We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Hydrogels Based on Chitosan and Chitosan Derivatives for Biomedical Applications

Kessily B. Rufato, Juliana P. Galdino, Kamila S. Ody,

Antonio G.B. Pereira, Elisangela Corradini,

Alessandro F. Martins, Alexandre T. Paulino,

André R. Fajardo, Fauze A. Aouada, Felipe A. La Porta,

Adley F. Rubira and Edvani C. Muniz

Abstract

Chitosan (CS) is a polymer obtained from chitin, being this, after the cellulose, the most abundant polysaccharide. The fact of (i) CS being obtained from renewable sources; (ii) CS to possess capability for doing interactions with different moieties being such capability dependent of pH; (iii) plenty of possibilities for chemical modification of CS; and (iv) tuning the final properties of CS derivatives makes this polymer very interesting in academic and technological points of view. In this way, hydrogels based on CS and on CS derivatives have been widely used for biomedical applications. Other important technological applications can be also cited, such as adsorbent of metals and dyes in wastewater from industrial effluents. In pharmaceutical field, hydrogels based on CS are often used as drugs' and proteins' carrier formulations due to the inherent characteristics such as the biocompatibility, nontoxicity, hydrophilicity, etc. This chapter is an attempt for updating and joining the plenty of available information regarding the preparation, characterization, and biomedical application of hydrogels based on chitosan and chitosan derivatives. More than 260 references are provided, being the majority of them published in the last 10 years.

Keywords: hydrogels, chitosan, chitosan derivatives, biomedical application, protein and drug delivery

1. Introduction

Among the diverse types of polymeric materials, the biopolymers represent an important class. Extensive research has been conducted on biodegradable polymeric materials, mainly because they are, in a huge majority, from renewable sources. Some of them present wide availability in nature and can be obtained for a low price. Frequently, they exhibit characteristics such as controlled reactivity, low toxicity, biocompatibility, biodegradability, and filmogenic properties [1, 2]. These

materials have gained attention due to environmental issues such as the reuse of organic waste and/or its reduction, preservation of natural resources [3–5].

There are a number of diverse naturally occurring polymers, such as those belonging to the class of polyesters, those obtained from bacterial sources, polymers of animal origin, proteins, and polypeptides. They can be easily used to produce fibers or particles at micro- or nanoscale for diverse and interesting pharmaceutical applications [6, 7]. Polysaccharides, in general, have some abundance in nature and are also biodegradable and nontoxic [8], making them an active subject of study. Because it is a vast field, many other polymers can also be used, and the decision must be according to the sought-after application. Chitosan (CS) is one of most important examples.

Chitosan is obtained from chitin (CT) that is the most abundant polysaccharide after cellulose [8]. CT is a linear, natural, biodegradable, biocompatible, and non-toxic polymer that is insoluble in most solvents [8, 9]. Chitosan is applied in several fields: agriculture, waste treatment, food, textile, and pharmaceutical industries, cosmetics development, and biomaterials such as gels, films, polymer membranes, and nanofibers [8, 9]. The vast and numerous applications are due to the chitosan's interesting properties. In addition to those mentioned, healing, antimicrobial, anti-fungal, and chelating properties may be also included [10, 11]. Besides, different methodologies and strategies for chitosan applications have been proposed in the literature. Development of hydrogels is one of such methodologies that have gained much attention. Hydrogels are three-dimensional structures formed by hydrophilic polymers that can absorb water or biological fluids [12]. The absorption capacity is due to the presence of cross-linking points, which can be chemical or physical, making these structures insoluble, with the possibility of controlling the pore size in the hydrogels during the preparation method [13]. Because of these characteristics, they have been widely used in various fields of science, such as pharmacy, environmental chemistry, and biomedicine [8, 14].

Hydrogels based on chitosan have attracted lot of attention since this biopolymer is degraded in humans by lysozyme [15], making it highly attractive for the fabrication of dressings. The chitosan dressings can, for example, aid in the absorption of wound secretions and control the hydration of the affected region. Furthermore, for another application in biomedicine, that is, skin regeneration, the polysaccharide chitosan, in addition to exhibiting the aforementioned several characteristics of interest, reduces the healing time of lesions caused by compromises, stimulates cell proliferation, and confers excellent mechanical resistance on the biomaterials [16–18].

In this chapter, the focus was on hydrogels based on chitosan and its derivatives. The study sought to harness the properties of chitosan toward hydrogel applications and to investigate its extensive use in the field of biomedicine.

1.1 Basic concepts and useful properties of hydrogels

Hydrogels are defined as three-dimensional (3D) polymer networks formed by cross-linking hydrophilic homopolymers (or copolymers) that have the ability to absorb large amounts of water and/or biological fluids [19]. Plenty of materials, both natural and synthetic, or mixture of them, fit the definition of hydrogels.

The physical, chemical, and biomechanical properties of hydrogels are dependent on intra- and intermolecular interactions among polymer groups or chain segments and solutes/solvents that may be present inside of 3D structure. The interest in this class of materials (hydrogels) has increased significantly in the academic and technological media due, mainly, to the inherent characteristics such as malleability, biocompatibility, nontoxicity, and swelling in the presence of water or biological fluids, without, however, dissolving [20]. The studies show that the most important

characteristic of hydrogels, the swelling, can be significantly altered through variation of external stimuli. Such change is accompanied by alteration in mechanical and morphological properties [21, 22].

Depending on the nature of the 3D network, hydrogels can be divided in two categories: (i) chemical hydrogels and (ii) physical hydrogels. The 3D matrix of chemical hydrogel has cross-links, which are formed by covalent bonds; in the other class, the 3D matrix is formed by physical interactions. Such physical interactions arise due to the presence of (i) groups with opposite electrical charges (electrostatic interactions, as in the case of polyelectrolyte complexes or PECs) and (ii) dipolar or hydrophobic groups (that works for physical hydrogel forming, as is the case of the hydrogels obtained by the process of freezing-thawing) [23, 24]. Thus, the use cross-linking agents is not needed for preparing physical hydrogels. Another characteristic that distinguishes physical hydrogels is the reversibility of cross-links, that is, the 3D matrix of a physical hydrogel can be destroyed by varying pH, temperature, ionic strength, etc. A typical example of physical hydrogel is that obtained by cross-linking alginate through complexation of carboxylic groups (existing in alginate chains) with calcium ions [19, 25]. A very widespread model known as egg box is used to explain this type of gelation [26]. The alginate/Ca^{2+} matrix may be broken down under acidic conditions and/or by the addition of EDTA [27].

