* gelation	Model molecule	Unspecified	[71]
Chlorpheniramine	Physical, *in situ* gelation	Antihistaminic for rhinitis, urticaria, allergy, asthma and fever treatment	Parenteral	[71]

Dipyridamole	Physical, *in situ* gelation	Antiplatelet	Sub-cutaneous	[70]
Dopamine	Physical and chemical, *in situ* gelation	Neurotransmitter	Parenteral *in situ*	[59]
Doxazosin	Chemical	Anticancer (cervical)	*In situ* implantation	[54]
Doxepin	Physical, *in situ* gelation	Antidepressant for bolt and moderate depressions and mycosis irritations	Intranasal	[74]
Doxorubicin	Physical, *in situ* gelation	Anticancer for glioma and carcinoma treatment	Intratumoral	[77]
	Chemical, *in situ* gelation			[64]
	Chemical			[58]
	Physical, *in situ* gelation			[78]
	Physical			[69]
Gentamicin	Physical, *in situ* gelation	Antibiotics for inner ear (Ménière's disease)	*In situ* in the inner ear	[76]
Lidocaine	Chemical Physical Chemical	Local and surface anaesthetics, dentistry	Oral	[108], [109], [111]
Metronidazole	Chemical and physical, *in situ*	Antibiotics (Parkinson's disease)	Parenteral	[59]

	gelation			
Papaverine	Chemical	Vasodilator	Unspecified	[79]
Thymol	Physical	Antioxidant, antimicrobial	Oral	[68]
Venlafaxine	Physical, *in situ* gelation	Antidepressant	Nasal or parenteral	[73]

All these studies showed that the CS hydrogels are very interesting systems for the delayed release of numerous hydrophilic drugs thanks to the hydrophilic behaviour of polymer chains, as well as the porous structure of hydrogels. After this part concerning hydrophilic drugs, the following part deals with the incorporation and the delivery of hydrophobic drugs.

3. Chitosan hydrogels for the controlled release of hydrophobic drugs

The major drawback of hydrophobic drugs is that they often have a low therapeutic index (*i.e.*, the ratio of the effective dose to the lethal dose of a drug [80]) in the organism. They are thus quickly absorbed by lymphatic system after administration, so that the dose has to be drastically increased in order to obtain the intended therapeutic effects (often at the expense of side effects). To overcome this low bioavailability of hydrophobic drugs, a possible alternative is to incorporate them into CS hydrogels to delay the diffusion, and thus the drug release. Nevertheless, the hydrophilic behaviour of chitosan hydrogels can make this operation difficult [81]. To this end, polymer chains are often chemically modified to enhance hydrophobic character of the CS hydrogel.

For cancer treatments, new strategies for hydrophobic anticancer drugs delivery have to be developed. Because of their low water-soluble property, higher concentrations of drugs are commonly used to obtain satisfactory results, but leading to an increase in side effects too. Obara *et al.* [37] embedded paclitaxel into CS chemical hydrogels before gelation process. Gelation was achieved by CS photo-crosslinking (condensation reaction), forming covalent bonds under UV irradiation between CS and lactobionic acid (that makes CS water-soluble at neutral pH) and azidebenzoic acid.

The hydrogel ability to limit the growth of mouse sub-cutaneous tumors (inhibition of Lewis lung cells (3LL) proliferation) was shown in this study. The therapeutic action lasts 2 times longer than free paclitaxel (almost 40% of incorporated paclitaxel were released in one day). *In vitro*, a progressive release was observed, with a plateau around 80% of paclitaxel

released 4 days after injection in the culture medium. Finally, this system was able to reduce angiogenesis [82] (*i.e.*, new blood vessels formation from pre-existing vessels promoting the metastasis formation), without preventing fibroblasts growth (*i.e.*, cells partially constituting the connective tissue).

Always for cancer treatments, implants based on CS chemical hydrogels were used for local radiotherapy, also called « brachytherapy » with controlled delivery of radioactive molecules. In this context, Azab *et al.* [83], [29] have incorporated, before hydrogel formation, a hydrophobic molecule, Sudan black, into the CS chemical solution. The resulting CS hydrogels were then injected into the mouse. Two hydrogels with different degradation properties were elaborated, a fast degradation hydrogel (FDG, prepared by dialysis against water) and a slow degradation hydrogel (SDG, prepared by dialysis against PBS, pH = 7.4), as a function of dialysis media used for the hydrogel elaboration. The authors showed that the Sudan black release was clearly influenced by the hydrogel degradation. Indeed, 100% of the initially incorporated Sudan black was released in less than one week, contrary to SDG that released less than 20% during almost one month. In conclusion, the drug release could be modulated by a better control of hydrogel degradation property, and this system could also be used for post-chirurgical brachytherapy treatments (after a lung tumour resection for example, in order to limit side effects of traditional radiotherapy).

Ruel-Gariépy and co-workers [84] have elaborated thermosensitive CS/GP chemical hydrogels to delay the release of a hydrophobic anticancer drug, paclitaxel, incorporated before the gelation process. With this *in situ* gelling formulation, only 32% of initially incorporated paclitaxel were released *in vitro* within 17 days. *In vivo*, an intratumoral injection of this hydrogel seemed to be as efficient as four intravenous injections of free paclitaxel. The incorporation of paclitaxel in the CS hydrogels constrained the tumour growth while reducing side effects of the drug itself. Similar results were obtained by Berrada *et al.* [85] after intratumoral injection of a thermosensitive formulation for the delivery of camptothecin, an anticancer drug too (incorporated before the gelation process). A sustained release of the drug has been observed during more than one month, and the sub-cutaneous tumour growth was delayed for more than 10 days *in vivo* on mouse model.

CS hydrogels can also be used for the cure of parasites thanks to the controlled delivery of antibiotics. As previously mentioned, some of CS hydrogels can release drugs as a function of environmental pH. For example, Vaghani and co-workers [86] elaborated a chemical hydrogel based on carboxymethyle-CS (CMC) for the delivery of ornidazole (an anti-infective drug

incorporated before gelation process) to treat amoebiasis (large intestine infection). They have shown that the most appropriate ornidazole:CMC weight ratio to delay the release was about 1:2.5, with a sustained release of ornidazole during 12h. They also noticed that the hydrogel swelling and the drug release were pH-sensitive. Indeed, the ratio above-mentioned (1:2.5 ornidazole:CMC) allowed the highest incorporation rate of ordinazole (92%). The highest swelling rate was obtained at a pH around 7.0, leading to the faster release. This team also worked on the controlled release of repaglinide (an antidiabetic drug) from CS/polyvinyl pyrrolidone (PVP) semi IPN hydrogels for non-insulin-dependent diabetes [87].

Another alternative for the incorporation of hydrophobic drugs consists in polymer chain modification to make it more hydrophobic. A chemical hydrogel based on carboxymethyl-hexanoyl-CS has been prepared by Liu *et al.* [88] for a better control of ibuprofen release (incorporated before gelation process). Some ammonium ions of CS were substituted by hexanoyl ones, enhancing the hydrophobicity of polymer chains and allowing the drug incorporation to be easier. This study also revealed that the higher the substitution degree, the slower the diffusion within the hydrogel.

CS hydrogels were also employed for ophthalmology applications, which often require repeated administrations (once a day or even more) of ocular drops. That can be very uncomfortable for patients and a non-negligible portion of drug is lost in tears. Cheng *et al.* [89] worked on the controlled release of latanoprost (an anti-glaucoma collyrium) of *in situ* gelling formulation of CS and gelatin for glaucoma treatment after an ocular pressure change. *In vitro,* the release of latanoprost (incorporated before gelation process) from this physical hydrogel was about 70% of initially incorporated drug over one month. *In vivo,* a decrease in ocular pressure was observed after the *in situ* hydrogel intraocular injection. After 8 days of treatment, ocular pressure was back to normal, and then stayed stable over almost a month. No immune response was detected up to 60 days after treatment, proof of its biocompatibility. For this particular disease, only one injection per month could thus be very interestingly sufficient (instead of once a day).

More recently, the same team worked on a less invasive alternative with a topical administration of eye drops [90]. One drop per week of *in situ* gelling solution incorporating latanoprost has seemed to be sufficient to reduce ocular pressure (contrary to a daily administration for free latanoprost).

Zhu *et al.* [91] elaborated a CS/HA/GP chemical hydrogel for the controlled delivery of kartogenin, an anti-inflammatory drug, incorporated before gelation process, for intervertebral

discs regeneration. Kartogenin is implied in mesenchymal stem cells differentiation into chondrocytes. In this study, the hydrogel containing kartogenin enhanced cell proliferation in comparison with the hydrogel without drug over 16 days. This represented promising results for intervertebral discs regeneration and wound filling. Thanks to that system, tissue regeneration was promoted *via* a better stem cells differentiation into *nucleus pulposus* specific cells (central part of the intervertebral disc).

Qu and co-workers [92] elaborated a polyaniline grafted CS chemical hydrogel, crosslinked by oxidized dextran for electrically-modulated delivery of amoxicillin and ibuprofen (incorporation of drugs before gelation process). Same release profiles were obtained for the two hydrophobic drugs: the higher the applied electrical current, the faster the release.

Furthermore, physical and chemical interactions combination can also be interesting for CS hydrogel elaboration. Songkroh *et al.* [93] worked on the elaboration of CS hydrogels chemically crosslinked with genipin in the presence of sodium salts. Covalent bonds were created between CS and genipin, but also electrostatic interactions took place between sodium salts and CS ammoniums. Curcumin, a natural pigment with anti-oxidative, anti-inflammatory and antibacterial properties, was incorporated before gelation process in these hydrogels. The authors mentioned that the higher the pore density, the faster the release with a faster diffusion. It has also been shown that the hydrogel porosity could be modified by the nature of added salt, influencing the drug incorporation rate and the drug release.

As presented above for hydrophilic drugs, the

Table 2 summaries the studies described above and others found in the literature about the delivery of hydrophobic drugs from CS hydrogels.

Table 2. Delivery of various hydrophobic drugs from chemical or physical CS hydrogels

Drug	Hydrogel type	Biomedical applications	Administration route	Reference
Camptothecin	Chemical, *in situ* gelation	Anticancer	Intratumoral	[85]
Curcumin	Chemical, *in situ*	Antibacterial, anti-oxidative, anti-inflammatory	Sub-cutaneous	[93]

	gelation			
Hydrocortisone	Chemical	Anti-inflammatory	Unspecified	[65]
Ibuprofen	Chemical, *in situ* gelation	Anti-inflammatory, analgesic	Sub-cutaneous	[92]
	Chemical		Unspecified	[88]
Indometacin	Chemical	Anti-inflammatory	Unspecified	[79]
I-norcholesterol	Chemical	Radioactive element for brachytherapy	Sub-cutaneous or intra-peritoneal	[83]
	Chemical			[29]
Latanoprost	Chemical, *in situ* gelation	Hypotensor	Intra-ocular Topical (eye drops)	[89]
	Chemical, *in situ* gelation			[90]
Ornidazole	Chemical	Anti-infectious	*In situ* in the colon	[86]
Paclitaxel	Chemical, *in situ* gelation	Anticancer	Intratumoral Sub-cutaneous	[84]
	Chemical			[37]
Repaglinide	Chemical	Antidiabetic	Parenteral	[87]
Sudan black	Chemical	Model molecule	Sub-cutaneous or intra-peritoneal	[83]

Although more complicated than hydrophilic drugs, the incorporation of hydrophobic drugs in CS hydrogels is thus possible. However, regardless of the hydrophilic or hydrophobic behavior of molecular drugs, the latters are often very quickly released from hydrogels because of their low molar mass. The following part is about the release of higher molar mass drugs (macromolecules with molar masses above 1,000 $g.mol^{-1}$) incorporated into chitosan hydrogels.

4. Chitosan hydrogels for the controlled release of macromolecular drugs

For a few years, macromolecules such as peptides and proteins have increasingly been used as therapeutic agents. However, they have a low bioavailability and a short half-life [94] because they are easily denatured or degraded in the organism. Consequently, their repeated administrations are necessary [95]. An alternative consists in incorporating them into hydrogels in order to protect them from enzyme degradation.

The following paragraphs are firstly dedicated to studies about the incorporation of a model protein, bovine serum albumin (BSA), in CS hydrogels. Secondly, the studies dealing with peptides and proteins with biomedical interest are presented and classified according to their targeted application (tissue engineering, wound healing, cancer and other disease treatments).

Release of a model protein (BSA)

With the aim of proving hydrogel efficiency for the protein delivery, several researchers have worked on the elaboration of CS hydrogel for the controlled delivery of BSA, a model protein (ca 66 000 $g.mol^{-1}$). In 2004, Chen *et al.* [96] have elaborated a carboxymethyl CS chemical hydrogel, crosslinked by genipin for a pH-sensitive delivery of BSA, incorporated before gelation process. The hydrogel elaboration was achieved at a neutral pH in order to protect the proteins. These authors showed that genipin concentration was directly related to hydrogel swelling and thus protein release (also depending on surrounding pH). The more acidic the pH, the lower the hydrogel swelling thanks to numerous hydrogen bonds. At neutral pH (pH = 7.4), carboxylic groups on the polymer chain are ionized, introducing electrostatic repulsions forces leading to a higher hydrogel swelling. This latter improved drug diffusion and protein release (with almost 80% of BSA released over 5h whereas only 20% at pH = 1.2, **Figure 5**).

Another study has been done with carboxymethyl CS chemical hydrogels crosslinked with glutaraldehyde [95], and similar results were obtained. The protein was protected by the hydrogel in the stomach environment, and was then progressively released in the intestine (pH close to 7.4). Similarly, at this neutral pH, there was a fast hydrogel swelling that released 30% of the initially incorporated BSA (burst release), followed by a sustained release of the protein.

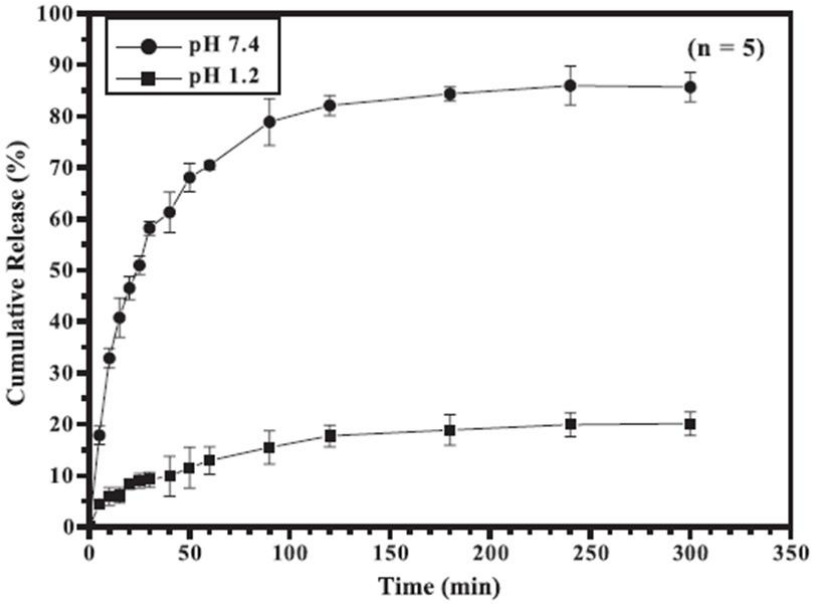

Figure 5. Cumulative release of BSA (% of total incorporated amount) at pH = 1.2 (squares) and at pH = 7,4 (circles) from CS/alginate/genipin hydrogels [96]

Thermosensitive CS hydrogels were also used for the controlled delivery of BSA. In 2012, Li and co-workers [97] worked on the association of carboxymethyl CS, GP, as well as oxidized alginate, for the elaboration of a thermosensitive chemical hydrogel for the controlled delivery of BSA (incorporated before gelation process). Covalent bonds took place between CS ammoniums and alginate aldehydes, forming a Schiff base (C=N bonds). BSA release was dependent on the pore size, swelling rate, and crosslinking rate of the hydrogel. First of all, a fast delivery of BSA was observed due to the protein diffusion. It was shown that this protein diffusion was limited by an increased alginate concentration, creating a denser and a less porous hydrogel, unfavourable to protein diffusion. In a second time, the protein release was governed by the hydrogel degradation, more or less fast according to the hydrogel composition.

Bhattarai *et al.* [98] used genipin as a crosslinking agent to delay the release of BSA. In this study, covalent bonds were created between CS and PEG chains, promoting CS solubility in aqueous medium at a neutral pH. Then, genipin was added to allow the hydrogel formation at body temperature in almost 10 min. This crosslinker addition also decreased the pore size, and slowed down the protein release (incorporated before gelation process). Less than 50% of BSA were released over one week for CS/PEG/genipin hydrogels, contrary to CS/PEG that released the same quantity over 5 hours.

Ruel-Gariépy *et al.* [71] elaborated thermosensitive physical hydrogels incorporating (before gelation process) FITC-albumin and dextran (between 12,000 and 148,000 g.mol^{-1}) and methylene blue (320 g/mol^{-1}). The authors also mentioned that the higher the molar mass of the incorporated molecule, the slower the release. Indeed, a plateau around 50% of released albumin was reached after 3 days, when 100% of methylene blue were released over 30h.