In the case of physical hydrogels, polymer-ion (for example, alginate/Ca^{2+} matrix) or polymer-polymer interactions (for example, complexation between anionic and cationic polymers) should prevail in relation to the polymer-solvent interactions. Without such prevalence, the gelation process would be prevented from occurring.

Several methods can be used for the production of chemical hydrogels. Those involve radiation polymerization by free radicals, for example, the polyacrylamide hydrogel made by acrylamide reaction (AAm) in the presence of methylenebisacrylamide (MBAAm); hydrogels can also be made by polycondensation such as the cross-linking of polysaccharides by reaction with dialdehyde that is very frequent in literature. Other important example of hydrogel by polycondensation is the obtainment of chitosan hydrogel by reaction with glutaraldehyde. The disadvantage of such methods is the need for using initiators and/or catalysts. Such a disadvantage ceases to exist if the polymerization/cross-linking is induced by irradiation, for example, gamma radiation, which produces pure, sterile, and residue-free hydrogels and, beyond this, no catalysts or additives are required to initiate the reaction [28]. Because of this, obtaining hydrogels is a very useful method in the preparation of hydrogels for medical applications, where even a small contamination is undesirable [29, 30]. In this case, the disadvantage is the use of high-cost and rigidly controlled equipment that makes this process of little accessibility in many research groups.

The history of hydrogels began in the 1950s when Wichterle and Lim synthesized hydrogels based on 2-hydroxyethyl methacrylate copolymer with ethylene dimethacrylate [31] and applied them as contact lenses. They were the first gelatinous contact lenses with proven biocompatibility. The great commercial success of gelatinous contact lenses stimulated enormous interest in this type of materials. Subsequently, a wide range of studies enabled the development of hydrogels with different chemical structures, morphologies, and properties through several methodologies. The state of the art in the area of hydrogels is the synthesis of "intelligent" or "smart" hydrogels that modifies their properties once exposed to change of external stimulus, such as pH, temperature, light, or electric field [32]. Hydrogels sensitive to pH and temperature have been played an important role in the control of the drug transport and the drug delivery systems, because temperature and pH are important environmental factors in biomedical systems [33–35].

The use of natural polymers in the preparation of hydrogels has attracted the attention of many researchers both for their natural abundance and for their better biocompatibility when used as biomaterials, with chitosan being one of the most commonly used [36]. Hydrogels derived from natural sources are advantageous because of their inherent biological properties and are widely researched for tissue engineering applications. However, as with other naturally occurring materials, variation in batch composition represents an important disadvantage [37].

1.2 Techniques often used for hydrogel characterization

The analysis of morphology, molecular structure, mechanical properties, and sensitivity to pH and temperature can be done through a great number of qualitative and quantitative techniques. The capability of absorbing liquids (swelling) is the main property of hydrogels, which depends on the hydrophilicity of the hydrogel matrix. From this characteristic, another important aspect arises: the liquid retention capacity of the hydrogel matrix. Changes associated to the swelling (or the shrinking), are controlled by various parameters such as temperature, pH, salinity, and ionic strength of the medium [38]. Superabsorbent hydrogels have the ability to absorb and retain large amount of liquid ($S \geq 100$) [39]. They are prepared using highly hydrophilic moieties (polymers or monomers) and have important technological applications, for instance, in environment (as ion and/or dye absorbent) [40] and agriculture (as soil conditioner or nutrient carriers) [41].

The absorbability of the liquid or the swelling (S) capability of the hydrogels is generally calculated by the following equation [39].

$$S = (W_s - W_d)/W_d \qquad (1)$$

where W_s is the weight of the swollen hydrogel and W_d is the weight of the dry hydrogel. Thus, this ratio relates the amount of the mass of liquid absorbed to the mass of dry hydrogel. The parameter S can be evaluated aiming to determine the maximum mass of fluid that a giving hydrogel is capable of absorbing (steady state) or can be evaluated at different time intervals to determine the kinetic swelling up to the equilibrium has been reached.

Evaluation of parameters related to swelling kinetics is important for hydrogel characterization during swelling. The swelling process is controlled by diffusion and/or relaxation. Different models for predicting the release behavior of hydrogel matrices dipped in pure solvent, solvent mixture, or solute-solvent solution have been developed. Brazel and Peppas [42, 43] developed a semiempirical equation that is widely used, even if it fits only for the initial 60% of the absorbed consumer liquid. In this way, other mathematical models have been proposed to fit the swelling profile in the whole time scale [44].

Aspects such as the type of functional groups present at polymer chains, the network type, the cross-link density, etc., as well as the intermolecular interactions (e.g., H-bond, ionic interactions, etc.), define the physical and chemical properties of hydrogels. They have been thoroughly investigated by infrared (FTIR) techniques [45, 46], X-ray photoelectron spectroscopy (XPS) [47], nuclear magnetic resonance (NMR), and Mossbauer spectroscopies [48]. Differential scanning calorimetry (DSC) and thermogravimetry (TGA) have been used for analysis of thermal behavior [49], while the crystallinity of the hydrogels have been evaluated mainly by small and wide X-ray diffraction measurements (SAXS and WAXS) [49–51]. Different methods have been effectively used for evaluating mechanical properties of the hydrogels and hydrogel composites. For example, the elastic modulus (E) can be evaluated using data collected on a texturometer equipment

that allows to correlate the necessary force (stress) to induce deformation of these soft materials during compressive tests (for example, strain-stress measurements of compression). In addition, rheological measurements should be cited here [52].

Very important parameters, such as the size of pore and the porous distribution of hydrogel and hydrogel composites, as well as the dispersion of the loads inside the matrix, can be obtained from the images obtained from sample under stress using microscopies techniques. Atomic force microscopy (AFM) in its different modalities is often used to evaluate the surface morphology and topography of hydrogel and hydrogel composites [53, 54]. Finally, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [54] are also very often techniques for analyzing the structure of hydrogels. In this way, lyophilization of swollen hydrogels and further fracture are important strategies for analysis of pore size and its distribution within the 3D matrix.