As Risbud *et al.* [61], Li and co-workers [97] elaborated a thermosensitive semi-IPN system based on carboxymethyl CS chemical hydrogels crosslinked by genipin and associated to non-crosslinked alginate chains. Alginate chains were expected to enhance i) the hydrogel swelling, ii) the interactions between aldehyde and hydroxyl groups coming from alginate, and with amino, hydroxyl and carboxymethyl groups from modified CS. The aim of the authors was to incorporate proteins before the gelation process, and then to release them in a very acidic environment such as stomach environment.

Tissue engineering applications

As previously mentioned, CS hydrogels can be used for tissue engineering applications. To improve their efficiency, a strategycan be the incorporation of proteins in the hydrogel network.

Muzzarelli *et al.* [99] and then Mattioli-Belmonte *et al.* [100] have worked on the elaboration of chemical hydrogels based on N, N-dicarboxymethyl chitosan (DCMC) associated to bone morphogenetic protein (BMP) (incorporated before gelation process). BMP are proteins implied in bone and cartilage morphogenesis after cartilage lesions. It has been shown that the complexes formed by electrostatic interactions between DCMC and BMP enhanced the cell density and the filling of cartilage lesions, while constraining inflammation of surrounding tissue. Furthermore, this system incorporating BMP and DCMC also leaded to an interesting *in vivo* cell proliferation and their differentiation into chondrocytes.

Ishihara *et al*. [101] have elaborated CS/lactose chemical hydrogels, photo-crosslinked by UV irradiation, and incorporating BSA and growth factors (FGF-A, FGF-2, $VEGF_{165}$, EGF, b-EGF, added before gelation process) in order to promote the extracellular matrix formation. The authors have shown that the higher the polymer concentration, the slower the release, probably because of a slower diffusion of the protein. Firstly, a burst effect was observed with the delivery of 15% of the initially incorporated growth factors during the first day. Then, this burst effect was followed by their sustained delivery thanks to the *in vivo* progressive hydrogel degradation over 10 to 14 days. These authors have also shown that this progressive

delivery allowed vascularization in less than 5 days, which is very interesting for tissue engineering applications.

Chenite and co-workers [102] have elaborated thermosensitive CS/GP physical hydrogels for controlled delivery of growth factors and chondrocytes (incorporated before gelation process) for regeneration of cartilage and intervertebral discs [103]. Gelation was achieved at pH = 7.15 at 37°C, and was demonstrated to be reversible (**Figure 6**). After an *in vivo* sub-cutaneous injection on rats, the formation of neo-tissues was also interestingly observed.

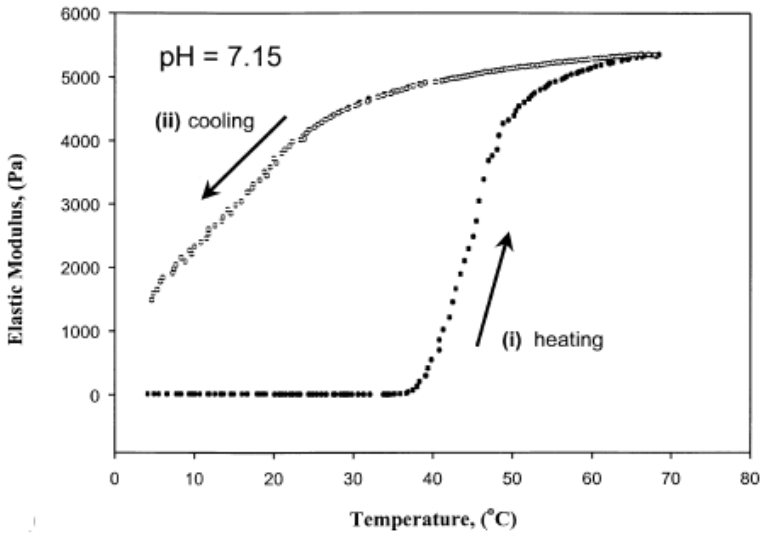

Figure 6. Elastic modulus G' variations of a CS/GP thermosensitive hydrogel as a function of temperature at pH = 7.15 [102]

Naderi *et al.* [104] examined the scaffold elaboration based on CS, GP, and hydroxyethylcellulose hydrogels for cartilage regeneration too. A hormone, insulin (protein of *ca* 5,800 $g.mol^{-1}$), was incorporated into these hydrogels before their gelation with the aim of replacing IGF-1 growth factor (implied in chondrocytes differentiation and thus in cartilage regeneration) because they are structurally analogous. It has been shown that these thermosensitive hydrogels could delay the release of insulin over 8 days compared to the reference.

Other studies [105] have shown that the incorporation of demineralized bone matrix (essentially composed of BMP and collagen) could also be performed (before gelation process) into thermosensitive CS/GP hydrogels to enhance osteoinduction process. Thanks to these systems, Tian *et al.* have revealed *in vivo* in rat models, the formation of a new cellular layer between the hydrogel and the pre-existing tissue in less than 7 days. Nevertheless, note

that an inflammatory response has been observed due to the presence of GP (*i.e.*, an increase of macrophage and lymphocyte concentrations, which decreased 3 weeks after injection).

Antibodies (immunoglobulin, IgG) or antibodies fragments (Fab antibodies fragments) have also been incorporated into CS/alginate physical hydrogels [106]. These bio-macromolecules (incorporated before gelation process) could be slowly released by a diffusion process. The authors mentioned that the higher CS/alginate ratio (not exceeding 50% v/v) the slower the release (**Figure 7**). According to the authors, electrostatic interactions between cationic CS chains and anionic charged antibodies could be a possible explanation. The authors also showed that antibodies (due to their higher size) were slower released than antibodies fragments.

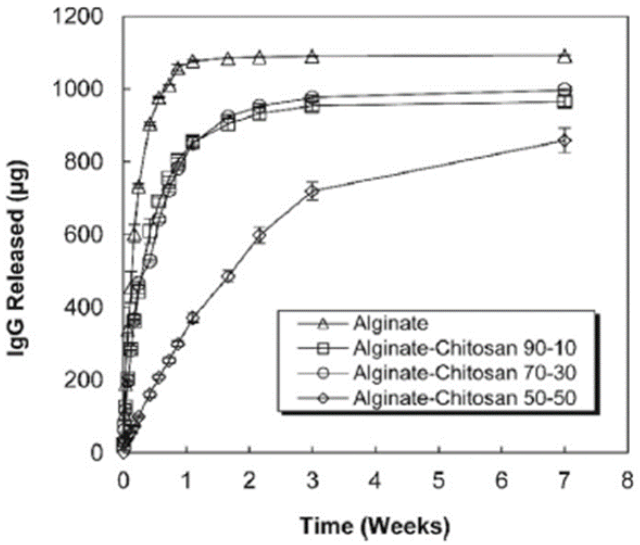

Figure 7. Cumulative release of immunoglobulin (IgG) from hydrogels with various alginate/CS ratios [106]

Wound healing

Three distinct and successive steps are implied in wound repair process: i) inflammation, ii) granulation and tissue formation, iii) matrix formation and remodelling [107]. FGF-2 (*ca* 18,000 $g.mol^{-1}$) is one of the growth factors that plays a role in proliferation of fibroblasts (dermis cells) [108], as well as in vascularization [109] (these two biological processes involved in steps ii) and iii). However, the half-life of FGF-2 is very short. Thus, its incorporation in CS hydrogels seemed to be interesting to improve the efficiency of FGF-2 *in situ* [110]. In this context, Obara and co-workers [111] have elaborated a photo-crosslinked chemical hydrogel based on CS chemically modified by lactose and azide (to make CS water-

soluble). The incorporation of FGF-2 into this CS hydrogel (before gelation process) has induced a better and faster wound healing than the same hydrogel but without FGF-2 (**Figure 8**), while maintaining humid environment necessary for wound healing. The progressive release of FGF-2, efficient over 7 days *in vivo* in mouse, was due to CS hydrogel degradation. Thanks to all these works, it was demonstrated that these hydrogels could be very interesting for wound healing applications, as well as growth factor delivery.

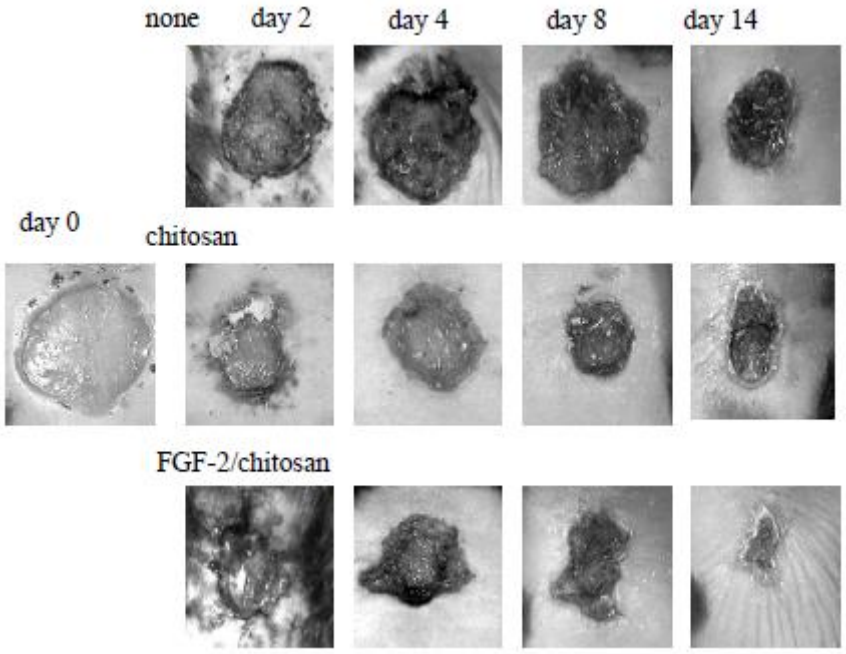

Figure 8. Wound closure of FGF-2-incorporated chitosan hydrogels in mice over 14 days without treatment (1st line), after the use of CS hydrogel (2nd line) or the use of FGF-2 incorporated CS hydrogel (3rd line) representative of eight mice in each group [111]

Finally, another different study has described a chemical modification of CS chains by macromolecules [112]. Chen and co-workers have indeed elaborated CS chains grafted with a SKYVAV peptide (6 amino acids) to improve wound healing. It has been shown that a better wound closure and a better vascularization around the wound were obtained thanks to these hydrogels, while constraining immune response of the organism. For example, the K1 marker (specific to keratinocytes differentiation in epidermis cells) was more expressed into wounds treated by peptide grafted-CS hydrogels (mean K1 density = 0.22) than unmodified CS hydrogels (mean K1 density = 0.16) after 14 days.

Cancer and other disease treatments

Chen *et al*. [113] have elaborated a thermosensitive CS/GP physical hydrogel to delay the release of Pingyangmycin (PYM, *ca* 1,440 g.mol^{-1}), an anticancer drug. This drug is also called Blomycin A5, and was incorporated before the gelation process in this study. *In vitro*, this drug was released over 12 days thanks to the slow hydrogel degradation by lysozymes. *In vivo*, the drug stability was enhanced by a longer half-life, and a prolonged action. The authors also mentioned that the gelation time could be modulated by varying GP concentration.

For cancer treatments, another strategy has consisted in over-expressing genes in the tumours, *via* transglutaminase, an enzyme implied in tumour cell proliferation. Han *et al.* [114] have elaborated a thermosensitive CS/GP physical hydrogel incorporating ribonucleic acid (RNA) and interfering RNA (SiRNA). A slowdown in cell proliferation has been shown with a decrease in TG2 gene expression (implied in tumour growth) about 60% after 48h *in vitro* injection. *In vivo*, intratumoral injection in mice has produced a tumour size reduction about 48%. The same authors have also shown that the efficiency of an anticancer drug (docetaxel) incorporated in hydrogel was increased without immune response on the injection site.

As previously mentioned, alginate chains can be associated to CS hydrogels to develop specific properties to resulting hydrogels such as a more compact structure or a pore size decrease. Xu and co-workers [115] have used this approach for the treatment of age related macular degeneration with the incorporation (before gelation process) of the bevacizumab antibody (Avastin®) in CS/alginate chemical hydrogels (involving a Schiff base reaction between glycol CS and oxidized alginate) for an intraocular administration. 30% of bevacizumab were released during the 4 first hours (diffusion process), followed by a sustained drug delivery over 3 days (slow hydrogel degradation). According to these authors, the higher the alginate concentration, the slower the degradation, and thus the slower the release.

Another thermosensitive CS/GP physical hydrogel has been elaborated by Nazar *et al.* [116] for the controlled delivery of insulin (*ca* 5,800 g.mol^{-1}, incorporated before gelation process) *via* nasal mycosis for the glycaemia regulation. Interesting results were obtained *in vitro*, and *in vivo* on diabetic mice, with a delayed release of initially incorporated insulin (50% over 90 min by intranasal route contrary to 50% over 60 min by sub-cutaneous route.

Furthermore, the insulin release was then sustained over 24 hours, which was very promising for diabete treatment.

Table 3 summaries the studies described above and others found in the literature about the delivery of macromolecular drugs from CS hydrogels.

Table 3. Delivery of various macromolecular drugs from chemical or physical CS hydrogels

Macromolecule	**Hydrogel type**	**Biomedical application**	**Administration route**	**Reference**
FITC-albumin (protein)	Physical, *in situ* gelation		*in situ* parenteral injection	[71]
Bevacizumab (Avastin®) (antibody fragments)	Chemical, *in situ* gelation	Age related macular degeneration treatment	Intra-ocular	[115]
BMP[1] (protein)	Chemical	Chondrocyte and osteoblast marker expression stimulation	*In situ* implantation or sub-cutaneous injection	[100]
	Physical, *in situ* gelation			[105]
	Chemical	Fibroblasts transformation into chondrocytes enhancement		[99]
BSA[2] (model protein)	Chemical	/	Oral or *in situ* parenteral	[96] [95]

[1] BMP = Bone morphogenetic protein
[2] BSA = Bovine serum albumin

			injection	[98]
	Chemical, *in situ* gelation			[97] [101] [117]
EGF[3] receptor (epidermal growth factor)	Unspecified	2nd degree burns therapy	Cutaneous	[118]
FGF[4], FGF-2 (Growth factor)	Chemical	Vascularization and fibroblast proliferation stimulation	Cutaneous	[111] [101]
IgG[5] (model antibody) IgG Fab fragments (antibody fragments)	Physical, *in situ* gelation	/	*In situ* parenteral	[106]
Insulin (protein hormone)	Physical, *in situ* gelation	Glycaemia regulation	*In situ* parenteral *In situ* implantation	[104] [116]
PYM[6] (glycopeptide antibiotics)	Physical, *in situ* gelation	Vascular malformation treatment	*In situ* parenteral	[113]
PYM (glycopeptide antibiotics)	Physical, *in situ* gelation	Cancer treatment	*In situ* parenteral	[113]
SiARN (interfering ARN)	Physical, *in situ* gelation	Cancer treatment	Intratumoral	[114]

Conclusion

[3] EGF = epidermal growth factor
[4] FGF = fibroblast growth factor
[5] IgG = immunoglobulin
[6] PYM = Pingyangmycin

As a conclusion, this review shows that chitosan hydrogels can be used for the delivery of various drugs, such as antibiotics, anaesthetics, hypotensors, or anticancer drugs. Indeed, chemical hydrogels, that is to say crosslinked with one or several chemical crosslinkers, and physical hydrogels, formed thanks to physical interactions, are revealed to be very efficient systems to incorporate hydrophilic, hydrophobic, as well as macromolecular drugs. Most of these studies deal with the incorporation of hydrophilic drugs, facilitated by the hydrophilic behaviour of CS hydrogel. Nevertheless, an increasing team number examine the incorporation of hydrophobic and macromolecular drugs. Indeed a lot of drugs, for example anticancer ones, present a hydrophobic behaviour. In general, they can be incorporated into CS hydrogels after chemical functionalization of CS chains. This increases their bioavailability in the organism. Furthermore, numerous hydrogels described in literature are pH-sensitive or thermosensitive, and so allow either a targeted delivery of drugs at a specific pH, or the development of injectable formulations. Whatever the incorporated drugs, the CS hydrogels act as a diffusion barrier, reducing the burst effect and side effects. Nevertheless, although the burst effect can be reduced, it can still remain non negligible and some research has to be done to design more efficient drug delivery systems. For example, an ongoing approach to improve this delayed release capacity is the incorporation of drugs into colloids, themselves entrapped within the hydrogel matrix. These colloids create a second diffusion barrier, the effect of which will add to that of the CS hydrogel.

References

[1] G. Skjak-Braek, T. Anthonsen, and P. A. Sandford, Eds., *Chitin and Chitosan: Sources, chemistry, biochemistry, physical properties and applications*. Springer Netherlands, 1989.

[2] N. K. Mathur and C. K. Narang, "Chitin and chitosan, versatile polysaccharides from marine animals," *J. Chem. Educ.*, vol. 67, no. 11, p. 938, Nov. 1990, doi: 10.1021/ed067p938.

[3] Y. D. Hang, "Chitosan production from Rhizopus oryzae mycelia," *Biotechnol. Lett.*, vol. 12, no. 12, pp. 911–912, Dec. 1990, doi: 10.1007/BF01022589.

[4] A. Clayer and A. Domard, "Élaboration d'hydrogels physiques de chitosane application à l'ingénierie tissulaire pour la régénération du cartilage," Université Claude Bernard, Lyon, 2004.