2. Chitosan obtainment through chitin deacetylation

Chitosan is a β-(1→4)-ᴅ-glucosamine copolymer obtained from chitin [55]. The main sources for chitin extraction and production are crustaceous shells, mollusks, insects, silkworm chrysalides, and microorganisms [56]. Chitin is commonly synthesized in six steps: (i) pretreatment, (ii) demineralization, (iii) deproteinization, (iv) bleaching, (v) deodorizing, and (vi) drying [57]. In the pretreatment processes, washes of the specific chitin source are carried with distilled water, aiming to remove vegetal compounds, organic tissues, contaminants, clays, and soil residues [58]. Demineralization process is carried out using diluted hydrochloric acid solutions under heating, aiming to decrease ash and mineral residues as calcium and magnesium. Other common reagents that may be used for the demineralization of chitin sources are nitric, sulfur, and acetic acids. The deproteinization process is performed using diluted sodium hydroxide solution for the removal of proteins. This process must be performed after the demineralization process. Reagents often employed in the deproteinization processes include sodium carbonate, potassium hydroxide, sodium phosphate, and calcium hydroxide [58]. Bleaching and deodorizing processes are responsible for removing color pigments and improving the taste of the prepared polysaccharide, respectively. Both processes can be performed by using diluted sodium hypochlorite solution [55]. Mixture containing sodium hypochlorite and hydrochloric acid needs sometimes to be used [55]. After each process, subsequent washes with distilled water must be performed, aiming to neutralize the final polysaccharide. Drying processes either by lyophilization under −60.0°C or in an oven at temperatures ranging from 60.0 to 80.0 ± 1.0°C are commonly performed [59].

Chitosan is widely prepared from the chitin deacetylation using concentrated sodium hydroxide solution containing sodium borohydride with the aim of avoiding the polysaccharide degradation [60]. Many primary amino groups are removed from the chitosan molecule during hydrolyze reaction, resulting in polysaccharide chains with different sizes [61]. In this case, the molar mass distribution is influenced for parameters such as extraction time, temperature, reagent concentration, and atmospheric conditions. So, chitosan molecules can have different molar masses as compared to parent chitin. Beyond this, deacetylation degrees and viscosities are influenced by deacetylation process that may significantly affect the performance of the final polysaccharide [62]. Some important parameters that are monitored during the obtainment of chitosan with high deacetylation degree are temperature [63], concentration of sodium hydroxide, and step numbers evolved on chitin modification reaction [64]. Enzymatic deacetylation of chitin for the

production of chitosan is an excellent alternative to offset some disadvantages of the chemical deacetylation process such as high energy consumption, generation of acid and alkaline residues, and environmental concerns [59]. Currently, 1 ton billion of chitin and chitosan are annually produced, around the world, by biological processes. The chitin and chitosan production is industrially important, as these polysaccharides are not found in high amounts in the natural environment due to their high biodegradability. Hydrolytic enzymes such as lysozyme, chitinase, chitin deacetylase, and chitosanase are commonly present in body tissue of animals, plants, and soil, avoiding the bioaccumulation of chitin and chitosan in the natural environment. It is estimated that approximately 50,000 tons per year of chitin is worldly produced with 30,000 ton per year from crustaceous shells, excluding the Krill that has potential to produce 56,000 tons per year of chitin. Depending on the extraction method of chitin and production of chitosan, it is possible to recovery proteins, astaxanthin and carotenoid [65].

The main source of chitosan production is from chitin. For producing 1 kg of chitosan from shrimp shells with 70% deacetylation degree, 6.3 kg of hydrochloric acid, 1.8 kg of sodium hydroxide, and 1400 L of water are necessary. The yield for producing chitin from insects, shrimp shells, and silkworm chrysalides ranges from 1.4 to 2.0, 10 to 15, and 6 to 10%, respectively [55]. Commercial chitosan has deacetylation degree ranging from 70 to 95% and molar mass from 1.0×10^4 to 1.0×10^6 g mol^{-1} [59]. The acetylation reaction can be used for obtaining chitosan with deacetylation degree around 50% and high solubility in water [66]. Chitin and chitosan are commercially produced in India, Japan, Poland, Norway, Australia, and China with costs that are dependent on the physical and chemical properties and desired application [55].

Chitin is extracted from different sources in α-chitin, β-chitin, or γ-chitin forms as indicated in **Figure 1a–c**. The α-chitin is the most abundant form of chitin that is commonly found in crab, shrimp, and lobster shells. It is rarely found in insects and fungi. β-Chitin is a rare form that can be also found in insects, chrysalides, crustaceous, and fungi. However, β-chitin is commonly found in squids. Finally, γ-chitin is found in cocoons of insects [55]. The α-chitin, β-chitin, and γ-chitin are identified by the position of acetyl groups in the molecular structure. The α-chitin is formed by repeating polymer units containing acetyl groups in opposite sides, alternating their position to each monomer (**Figure 1a**). The β-chitin has acetyl groups alternating to each two monomers (**Figure 1b**), while the γ-chitin has two acetyl groups in the same side of two monomers, followed by one acetyl group of the opposite side in the third monomer (**Figure 1c**) [67].

2.1 Properties of chitosan

CS is a nontoxic (DL$_{50}$ of 16 g kg^{-1}, studies *in vivo* using rats), biodegradable, biocompatible, antiallergenic, anticoagulant, antifungal, and antimicrobial polysaccharide. These properties are important to apply CS as a biomaterial in medicine, pharmacy, and so forth [68]. Many biomaterials are developed by using CS as solid support due to its feasible biological properties. For instance, capsules of controlled drug release and adhesive films have been registered by the US Food and Drug Administration (FDA) for human applications. Moreover, CS-based biomaterials can be employed in controlled drug release systems, wound healing, filtration membranes, and so forth [66]. CS has been studied for the synthesis of medical biomaterial [69] due to its alkaline characteristic. As CS can be a zwitterionic polysaccharide that contains cationic/anionic groups in its molecular structure, it is efficient for the controlled drug release systems [69], water and wastewater treatment [70], and immobilization of enzymes [71]. The amino groups in the CS
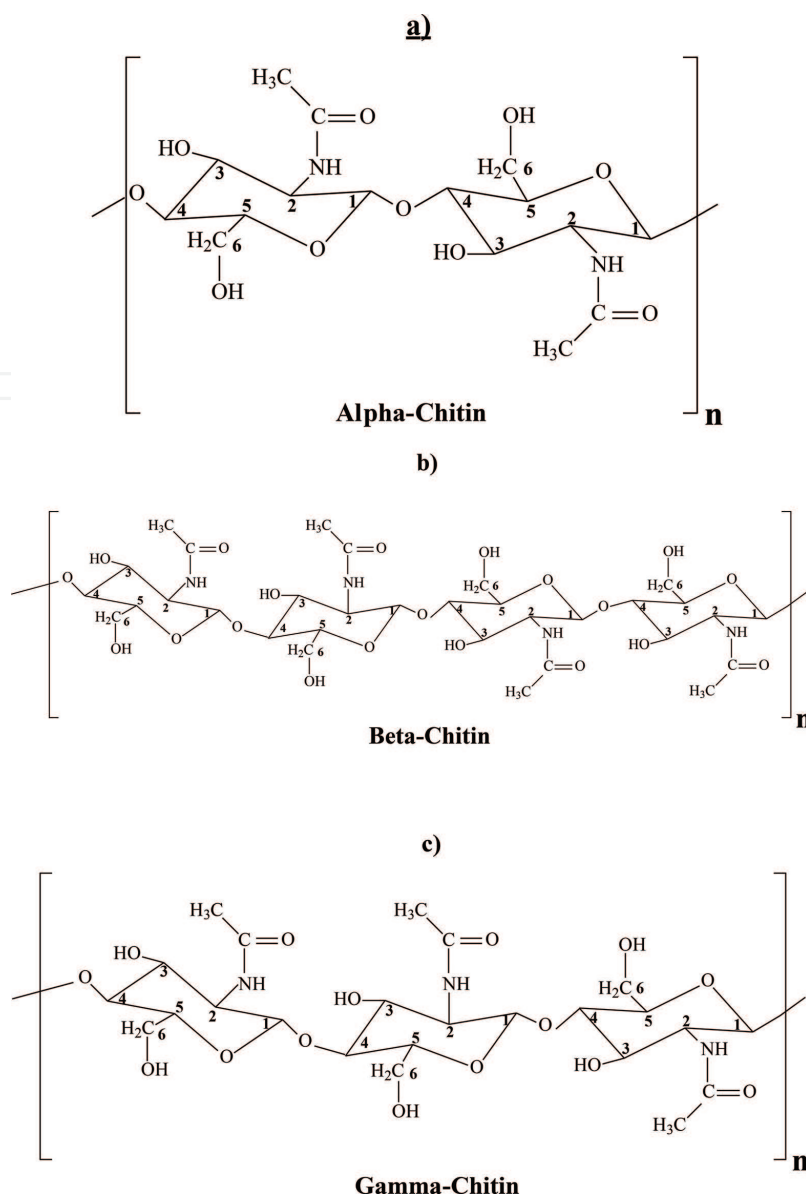
Figure 1.
(a–c) Molecular structures of α-chitin (a), β-chitin (b), and γ-chitin (c).

molecules have pKa values of approximately 6.5, which are deprotonated in higher pHs. Thus, the charge density depends on the pH value and deacetylation degree [55]. CS/silver zeolite composite films can be used for producing burn dressings [72], CS-hydroxyapatite composites can be used for hard tissue regeneration [73], and composite of CS/montmorillonite can be used as matrix for prolonged delivery of some nitric oxide donor compounds [74]. The application of CS as support for the synthesis of composite biomaterials depends on its physiochemical properties such as deacetylation degree and molar mass [75]. Composite materials based on polysaccharides are generally formed by blending either inorganic or organic species to polymer molecules. These composites may be synthesized by electrospray [76], sol-gel process [77], thermomechanical process [78], solvothermal method [79], precipitation and coprecipitation methods [80], solvent casting and evaporation process [81], simple mixing and heating method [75], biomimetic method [82], alternate soaking method [83], and so forth. Composite materials have physiochemical properties different from those ones of initially individual materials and depend on the type of the formed product. For instance, CS-magnetite composite has biodegradability, biocompatibility, mechanical resistance, and magnetic sensibility [84], and the CS-hydroxyapatite composite is pH-sensitive

[85]. CS-iron (III) chloride composite membrane has excellent permeability for different fluids [86]. CS-carbon nanotube composite is mechanically resistant [87]. Carboxymethylated-CS composite protected with ruthenium nanoparticles was synthesized with good thermal stability [88]. Graphene oxide-CS nanocomposite films are formed with excellent mechanical and thermal properties [89], and montmorillonite-CS nanocomposites are produced with excellent biocompatibility and antimicrobial properties [89]. Moreover, CS-based composites were synthesized to be used as microcapsules in drug release systems [90], CS-porous-silica [91] and nanohydroxyapatite-CT derivative composites for bone regeneration [92], CS-zirconium composite as precursor of zirconium carbide [93], CS-barium sulfate composite fibers for endovascular prosthesis, fibrous embolic agent, bone substitution, and X-ray marker [89], CS composite reinforced with carbon nanotubes for biomedicine [78], and CS-hydroxyapatite-glycopolymer/cloisite composite for biomedical applications [94].

2.2 Main chitosan derivatives of technological interest

Considering the vast number of functional groups available on the CS chains and the myriad of chemical compounds that can react with such groups, it is consensual that a plenty of possibilities regarding the use of CS derivatives as precursor materials for the synthesis of hydrogels applicable in drug delivery. There are some methodologies that allow these modifications to occur in CS, such as carboxymethylation, acetylation, and alkylation [7]. In this way, CS derivatives may appear, which seek to improve some properties of this polymer. Among them, the improvement in water solubility is often investigated [6, 95].

From the carboxymethylation reaction, a water-soluble CS derivative can be obtained over a wide pH range [95], and among those obtained so far, carboxymethyl CS stands out because it contains ether groups, and the -COOH and -NH_2 groups [95]. Similarly to CS, the carboxymethylated-CS, also stands out because it exhibits interesting characteristics, such as antifungal activity, low toxicity, and membrane properties, which allows its application for drug release [96].

Quaternized CS through successive methylation in -NH_2 groups [97] has been proposed using different methodologies, methacrylated glycol CS [98] and sulfated CS [99].

The methylation in -NH_2 groups is a very interesting strategy allowing the N atoms be permanently charged with positive charges as -$N(CH_3)_3^+$. Such increase in positive charges allows the material (N,N,N-trimethylchitosan, TMC) be soluble in water in the whole range of pH [100]. Beyond this, the positive charges help for the occurrence of interactions of TMC and the membrane of gram-negative bacteria. So, the TMC has pronounced antibacterial activity [101]. There are several methodologies for synthesis of TMC [101–103]. The following methodology is often used for quaternization of CS: the dispersion of CS in N-methylpyrrolidone containing sodium iodide/methyl iodide in the presence of sodium hydroxide. At final, the iodide counterions of the reaction product are exchanged with chloride for obtaining a more stable salt. Some reviews of CS chemistry are recently published [101–103].