[5] M. Rinaudo, "Chitin and chitosan: Properties and applications," *Prog. Polym. Sci.*, vol. 31, no. 7, pp. 603–632, Jul. 2006, doi: 10.1016/j.progpolymsci.2006.06.001.

[6] E. P. D. Azevedo, "Chitosan hydrogels for drug delivery and tissue engineering applications," *Int. J. Pharm. Pharm. Sci.*, vol. 7, no. 12, pp. 8–14, Oct. 2015.

[7] M. Thanou, J. C. Verhoef, and H. E. Junginger, "Oral drug absorption enhancement by chitosan and its derivatives," *Adv. Drug Deliv. Rev.*, vol. 52, no. 2, pp. 117–126, Nov. 2001, doi: 10.1016/S0169-409X(01)00231-9.

[8] S. Hirano, "Bio-compatibility of chitosan by oral and intravenous administrations," *Polym. Mater. Eng. Sci.*, vol. 59, pp. 897–901, 1988.

[9] L. Elviri, M. Asadzadeh, R. Cucinelli, A. Bianchera, and R. Bettini, "Macroporous chitosan hydrogels: Effects of sulfur on the loading and release behaviour of amino acid-based compounds," *Carbohydr. Polym.*, vol. 132, pp. 50–58, Nov. 2015, doi: 10.1016/j.carbpol.2015.06.048.

[10] M. Jumaa, F. H. Furkert, and B. W. Müller, "A new lipid emulsion formulation with high antimicrobial efficacy using chitosan," *Eur. J. Pharm. Biopharm.*, vol. 53, no. 1, pp. 115–123, Jan. 2002, doi: 10.1016/S0939-6411(01)00191-6.

[11] M. Kong, X. G. Chen, K. Xing, and H. J. Park, "Antimicrobial properties of chitosan and mode of action: A state of the art review," *Int. J. Food Microbiol.*, vol. 144, no. 1, pp. 51–63, Nov. 2010, doi: 10.1016/j.ijfoodmicro.2010.09.012.

[12] Z. Guo *et al.*, "Novel derivatives of chitosan and their antifungal activities in vitro," *Carbohydr. Res.*, vol. 341, no. 3, pp. 351–354, Feb. 2006, doi: 10.1016/j.carres.2005.11.002.

[13] N. Bhattarai, J. Gunn, and M. Zhang, "Chitosan-based hydrogels for controlled, localized drug delivery," *Adv. Drug Deliv. Rev.*, vol. 62, no. 1, pp. 83–99, Jan. 2010, doi: 10.1016/j.addr.2009.07.019.

[14] T. M. M. Ways, W. M. Lau, and V. V. Khutoryanskiy, "Chitosan and Its Derivatives for Application in Mucoadhesive Drug Delivery Systems," *Polymers*, vol. 10, no. 3, p. 267, Mar. 2018, doi: 10.3390/polym10030267.

[15] H. Ueno, T. Mori, and T. Fujinaga, "Topical formulations and wound healing applications of chitosan," *Adv. Drug Deliv. Rev.*, vol. 52, no. 2, pp. 105–115, Nov. 2001, doi: 10.1016/S0169-409X(01)00189-2.

[16] M. Dash, F. Chiellini, R. M. Ottenbrite, and E. Chiellini, "Chitosan—A versatile semi-synthetic polymer in biomedical applications," *Prog. Polym. Sci.*, vol. 36, no. 8, pp. 981–1014, Aug. 2011, doi: 10.1016/j.progpolymsci.2011.02.001.

[17] A. Anitha *et al.*, "Chitin and chitosan in selected biomedical applications," *Prog. Polym. Sci.*, vol. 39, no. 9, pp. 1644–1667, Sep. 2014, doi: 10.1016/j.progpolymsci.2014.02.008.

[18] F. C. Mooren, A. Berthold, W. Domschke, and J. Kreuter, "Influence of Chitosan Microspheres on the Transport of Prednisolone Sodium Phosphate Across HT-29 Cell Monolayers," *Pharm. Res.*, vol. 15, no. 1, pp. 58–65, Jan. 1998, doi: 10.1023/A:1011996619500.

[19] L. Vodná, S. Bubeníková, and D. Bakoš, "Chitosan Based Hydrogel Microspheres as Drug Carriers," *Macromol. Biosci.*, vol. 7, no. 5, pp. 629–634, May 2007, doi: 10.1002/mabi.200600290.

[20] K. Kofuji, K. Shibata, Y. Murata, E. Miyamoto, and S. Kawashima, "Preparation and Drug Retention of Biodegradable Chitosan Gel Beads," *Chem. Pharm. Bull. (Tokyo)*, vol. 47, no. 10, pp. 1494–1496, 1999, doi: 10.1248/cpb.47.1494.

[21] K. Kofuji, Y. Murata, and S. Kawashima, "Sustained insulin release with biodegradation of chitosan gel beads prepared by copper ions," *Int. J. Pharm.*, vol. 303, no. 1–2, pp. 95–103, Oct. 2005, doi: 10.1016/j.ijpharm.2005.07.011.

[22] T. Horio *et al.*, "Effect of Photocrosslinkable Chitosan Hydrogel and Its Sponges to Stop Bleeding in a Rat Liver Injury Model," *Artif. Organs*, vol. 34, no. 4, pp. 342–347, Apr. 2010, doi: 10.1111/j.1525-1594.2009.00855.x.

[23] K. Mizuno *et al.*, "Effect of chitosan film containing basic fibroblast growth factor on wound healing in genetically diabetic mice," *J. Biomed. Mater. Res. A*, vol. 64A, 2003, doi: 10.1002/jbm.a.10396.

[24] C. Chatelet, O. Damour, and A. Domard, "Influence of the degree of acetylation on some biological properties of chitosan films," *Biomaterials*, vol. 22, no. 3, pp. 261–268, Feb. 2001, doi: 10.1016/S0142-9612(00)00183-6.

[25] A. Montembault, C. Viton, and A. Domard, "Physico-chemical studies of the gelation of chitosan in a hydroalcoholic medium," *Biomaterials*, vol. 26, no. 8, pp. 933–943, Mar. 2005, doi: 10.1016/j.biomaterials.2004.03.033.

[26] A. Montembault, C. Viton, and A. Domard, "Rheometric Study of the Gelation of Chitosan in Aqueous Solution without Cross-Linking Agent," *Biomacromolecules*, vol. 6, no. 2, pp. 653–662, Mar. 2005, doi: 10.1021/bm049593m.

[27] K. Almdal, J. Dyre, S. Hvidt, and O. Kramer, "Towards a phenomenological definition of the term 'gel,'" *Polym. Gels Netw.*, vol. 1, no. 1, pp. 5–17, Jan. 1993, doi: 10.1016/0966-7822(93)90020-I.

[28] A. Singh, S. S. Narvi, P. K. Dutta, and N. D. Pandey, "External stimuli response on a novel chitosan hydrogel crosslinked with formaldehyde," *Bull. Mater. Sci.*, vol. 29, no. 3, pp. 233–238, 2006.

[29] A. K. Azab *et al.*, "Prevention of tumor recurrence and distant metastasis formation in a breast cancer mouse model by biodegradable implant of 131I-norcholesterol," *J. Controlled Release*, vol. 123, no. 2, pp. 116–122, Nov. 2007, doi: 10.1016/j.jconrel.2007.07.014.

[30] A. K. Azab *et al.*, "Crosslinked chitosan implants as potential degradable devices for brachytherapy: In vitro and in vivo analysis," *J. Controlled Release*, vol. 111, no. 3, pp. 281–289, Apr. 2006, doi: 10.1016/j.jconrel.2005.12.014.

[31] F.-L. Mi, C.-Y. Kuan, S.-S. Shyu, S.-T. Lee, and S.-F. Chang, "The study of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release," *Carbohydr. Polym.*, vol. 41, no. 4, pp. 389–396, Apr. 2000, doi: 10.1016/S0144-8617(99)00104-6.

[32] B. Hoffmann, D. Seitz, A. Mencke, A. Kokott, and G. Ziegler, "Glutaraldehyde and oxidised dextran as crosslinker reagents for chitosan-based scaffolds for cartilage tissue

engineering," *J. Mater. Sci. Mater. Med.*, vol. 20, no. 7, pp. 1495–1503, Mar. 2009, doi: 10.1007/s10856-009-3707-3.

[33] H. Du, M. Liu, X. Yang, and G. Zhai, "The design of pH-sensitive chitosan-based formulations for gastrointestinal delivery," *Drug Discov. Today*, vol. 20, no. 8, pp. 1004–1011, Aug. 2015, doi: 10.1016/j.drudis.2015.03.002.