2.3 Methodologies used for obtaining chitosan derivatives aiming at biomedical applications as hydrogels

A large variety of synthetic methods have been utilized for the fabrication of hydrogels based on CS and CS derivatives. The degree of acetylation and/or polymerization of CS-based materials have been widely considered as the critical parameters for controlling their outstanding properties [104–106]. These

structural changes, in principle, lead to completely new properties as well as a significant improvement in water solubility [6, 95]. From the carboxymethylation reaction, a water-soluble CS derivative can be obtained over a wide pH range [95], and among those obtained thus far, carboxymethyl CS stands out because it contains ether, -COOH, and -NH$_2$ groups [95]. Similar to CS, the carboxymethyl CS derivative also stands out because it exhibits interesting characteristics such as antifungal activity, low toxicity, and membrane properties, which allow its application in drug delivery systems [96]. In general, it is well known that a high degree of deacetylation and a narrow polymer molecular weight distribution are critical parameters for controlling the particle size distribution [107]. Evidently, the size of the particles has significant influence on the biomedical applicability of CS-based hydrogels [106, 108–111].

It is well known that the hydrogel matrix structure is generally created from the hydrophilic groups (or domains) present in a natural or synthetic polymeric network upon hydration in an entirely aqueous environment [112]. On the other hand, it is also well known that the various properties exhibited by hydrogels (such as self-healing, biodegradability, swelling degree, mechanical resistance, and so on) are intrinsically related to the physical or chemical cross-linking methods [113, 114].

Hence, the physical cross-linking of hydrogels leads to the formation of a non-permanent network due to noncovalent interactions (e.g., hydrogen or electrostatic bonds and physical entanglements) [22, 115–117]. Consequently, physically cross-linked hydrogels can be formed via ionic interactions of charges, that is, by utilizing the graft copolymers, crystallization, as well as the formation of different stereo-complex forms [117, 118]. Notably, CS can be self-cross-linked when the initial polymer concentration is beyond the critical concentration (C*) for chain entanglement. In this condition, a precise balance between the hydrophilic and hydrophobic interactions is reached [114]. These values may be achieved after decreasing the apparent charge density by solvent evaporation or changing the dielectric constant of the medium [119]. Phosphate-bearing molecules such as polyacrylic acid, sodium alginate, heparin, and polyglutamic acid are the common anions used for physically cross-linked CS [120]. Sarmento et al. have reported the preparation of alginate/CS nanoparticle hydrogels by ionotropic pregelation of an alginate core followed by CS polyelectrolyte complexation, for biomedical applications [121].

On the other hand, it is well known that chemical cross-linking methods, including free-radical polymerization, condensation reactions, and addition reactions, provide good mechanical strength while preserving the hydrogel properties [114, 122–125]. Particularly, chemically cross-linked hydrogels have more uniform properties as compared with those of physically cross-linked hydrogels. For biomedical applications, immense attention must be paid to the cleavage of the cross-linker (which can be done either by chemical or enzymatic methods) to avoid the release of toxic compounds [114]. However, the concentration of the cross-linking agent and the cross-linking reaction time are the main factors that could affect such an approach [126]. For example, the photopolymerization process is started by free radicals produced by radiation (e.g., UV, visible light irradiation, gamma irradiation, or electron beam) that, in turn, promote attacks on the double bonds of monomers and propagate the radical attack, creating a chemically cross-linked polymer network [114, 127–129]. Furthermore, the cross-linking reactions can occur more efficiently on the surface than in the polymer center, probably due to the steric effects [130]. However, the principal disadvantage of using CS-based hydrogels, mainly with regard to their biomedical applicability, is probably the poor reproducibility of the particles formed [106]. Hence, it is believed that a deeper understanding of these methods as well as the development of new strategies is fundamentally necessary and represents

a prerequisite to obtaining optimized CS-based hydrogels with entirely new functionality and properties for a broad variety of applications in emerging biomedical technologies.

3. Uses of hydrogels based on chitosan and chitosan derivatives

The well-known and attractive properties of CS rank this polysaccharide as a safe choice to engineer novel materials applicable as biomaterials. Proof of this is the great volume of studies and review papers published in the literature dealing with this subject [131–133]. As noticed, various research groups are focused on the development of materials based on CS or CS derivatives, aiming their use in the most varied areas and subareas of pharmacy, medicine, and biochemistry [134–136]. In particular, hydrogels synthesized from CS or CS derivatives have been extensively utilized as delivery systems (for drugs, gene factors, and/or protein delivery), dressing devices, and scaffolds for cell/tissue culture [134–137]. Considering these applications, some notable finds and characteristics related to CS and CS-derivative-based hydrogels will be discussed below.

3.1 As carriers of drug

Drug delivery systems that make use of carriers based on CS are of particular interest because this polysaccharide exhibits three paramount features: a mucoadhesive nature, ability to transiently open epithelial tight junctions, and biodegradability [138]. The mucoadhesion ability can be assigned to different interactions (electrostatic attraction, hydrogen bonding, and hydrophobic effects) that take place between CS and mucosa [139]. Biodegradability property is attributed to CS because it is degraded by the two naturally produced enzymes: lysozyme (present in various mucosal surfaces) and chitinase (present in the intestinal flora) [117]. Due to this, CS-based carriers are able to deliver drugs across various well-organized epithelia (e.g., ocular, nasal, buccal, pulmonary, and intestinal) in a controlled manner [140].

In light of this, carrier systems based on CS have been formulated in different forms (tablets, particles, films, membranes, gels, and so forth) using a vast number of protocols [141, 142]. CS hydrogels can be prepared via physical and/or chemical cross-linking processes, and its functional groups allow grafting synthetic monomers on its backbone [143, 144]. Generally, the association of CS with other synthetic polymer or its grafting with vinylic monomers (acrylic acid, acrylamide, etc.) is architected in order to increase the liquid uptake capacity and to enhance the mechanical properties of the hydrogel [117, 145]. Of course, the cross-linking process has a direct relationship with the final properties of the hydrogel, which allows, for example, tailoring hydrogel properties according to the application. Furthermore, CS hydrogels usually show responsive properties, mainly pH-dependent properties [146]. In acidic condition, CS hydrogels show high liquid uptake capacity favoring the drug release by diffusional processes [147]. On the other hand, hydrogels synthesized from the polyelectrolyte complexation of CS (polycationic) with polyanionic polymers (e.g., alginate, pectin, chondroitin sulfate, among others) or anionic salts (e.g., potassium tripolyphosphate) can be disrupted by changing the pH of the release medium [148–150]. In this case, the drug loaded into the hydrogel is released owing to erosion/disruption process. This pH-sensitive is useful to modulate the drug release profile, which prevents unwanted side effects such as burst or time lag release, and to promote targeted drug release.