[34] H. Tan, C. R. Chu, K. A. Payne, and K. G. Marra, "Injectable in situ forming biodegradable chitosan–hyaluronic acid based hydrogels for cartilage tissue engineering," *Biomaterials*, vol. 30, no. 13, pp. 2499–2506, May 2009, doi: 10.1016/j.biomaterials.2008.12.080.

[35] Ono Katsuaki *et al.*, "Photocrosslinkable chitosan as a biological adhesive," *J. Biomed. Mater. Res.*, vol. 49, no. 2, pp. 289–295, Nov. 1999, doi: 10.1002/(SICI)1097-4636(200002)49:2<289::AID-JBM18>3.0.CO;2-M.

[36] C. M. Valmikinathan, V. J. Mukhatyar, A. Jain, L. Karumbaiah, M. Dasari, and R. V. Bellamkonda, "Photocrosslinkable chitosan based hydrogels for neural tissue engineering," *Soft Matter*, vol. 8, no. 6, pp. 1964–1976, Jan. 2012, doi: 10.1039/C1SM06629C.

[37] K. Obara *et al.*, "Controlled release of paclitaxel from photocrosslinked chitosan hydrogels and its subsequent effect on subcutaneous tumor growth in mice," *J. Controlled Release*, vol. 110, no. 1, pp. 79–89, Dec. 2005, doi: 10.1016/j.jconrel.2005.09.026.

[38] K. Ono *et al.*, "Experimental evaluation of photocrosslinkable chitosan as a biologic adhesive with surgical applications," *Surgery*, vol. 130, no. 5, pp. 844–850, Nov. 2001, doi: 10.1067/msy.2001.117197.

[39] M. Ishihara *et al.*, "Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process," *Biomaterials*, vol. 23, no. 3, pp. 833–840, Feb. 2002, doi: 10.1016/S0142-9612(01)00189-2.

[40] S. Zhao, F. Zhou, L. Li, M. Cao, D. Zuo, and H. Liu, "Removal of anionic dyes from aqueous solutions by adsorption of chitosan-based semi-IPN hydrogel composites," *Compos. Part B Eng.*, vol. 43, no. 3, pp. 1570–1578, Apr. 2012, doi: 10.1016/j.compositesb.2012.01.015.

[41] J. Berger, M. Reist, J. M. Mayer, O. Felt, and R. Gurny, "Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications," *Eur. J. Pharm. Biopharm.*, vol. 57, no. 1, pp. 35–52, Jan. 2004, doi: 10.1016/S0939-6411(03)00160-7.

[42] A. S. Hoffman, "Hydrogels for biomedical applications," *Adv. Drug Deliv. Rev.*, vol. 64, Supplement, pp. 18–23, Dec. 2012, doi: 10.1016/j.addr.2012.09.010.

[43] J. Berger, M. Reist, J. M. Mayer, O. Felt, N. A. Peppas, and R. Gurny, "Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications," *Eur. J. Pharm. Biopharm.*, vol. 57, no. 1, pp. 19–34, Jan. 2004, doi: 10.1016/S0939-6411(03)00161-9.

[44] D. Chitkara, A. Shikanov, N. Kumar, and A. J. Domb, "Biodegradable Injectable In Situ Depot-Forming Drug Delivery Systems," *Macromol. Biosci.*, vol. 6, no. 12, pp. 977–990, Dec. 2006, doi: 10.1002/mabi.200600129.

[45] R. Dimatteo, N. J. Darling, and T. Segura, "In situ forming injectable hydrogels for drug delivery and wound repair," *Adv. Drug Deliv. Rev.*, Mar. 2018, doi: 10.1016/j.addr.2018.03.007.

[46] E. Ruel-Gariépy and J.-C. Leroux, "In situ-forming hydrogels—review of temperature-sensitive systems," *Eur. J. Pharm. Biopharm.*, vol. 58, no. 2, pp. 409–426, Sep. 2004, doi: 10.1016/j.ejpb.2004.03.019.

[47] G. Molinaro, J.-C. Leroux, J. Damas, and A. Adam, "Biocompatibility of thermosensitive chitosan-based hydrogels: an in vivo experimental approach to injectable

biomaterials," *Biomaterials*, vol. 23, no. 13, pp. 2717–2722, Jul. 2002, doi: 10.1016/S0142-9612(02)00004-2.

[48] L. Liu, Q. Gao, X. Lu, and H. Zhou, "In situ forming hydrogels based on chitosan for drug delivery and tissue regeneration," *Asian J. Pharm. Sci.*, 2016, doi: 10.1016/j.ajps.2016.07.001.

[49] T. Chen, H. D. Embree, E. M. Brown, M. M. Taylor, and G. F. Payne, "Enzyme-catalyzed gel formation of gelatin and chitosan: potential for in situ applications," *Biomaterials*, vol. 24, no. 17, pp. 2831–2841, Aug. 2003, doi: 10.1016/S0142-9612(03)00096-6.

[50] S. Sakai, Y. Yamada, T. Zenke, and K. Kawakami, "Novel chitosan derivative soluble at neutral pH and in-situ gellable via peroxidase-catalyzed enzymatic reaction," *J. Mater. Chem.*, vol. 19, no. 2, pp. 230–235, Dec. 2008, doi: 10.1039/B812086B.

[51] G. Chang *et al.*, "Self-healable hydrogel on tumor cell as drug delivery system for localized and effective therapy," *Carbohydr. Polym.*, vol. 122, pp. 336–342, May 2015, doi: 10.1016/j.carbpol.2014.12.077.

[52] H. Y. Zhou, L. J. Jiang, P. P. Cao, J. B. Li, and X. G. Chen, "Glycerophosphate-based chitosan thermosensitive hydrogels and their biomedical applications," *Carbohydr. Polym.*, vol. 117, pp. 524–536, Mar. 2015, doi: 10.1016/j.carbpol.2014.09.094.

[53] A. Chenite, M. Buschmann, D. Wang, C. Chaput, and N. Kandani, "Rheological characterisation of thermogelling chitosan/glycerol-phosphate solutions," *Carbohydr. Polym.*, vol. 46, no. 1, pp. 39–47, Sep. 2001, doi: 10.1016/S0144-8617(00)00281-2.

[54] A. Jamal *et al.*, "Identification of anti-cancer potential of doxazocin: Loading into chitosan based biodegradable hydrogels for on-site delivery to treat cervical cancer," *Mater. Sci. Eng. C*, vol. 82, pp. 102–109, Jan. 2018, doi: 10.1016/j.msec.2017.08.054.

[55] J. Xu, S. Strandman, J. X. X. Zhu, J. Barralet, and M. Cerruti, "Genipin-crosslinked catechol-chitosan mucoadhesive hydrogels for buccal drug delivery," *Biomaterials*, vol. 37, pp. 395–404, Jan. 2015, doi: 10.1016/j.biomaterials.2014.10.024.

[56] J. Kristl, J. Šmid-Korbar, E. Štruc, M. Schara, and H. Rupprecht, "Hydrocolloids and gels of chitosan as drug carriers," *Int. J. Pharm.*, vol. 99, no. 1, pp. 13–19, Sep. 1993, doi: 10.1016/0378-5173(93)90317-9.

[57] H. Zhang, H. Xia, and Y. Zhao, "Poly(vinyl alcohol) Hydrogel Can Autonomously Self-Heal," *ACS Macro Lett.*, vol. 1, no. 11, pp. 1233–1236, Nov. 2012, doi: 10.1021/mz300451r.

[58] L. Wang *et al.*, "UV-crosslinkable and thermo-responsive chitosan hybrid hydrogel for NIR-triggered localized on-demand drug delivery," *Carbohydr. Polym.*, vol. 174, pp. 904–914, Oct. 2017, doi: 10.1016/j.carbpol.2017.07.013.

[59] Y. Ren, X. Zhao, X. Liang, P. X. Ma, and 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.*, Jul. 2017, doi: 10.1016/j.ijbiomac.2017.07.130.

[60] A. Islam, T. Yasin, I. Bano, and M. Riaz, "Controlled release of aspirin from pH- sensitive chitosan/poly(vinyl alcohol) hydrogel," *J. Appl. Polym. Sci.*, vol. 124, no. 5, pp. 4184–4192, Jun. 2012, doi: 10.1002/app.35392.

[61] M. V. Risbud, A. A. Hardikar, S. V. Bhat, and R. R. Bhonde, "pH-sensitive freeze-dried chitosan–polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery," *J. Controlled Release*, vol. 68, no. 1, pp. 23–30, Jul. 2000, doi: 10.1016/S0168-3659(00)00208-X.

[62] V. R. Patel and M. M. Amiji, "Preparation and Characterization of Freeze-dried Chitosan-Poly(Ethylene Oxide) Hydrogels for Site-Specific Antibiotic Delivery in the

Stomach," *Pharm. Res.*, vol. 13, no. 4, pp. 588–593, Apr. 1996, doi: 10.1023/A:1016054306763.