CS hydrogels also show versatility regarding the drug loading process because drugs can be encapsulated *in situ* during the hydrogel synthesis procedure (this increases the loading efficiency) or they can be loaded in the hydrogel by sorption processes. Generally, drugs adsorbed in the hydrogel are easily released due to the weak interaction forces between the drug and the hydrogel matrix [151]. More recently, the incorporation of filler materials (e.g., clays, metallic particles, graphene, etc.) within the CS matrix has been investigated, aiming to increase the drug encapsulation efficiency (mainly for hydrophobic drugs) and a better control of the release profile [114, 152, 153]. Another strategy adopted by several researchers to enhance the encapsulation efficiency and to improve the release profile is the synthesis of hydrogels using CS derivatives. In general lines, CS derivatives have been synthesized in order to solve some issues related to the use of raw CS in the design of delivery systems. For example, CS shows poor solubility under neutral conditions, which limit its processability and reactivity in biological solutions (pH 7.4) [154]. Moreover, CS is insoluble in the most part of organic solvents hindering the loading and delivery of hydrophobic drugs from CS-based hydrogels [58].

Currently, various studies report the synthesis of hydrogels from water-soluble CS derivatives, such as carboxymethyl CS, quaternized CS, (such as TMC) zwitterionic CS, oligomerized CS, and so on, and their use as drug delivery systems [155, 156]. The chemical modification of the CS backbone without modification of its initial backbone (to preserve the original properties) is a reliable strategy to overcome the shortcomings related to the use of raw CS. For instance, owing to their properties, CS derivative hydrogels can offer prolongation of the contact time between the drug and the absorptive sites in the mucosa and slow and continuous drug release [157]. Furthermore, the grafting of specific chemical modifiers on CS makes possible new cross-linking routes (click reactions, self-assembling, etc.) allowing, for example, the synthesis of *in situ* hydrogels [158, 159]. The use of CS derivatives to synthesize hydrogels may impart new properties to this material (antioxidant or bactericidal properties, for instance), which enlarge the field of the potential applications of such materials, especially as carriers for drug delivery [160, 161].

3.2 As wound healing

Any internal or external stimulus that damages the anatomy of a tissue and compromises its function generates a wound [162]. In general, the healing process of a wound can be described in four phases named vascular response, inflammatory response, proliferation, and maturation [163]. Therefore, a proper wound dressing should present specific features to act in each abovementioned phase to promote a satisfactory healing process. Such features include blood clotting, inflammation fluids absorption, barrier against infection, protection against friction, support for cell attachment and growth, hydration, air permeability, and others [164].

CT, CS, and CS derivatives have been studied in several preparations for treatment of wounds and in tissue regeneration especially due to their biological properties including hemostatic, antibacterial, antifungal, biocompatibility, biodegradability, lack of toxicity, adhesive, and more [165, 166], all those required by a proper dressing. Currently, distinct manufacturers make available wound dressings based on CT and its derivatives under many trade names (Syvek-Patch®, Beschitin®, Tegaderm®, Chitodine®, Trauma DEX®, and Talymed®, among several others) [164].

CT/CS-based wound dressings have been reported in a variety of forms, for example, membranes, sponges, scaffold, fibers, and so on. However, hydrogels are probably the most promising materials for wound dressing because of their similarity and physical chemical properties to the extracellular matrix, which allows cell

diffusion and proliferation. Either chemically or physically, hydrogels can be readily prepared from CS, as extensively reported [167–169].

Zhang et al. demonstrated the wound dressing based on CS loaded with super-oxide dismutase (SOD) enzyme could effectively improve the healing process in chronic wounds in rat models [170]. Hydrogels based on electrostatic interaction of the cationic CS and the anionic heparin and poly(γ-glutamic acid) were successfully prepared and characterized. The best composition was loaded with SOD and applied to the wounds. The continuous release of SOD prevented cell oxidative damage due to excess of O_2^- and improved the healing process.

Hydrogels based on CS and Ag nanoparticles were proved to be efficient restructuration of epithelium and collagen deposition, effectively accelerating the wound healing [171]. CS hydrogel was prepared by the freeze-thawing process using the system LiOH:KOH:urea:H_2O as solvent. In this case, the Ag nanoparticles worked as both fillers for improving the mechanical properties of the hydrogel and anti-microbial agent. The mechanism of bactericidal activity was a combination of cell membrane disruption and DNA binding, preventing bacteria replication.

Other study reported hydrogels based on freeze-thawing of solution of CS with poly(vinyl alcohol) PVA, sodium alginate (SA) or Pluronic F68 [172]. The dressings based on CS and Pluronic were more effective in the healing probably due to its porous morphology and the level of moisture based on comparative histology of healing effects of hydrogels after 15 days of inflicted wounds. Chemically cross-linked hydrogel based on glycol CS and glycidyl methacrylate was obtained via visible light radiation [173]. The endothelial and fibroblast growth factors-loaded hydrogel accelerated the wound healing *in vivo* models. In general lines, several reports have demonstrated the CS-based hydrogels either unloaded or loaded (bactericidal agents, growth factors, etc.) played an important role in the wound by direct acting in different phases of the healing process. The authors also refer to the following review papers focusing on CT/CS hydrogels as wound dressing for further reading [162, 174, 175].

3.3 As protein delivering

Mucoadhesive systems such as CS-based matrix are used in order to increase the protein residence time at the activity site [176]. However, CS can be dissolved in the stomach due its solubility at low pH condition (pKa 6.5) [177], causing release and denaturation of protein [178]. Therefore, the solubility of CS can be prevented by its association with anionic polymers including alginate [179], pectin [180], gelatin [181], and carrageenan [182] to create hydrogels as oral protein delivery carriers.

When proteins are physically incorporated in CS-based hydrogels, their release can occur by diffusion, erosion/degradation, swelling, or a combination of these mechanisms [183]. In order to slow down the degradation rate of hydrogels and prevent burst protein release, polycaprolactone can be incorporated in CS-based hydrogels. Shamloo et al. [181] developed poly(vinyl alcohol)/CS/gelatin hydrogel incorporating polycaprolactone microspheres for delivery of basic fibroblast growth factor (bFGF). Poly(vinyl alcohol) and gelatin were used to improve mechanical properties and increase cell adhesion, respectively. The bFGF release accelerated the wound healing process with polycaprolactone incorporation into hydrogel [181].