[63] R. Hejazi and M. Amiji, "Chitosan-based gastrointestinal delivery systems," *J. Controlled Release*, vol. 89, no. 2, pp. 151–165, Apr. 2003, doi: 10.1016/S0168-3659(03)00126-3.

[64] W. Zhang, X. Jin, H. Li, R. Zhang, and C. Wu, "Injectable and body temperature sensitive hydrogels based on chitosan and hyaluronic acid for pH sensitive drug release," *Carbohydr. Polym.*, vol. 186, pp. 82–90, Apr. 2018, doi: 10.1016/j.carbpol.2018.01.008.

[65] S. Ramanathan and L. H. Block, "The use of chitosan gels as matrices for electrically-modulated drug delivery," *J. Controlled Release*, vol. 70, no. 1, pp. 109–123, Jan. 2001, doi: 10.1016/S0168-3659(00)00333-3.

[66] J. Qu, X. Zhao, P. X. Ma, and B. Guo, "Injectable antibacterial conductive hydrogels with dual response to an electric field and pH for localized 'smart' drug release," *Acta Biomater.*, doi: 10.1016/j.actbio.2018.03.018.

[67] L. Li, J. Ge, P. X. Ma, and B. Guo, "Injectable conducting interpenetrating polymer network hydrogels from gelatin-graft-polyaniline and oxidized dextran with enhanced mechanical properties," *RSC Adv.*, vol. 5, no. 112, pp. 92490–92498, Oct. 2015, doi: 10.1039/C5RA19467A.

[68] M. I. Alvarez Echazú, C. E. Olivetti, C. Anesini, C. J. Perez, G. S. Alvarez, and M. F. Desimone, "Development and evaluation of thymol-chitosan hydrogels with antimicrobial-antioxidant activity for oral local delivery," *Mater. Sci. Eng. C*, vol. 81, pp. 588–596, Dec. 2017, doi: 10.1016/j.msec.2017.08.059.

[69] R. Chen, Q. Chen, D. Huo, Y. Ding, Y. Hu, and X. Jiang, "In situ formation of chitosan–gold hybrid hydrogel and its application for drug delivery," *Colloids Surf. B Biointerfaces*, vol. 97, pp. 132–137, Sep. 2012, doi: 10.1016/j.colsurfb.2012.03.027.

[70] L. Liu, X. Tang, Y. Wang, and S. Guo, "Smart gelation of chitosan solution in the presence of NaHCO3 for injectable drug delivery system," *Int. J. Pharm.*, vol. 414, no. 1, pp. 6–15, Jul. 2011, doi: 10.1016/j.ijpharm.2011.04.052.

[71] E. Ruel-Gariépy, A. Chenite, C. Chaput, S. Guirguis, and J.-C. Leroux, "Characterization of thermosensitive chitosan gels for the sustained delivery of drugs," *Int. J. Pharm.*, vol. 203, no. 1–2, pp. 89–98, Aug. 2000, doi: 10.1016/S0378-5173(00)00428-2.

[72] H. Nazar *et al.*, "Thermosensitive hydrogels for nasal drug delivery: The formulation and characterisation of systems based on N-trimethyl chitosan chloride," *Eur. J. Pharm. Biopharm.*, vol. 77, no. 2, pp. 225–232, Feb. 2011, doi: 10.1016/j.ejpb.2010.11.022.

[73] Y. Peng, J. Li, J. Li, Y. Fei, J. Dong, and W. Pan, "Optimization of thermosensitive chitosan hydrogels for the sustained delivery of venlafaxine hydrochloride," *Int. J. Pharm.*, vol. 441, no. 1, pp. 482–490, Jan. 2013, doi: 10.1016/j.ijpharm.2012.11.005.

[74] A. Naik, H. Nair, A. Naik, and H. Nair, "Formulation and Evaluation of Thermosensitive Biogels for Nose to Brain Delivery of Doxepin, Formulation and Evaluation of Thermosensitive Biogels for Nose to Brain Delivery of Doxepin," *BioMed Res. Int. BioMed Res. Int.*, vol. 2014, 2014, p. e847547, Jun. 2014, doi: 10.1155/2014/847547, 10.1155/2014/847547.

[75] S. Rossi *et al.*, "Thermally sensitive gels based on chitosan derivatives for the treatment of oral mucositis," *Eur. J. Pharm. Biopharm.*, vol. 74, no. 2, pp. 248–254, Feb. 2010, doi: 10.1016/j.ejpb.2009.10.003.

[76] S. A. Lajud *et al.*, "A regulated delivery system for inner ear drug application," *J. Controlled Release*, vol. 166, no. 3, pp. 268–276, Mar. 2013, doi: 10.1016/j.jconrel.2012.12.031.

[77] J. Wu, Z.-G. Su, and G.-H. Ma, “A thermo- and pH-sensitive hydrogel composed of quaternized chitosan/glycerophosphate,” *Int. J. Pharm.*, vol. 315, no. 1, pp. 1–11, Jun. 2006, doi: 10.1016/j.ijpharm.2006.01.045.

[78] H. T. Ta, C. R. Dass, I. Larson, P. F. M. Choong, and D. E. Dunstan, “A chitosan–dipotassium orthophosphate hydrogel for the delivery of Doxorubicin in the treatment of osteosarcoma,” *Biomaterials*, vol. 30, no. 21, pp. 3605–3613, Jul. 2009, doi: 10.1016/j.biomaterials.2009.03.022.

[79] S. Miyazaki, K. Ishii, and T. Nadai, “The use of chitin and chitosan as drug carriers,” *Chem. Pharm. Bull. (Tokyo)*, vol. 29, no. 10, pp. 3067–3069, Jun. 1981.

[80] “index thérapeutique : Dictionnaire pharmaceutique - Pharmacologie et chimie des médicaments.” [Online]. Available: https://cisr-sslvpn-out1.insa-lyon.fr/+CSCO+00756767633A2F2F6A6A6A2E76617362657A6E677662617562666376676E79767265722E70627A++/pharma-7206-index-therapeutique.html. [Accessed: 15-Aug-2018].

[81] J. Li and D. J. Mooney, “Designing hydrogels for controlled drug delivery,” *Nat. Rev. Mater.*, vol. 1, no. 12, Dec. 2016, doi: 10.1038/natrevmats.2016.71.

[82] “Angiogenèse.” [Online]. Available: http://www.inserm.fr/dossiers-d-information/angiogenese. [Accessed: 02-Nov-2016].

[83] A. K. Azab *et al.*, “Crosslinked chitosan implants as potential degradable devices for brachytherapy: In vitro and in vivo analysis,” *J. Controlled Release*, vol. 111, no. 3, pp. 281–289, Apr. 2006, doi: 10.1016/j.jconrel.2005.12.014.

[84] E. Ruel-Gariépy *et al.*, “A thermosensitive chitosan-based hydrogel for the local delivery of paclitaxel,” *Eur. J. Pharm. Biopharm.*, vol. 57, no. 1, pp. 53–63, Jan. 2004, doi: 10.1016/S0939-6411(03)00095-X.

[85] M. Berrada, A. Serreqi, F. Dabbarh, A. Owusu, A. Gupta, and S. Lehnert, “A novel non-toxic camptothecin formulation for cancer chemotherapy,” *Biomaterials*, vol. 26, no. 14, pp. 2115–2120, May 2005, doi: 10.1016/j.biomaterials.2004.06.013.

[86] S. S. Vaghani, M. M. Patel, and C. S. Satish, “Synthesis and characterization of pH-sensitive hydrogel composed of carboxymethyl chitosan for colon targeted delivery of ornidazole,” *Carbohydr. Res.*, vol. 347, no. 1, pp. 76–82, Jan. 2012, doi: 10.1016/j.carres.2011.04.048.

[87] S. S. Vaghani and M. M. Patel, “Hydrogels Based on Interpenetrating Network of Chitosan and Polyvinyl Pyrrolidone for pH-Sensitive Delivery of Repaglinide,” *Curr. Drug Discov. Technol.*, vol. 8, no. 2, pp. 126–135, Jun. 2011, doi: 10.2174/157016311795563848.

[88] T.-Y. Liu and Y.-L. Lin, “Novel pH-sensitive chitosan-based hydrogel for encapsulating poorly water-soluble drugs,” *Acta Biomater.*, vol. 6, no. 4, pp. 1423–1429, Apr. 2010, doi: 10.1016/j.actbio.2009.10.010.

[89] Y.-H. Cheng *et al.*, “Sustained delivery of latanoprost by thermosensitive chitosan–gelatin-based hydrogel for controlling ocular hypertension,” *Acta Biomater.*, vol. 10, no. 10, pp. 4360–4366, Oct. 2014, doi: 10.1016/j.actbio.2014.05.031.