The control protein release can also be enhanced with use of mineralized inorganic compounds combined with CS-based hydrogel network [184]. Salama et al. [184] reported the synthesis of CS-g-poly(3-sulfopropyl methacrylate) hydrogel mineralized with calcium phosphate for bovine serum albumin (BSA) release. The mineralization decreased the permeability of the loaded protein and controlled the release proteins [184].

Hydrogels prepared from polysaccharides containing -COOH groups such as carboxymethyl CS may undergo shrinking in acidic condition (e.g., gastric juice) and swelled in neutral or alkaline environment (e.g., intestinal juice) due to electrostatic repulsion between the ionized acid groups present on polysaccharides chains [185]. Therefore, carboxymethyl CS-based pH-sensitive hydrogels can be used to direct protein release, including BSA and insulin (INS), into intestinal region [178]. Zhang et al. [185] synthesized carboxymethyl CS-g-polyacrylic acid for insulin delivery. The results showed that 16.3 and 93.2% of insulin was released at pH 1.2 and 7.4, respectively [185].

Carboxymethyl CS can also be associated with xanthan to prepare hydrogel to fluorescein-isothiocyanate-labeled bovine serum albumin (FITC-BSA) release [186]. Huang et al. [186] developed aldehyde xanthan/carboxymethyl CS hydrogel to FITC-BSA release. Moreover, a signaling protein, endothelial growth factor (VEGF), was loaded to accelerate abdominal wall reconstruction. The BSA-FITC release was stable within 10 h. After VEGF incorporation, the abdominal wall reconstruction was accelerated [186].

Besides carboxymethyl CS, other CS derivatives such as quaternized CS [97], methacrylated glycol CS [98], and sulfated CS [99] have been used as oral protein delivery carriers. Quaternized CS has been used in hydrogel preparation for protein delivering due to its several properties such as absorption enhanced across intestinal epithelial for hydrophilic drug delivery [187], low toxicity [188], capacity to open the junctions between epithelial cells, which allow greater transport of hydrophilic compounds [187], better mucoadhesive [189], and antibacterial activity [190] than CS. Wu et al. [97] developed hydrogel-based N-[(2-hydroxy-3-trimethylammonium)propyl]chitosan chloride (HTCC) and poly(ethylene glycol) (PEG) for insulin release. Hydrogen bonds among amino groups present in insulin and hydroxyl groups present in PEG or HTCC allowed slowed drug release. The results showed that the hydrogel can be used as nasal delivery carrier for protein or peptide drugs [97].

The controlled protein release from heparin-based hydrogels has also been studied due to its strong binding capacity, which attenuates the burst protein release such as rhBMP2 [191]. Therefore, some CS derivatives have been used to mimic heparin to protein delivery, including sulfonated molecules incorporated into methacrylated glycol CS hydrogels [98] and sulfated CS hydrogels [99]. Kim and Chung [98] developed methacrylated glycol CS (MeGC) hydrogel used to mimic heparin to stabilize bone morphogenetic protein-2 (BMP-2) and to enhance osteogenesis by the addition of poly-4-styrenesulfonic acid (PSS) or poly-vinylsulfonic acid (PVSA) into hydrogel. The addition of PSS or PVSA reduced the initial burst and increased the recombinant human BMP-2-induced osteogenesis differentiation, indicating efficient protein delivery [98].

However, sulfated CS, 2-N,6-O-sulfated CS, besides to mimic heparin, has been shown to enhance BMP-2 bioactivity than heparin [192]. In this context, Cao et al. developed rhBMP-2-loaded 2-N,6-O-sulfated CS nanoparticles and hydrogel photopolymerizable incorporating rhBMP-2-loaded 2-N,6-O-sulfated CS nanoparticles. The composite gel system showed gradual and more release than nanoparticle system. The use of 2-N,6-O-sulfated CS enhanced the bioactivity of released rhBMP-2 [99].

The protein residence time at activity can also be improved by thiolation of CS due to the covalent bond formation between subdomains with high cysteine content in the mucus glycoproteins and thiol groups [193]. Liu et al. [194] developed thiolated CS-TBA/hydroxyapatite(HA)/beta-glycerophosphate (β-GP) hydrogel. The BSA protein residence time at activity using such hydrogel was higher than unmodified CS system CS/HA/β-GP [194].

In order to increase protein half-life and to improve the biocompatibility of CS, polyethylene glycol (PEG) can be used to prepare chemically modified CS hydrogels [195, 196]. Farahani et al. [197] developed semi-interpenetrating polymer network CS-PEG-acrylamide hydrogels for closed-loop insulin delivery. Moreover, catalase and glucose oxidase were loaded into hydrogel to make an intelligent protein carrier. The increasing of PEG increased the swelling ratio, protein loading capacity, and entrapment efficiency. The increase in insulin release was observed with increase in the glucose level, indicating that the hydrogel has a good responsiveness to the glucose concentration [197].

3.4 As scaffolds for cell growth in tissue engineering

Scaffolds provide an intermediary template for neotissue/organ formation as well as temporary artificial extracellular matrices [198, 199]. Scaffolds-based extra-cellular matrices are characterized by pore volume fraction of typically 0.90–0.95 or higher and pore diameter in the range of 5–500 μm [199]. Generally, the use of scaffolds in tissue engineering should have several properties, such as biocompat-ibility, cell proliferation, controlled swelling, antimicrobial, biomineralization, biodegradability, stability, porosity, adhesion, and protein absorption [200–202].

3.4.1 Chitosan hydrogels in bone tissue engineering

CS has been widely used for bone tissue engineering due to its capacity in providing growth and deposition of matrix with high mineral content by osteoblast cell culture [203]. As already mentioned in this chapter, CS can be associated with anionic polymers, such as pectin to create physical hydrogels [204]. Moreover, metallic nanoparticles (NPs) coupled to hydrogels can be used to induce cell growth [205, 206]. Tentor et al. related scaffolds-based CS/pectin/gold nanoparticles for bone tissue engineering [204]. The hydrogels were cytocompatible with several cell types, such as normal kidney epithelial cell, HPV-16-positive human cervical tumor cells, epithelial colorectal adenocarcinoma cells, and murine macrophage cells. Regarding the cell viability assay, such hydrogels possess potential for applications in the bone tissue engineering to promote proliferation and growth of bone cells (e.g., MC3T3-E1).