[90] Y.-H. Cheng *et al.*, “Thermosensitive chitosan-based hydrogel as a topical ocular drug delivery system of latanoprost for glaucoma treatment,” *Carbohydr. Polym.*, vol. 144, pp. 390–399, Jun. 2016, doi: 10.1016/j.carbpol.2016.02.080.

[91] Y. Zhu *et al.*, “Development of kartogenin-conjugated chitosan–hyaluronic acid hydrogel for nucleus pulposus regeneration,” *Biomater. Sci.*, vol. 5, no. 4, pp. 784–791, Mar. 2017, doi: 10.1039/C7BM00001D.

[92] J. Qu, X. Zhao, P. X. Ma, and B. Guo, “Injectable antibacterial conductive hydrogels with dual response to an electric field and pH for localized ‘smart’ drug release,” *Acta Biomater.*, vol. 72, pp. 55–69, May 2018, doi: 10.1016/j.actbio.2018.03.018.

[93] T. Songkroh *et al.*, "Injectable In situ Forming Chitosan-Based Hydrogels For Curcumin Delivery," *Macromol. Res.*, vol. 23, no. 1, pp. 53–59, Jan. 2015, doi: 10.1007/s13233-015-3006-4.

[94] P. Agarwal and I. D. Rupenthal, "Injectable implants for the sustained release of protein and peptide drugs," *Drug Discov. Today*, vol. 18, no. 7, pp. 337–349, Apr. 2013, doi: 10.1016/j.drudis.2013.01.013.

[95] L. Chen, Z. Tian, and Y. Du, "Synthesis and pH sensitivity of carboxymethyl chitosan-based polyampholyte hydrogels for protein carrier matrices," *Biomaterials*, vol. 25, no. 17, pp. 3725–3732, Aug. 2004, doi: 10.1016/j.biomaterials.2003.09.100.

[96] S.-C. Chen, Y.-C. Wu, F.-L. Mi, Y.-H. Lin, L.-C. Yu, and H.-W. Sung, "A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery," *J. Controlled Release*, vol. 96, no. 2, pp. 285–300, Apr. 2004, doi: 10.1016/j.jconrel.2004.02.002.

[97] X. Li *et al.*, "A covalently crosslinked polysaccharide hydrogel for potential applications in drug delivery and tissue engineering," *J. Mater. Sci. Mater. Med.*, vol. 23, no. 12, pp. 2857–2865, Oct. 2012, doi: 10.1007/s10856-012-4757-5.

[98] N. Bhattarai, H. R. Ramay, J. Gunn, F. A. Matsen, and M. Zhang, "PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release," *J. Controlled Release*, vol. 103, no. 3, pp. 609–624, Apr. 2005, doi: 10.1016/j.jconrel.2004.12.019.

[99] R. A. A. Muzzarelli *et al.*, "Osteoinduction by Chitosan-Complexed BMP: Morpho-Structural Responses in an Osteoporotic Model," *J. Bioact. Compat. Polym.*, vol. 12, no. 4, pp. 321–329, Oct. 1997, doi: 10.1177/088391159701200405.

[100] M. Mattioli-Belmonte *et al.*, "N,N-dicarboxymethyl chitosan as delivery agent for bone morphogenetic protein in the repair of articular cartilage," *Med. Biol. Eng. Comput.*, vol. 37, no. 1, pp. 130–134, Jan. 1999, doi: 10.1007/BF02513279.

[101] M. Ishihara *et al.*, "Controlled release of fibroblast growth factors and heparin from photocrosslinked chitosan hydrogels and subsequent effect on in vivo vascularization," *J. Biomed. Mater. Res. A*, vol. 64A, no. 3, pp. 551–559, Mar. 2003, doi: 10.1002/jbm.a.10427.

[102] A. Chenite *et al.*, "Novel injectable neutral solutions of chitosan form biodegradable gels in situ," *Biomaterials*, vol. 21, no. 21, pp. 2155–2161, Nov. 2000, doi: 10.1016/S0142-9612(00)00116-2.

[103] S. M. Richardson, N. Hughes, J. A. Hunt, A. J. Freemont, and J. A. Hoyland, "Human mesenchymal stem cell differentiation to NP-like cells in chitosan–glycerophosphate hydrogels," *Biomaterials*, vol. 29, no. 1, pp. 85–93, Jan. 2008, doi: 10.1016/j.biomaterials.2007.09.018.

[104] H. Naderi-Meshkin *et al.*, "Chitosan-based injectable hydrogel as a promising in situ forming scaffold for cartilage tissue engineering," *Cell Biol. Int.*, vol. 38, no. 1, pp. 72–84, Jan. 2014, doi: 10.1002/cbin.10181.

[105] M. Tian, Z. Yang, K. Kuwahara, M. E. Nimni, C. Wan, and B. Han, "Delivery of demineralized bone matrix powder using a thermogelling chitosan carrier," *Acta Biomater.*, vol. 8, no. 2, pp. 753–762, Feb. 2012, doi: 10.1016/j.actbio.2011.10.030.

[106] N. A. Fletcher, L. R. Babcock, E. A. Murray, and M. D. Krebs, "Controlled delivery of antibodies from injectable hydrogels," *Mater. Sci. Eng. C*, vol. 59, pp. 801–806, Feb. 2016, doi: 10.1016/j.msec.2015.10.096.

[107] R. A. F. Clark, "Cutaneous tissue repair: Basic biologic considerations. I," *J. Am. Acad. Dermatol.*, vol. 13, no. 5, Part 1, pp. 701–725, Nov. 1985, doi: 10.1016/S0190-9622(85)70213-7.

[108] G. S. McGee *et al.*, "Recombinant basic fibroblast growth factor accelerates wound healing," *J. Surg. Res.*, vol. 45, no. 1, pp. 145–153, Jul. 1988, doi: 10.1016/0022-4804(88)90034-0.

[109] R. Montesano, J. D. Vassalli, A. Baird, R. Guillemin, and L. Orci, "Basic fibroblast growth factor induces angiogenesis in vitro.," *Proc. Natl. Acad. Sci.*, vol. 83, no. 19, pp. 7297–7301, Oct. 1986, doi: 10.1073/pnas.83.19.7297.

[110] G. Gainza, S. Villullas, J. L. Pedraz, R. M. Hernandez, and M. Igartua, "Advances in drug delivery systems (DDSs) to release growth factors for wound healing and skin regeneration," *Nanomedicine Nanotechnol. Biol. Med.*, vol. 11, no. 6, pp. 1551–1573, Aug. 2015, doi: 10.1016/j.nano.2015.03.002.

[111] K. Obara *et al.*, "Photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired db/db mice," *Biomaterials*, vol. 24, no. 20, pp. 3437–3444, Sep. 2003, doi: 10.1016/S0142-9612(03)00220-5.

[112] X. Chen *et al.*, "Peptide-modified chitosan hydrogels promote skin wound healing by enhancing wound angiogenesis and inhibiting inflammation," *Am. J. Transl. Res.*, vol. 9, no. 5, p. 2352, 2017.

[113] F. Chen *et al.*, "Injectable chitosan thermogels for sustained and localized delivery of pingyangmycin in vascular malformations," *Int. J. Pharm.*, vol. 476, no. 1–2, pp. 232–240, Dec. 2014, doi: 10.1016/j.ijpharm.2014.09.058.

[114] H. D. Han *et al.*, "Chitosan hydrogel for localized gene silencing," *Cancer Biol. Ther.*, vol. 11, no. 9, pp. 839–845, May 2011, doi: 10.4161/cbt.11.9.15185.

[115] X. Xu, Y. Weng, L. Xu, and H. Chen, "Sustained release of avastin® from polysaccharides cross-linked hydrogels for ocular drug delivery," *Int. J. Biol. Macromol.*, vol. 60, pp. 272–276, Sep. 2013, doi: 10.1016/j.ijbiomac.2013.05.034.

[116] H. Nazar *et al.*, "A once-a-day dosage form for the delivery of insulin through the nasal route: in vitro assessment and in vivo evaluation," *Biomater. Sci.*, vol. 1, no. 3, pp. 306–314, Feb. 2013, doi: 10.1039/C2BM00132B.

[117] M. F. Shamji, P. Hwang, R. W. Bullock, S. B. Adams, D. L. Nettles, and L. A. Setton, "Release and activity of anti- TNFα therapeutics from injectable chitosan preparations for local drug delivery," *J. Biomed. Mater. Res. B Appl. Biomater.*, vol. 90B, no. 1, pp. 319–326, Jul. 2009, doi: 10.1002/jbm.b.31289.

[118] C. Alemdaroğlu, Z. Değim, N. Çelebi, F. Zor, S. Öztürk, and D. Erdoğan, "An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor," *Burns*, vol. 32, no. 3, pp. 319–327, May 2006, doi: 10.1016/j.burns.2005.10.015.