Hydroxyapatite (HA) is the major inorganic component of bone [207]. So, nanohydroxyapatite (n-HA) has been used in bone tissue engineering due to its osteoconductivity and bioactivity [208]. However, n-HA has poor shape ability [208]. Mechanical property of HA can be improved by its association with hyal-uronic acid [209] and glycol CS [210], for fabricating scaffold hydrogels to apply in bone tissue engineering. Huang et al. [210] developed n-HA/glycol CS/hyaluronic acid hydrogel composite as scaffold for bone tissue engineering. The porosity of hydrogel increased with increase in the HA concentration. *In vitro* cytocompat-ibility tests were carried out using MC-3T3-E1 cells. After 7 days co-incubation, cells were attached, and spreading on scaffolds and increasing in cell aggregation were observed. The scaffolds were cytocompatible and nontoxic, so these results are suitable for bone tissue engineering application [210].

3.4.2 Cardiac and nerve tissue engineering

CS and CS associated with biopolymers [211], including gelatin [212], collagen [213], and alginate [214], have been used in developing hydrogel scaffolds for cardiac tissue engineering applications [215]. Gelatin scaffolds are susceptible to fast degradation, while gelatin/CS composite scaffolds are structurally stable in cell

culture media [212]. However, these polymers may be associated with polycaprolactone (PCL), to provide sufficient tensile strength to work in the ventricular wall [216]. Pok et al. [216] developed 3D scaffolds composed of self-assembled PCL sandwiched in a gelatin-CS hydrogel for reconstruction of congenital heart defects. The compressive modulus of the hydrogel was similar to native tissue, and migration of neonatal rat ventricular myocytes (NRVMs) was observed [216].

Quaternized CS can also be used in tissue engineering due to its enhanced antibacterial activity and more solubility than CS [217]. Similar to CS, quaternized CS possesses properties of biocompatibility, low toxicity, biocompatibility, and biodegradability [188]. Zhao et al. [218] developed antibacterial conductive hydrogel scaffolds using quaternized CS-grafted polyaniline with oxidized dextran as cross-linker. The use of polyaniline into quaternized CS copolymer decreased the cytotoxicity, enhanced the antibacterial activity, and stimulated proliferation of C2C12 myoblast cells, by a synergistic effect, as compared with quaternized CS hydrogel. These scaffold showed great potential as scaffold for muscle, nerve, and cardiovascular repair [218].

3.4.3 Cartilage and skin tissue engineering

The cartilage tissue engineering can involve the seeding chondrogenic cells in scaffolds for cartilage repair [136]. Glycosaminoglycans and type II collagen are components commonly found in the cartilage-specific extracellular matrix, which may stimulate the chondrogenesis [219, 220]. CS has structural characteristics similar to glycosaminoglycans and can mimic their functional behavior [221]. Several polymers, such as alginate [222], glycosaminoglycans [223], collagen [224], and carrageenan [225], have been associated with CS for cartilage engineering tissue applications. Hong et al. [224] developed an injectable composite scaffold obtained from collagen-coated polylactide microcarriers/CS hydrogel. Collagen-coated polylactide microcarriers enhanced the mechanical properties of the scaffold. The cell metabolic activity increased rapidly before 9 days of *in vitro* chondrocytes growth within the scaffold. After 9–12 days, confluent cell layers were formed. The composite scaffolds showed great potential for tissue engineering applications, particularly in orthopedics [224]. Liang et al. [225] developed rubbery CS/carrageenan hydrogels prepared by electroneutrality system as cartilage scaffold. The results showed pH- and salt-responsiveness, hierarchically porous architecture, and great mechanical properties. The hydrogels enhanced the viability and the adhesion of TDC5 cells [225].

Mechanical properties and biological activities may be altered by chemical modification of CS. *N*-succinyl CS possess biocompatibility and long-term retention *in vivo* [226]. Kamoun [227] developed *N*-succinyl CS-dialdehyde starch hydrogels for cartilage repair. The hydrogel was relatively stable, and the hydrolysis rate was limited with a high N-succinyl hydrogel composition without any by-products in physiological conditions. The adhered human gingival fibroblast cell number on hydrogel surface was improved by *N*-succinyl CS content in hybrid hydrogels. This hydrogel showed great potential to be used as injectable scaffold for cartilage repair [227]. *N*-succinyl CS can also be associated with other polysaccharides such as hyaluronic acid to prepare hydrogels, as an injectable scaffold, to improve biocompatibility and biodegradation [228].

Regarding skin tissue engineering, CS has been used in the preparation of scaffold hydrogel due to its biocompatibility [229], biodegradability [230], antibacterial properties [231], and hemostatic activity [232], stimulating fibroblast growth and accelerating tissue regeneration [233]. Franco et al. [234] developed CS/gelatin hydrogel scaffolds for skin engineering. The hydrogel showed a high porosity and

supported fibroblast cell proliferation. Notably, the scaffold with lowest cross-linker content swelled more than 600% of its dry weight [234].

Carboxymethyl CS can also be used in skin tissue engineering. This polysaccharide is biodegradable, biocompatible, and more bioactive than the raw CS [235]. Jaikumar et al. [236] developed alginate/O-carboxymethyl CS composite hydrogel scaffolds for adipose tissue regeneration with incorporation of fibrin nanoparticles. The use of fibrin nanoparticles promoted adhesion and proliferation on hydrogel. Human adipose-derived stem cells cultured on this hydrogel scaffold supported cell growth [236].

3.5 Other technological applications

The main reasons to propose different applications of hydrogels is attributed to their considerable volume change capacity in response to little surrounding alterations, such as electric and magnetic fields, solvent, pH, ionic strength, and temperature. Specifically, in biomedical application, it is desirable that the hydrogels may mimic the performance of human organs in response to some alterations in the environmental conditions such as pH, temperature, enzymes, and electric field [237].

Beyond technological applications mentioned in this session, hydrogel and nanocomposites based on CS and their derivatives have been studied for other several applications, such as contact lenses [238, 239], enzyme and cell separations and immobilizations [71, 240–243], DNA delivering [244], cartilage and skin regenerations [245–249], biosensors [250–252], submucosal fluid or injections [253–257], tissue engineering [258–263], postoperative adhesion prevention [264–266], cancer treatment [267–270], orthopedic applications [271, 272], artificial muscles [273], and others. Important characteristics of some cited applications are discussed as follows.

Among the different areas that the hydrogels would be used, the chemotherapy treatment is one of the most important due to great side effects that chemotherapy treatment provokes for patients, such as highly toxic, poor specific drugs, insufficient availability of drugs to the tumor, and others. In this way, the usual chemotherapy treatments have been changed to controlled/localized drug release technology using polysaccharide hydrogel as carrier vehicles [274]. In that work, the authors concluded that the CS/polyvinyl alcohol hydrogels have great potential to be used for the treatment of cancer because these 3D matrices had an antiproliferative effect and great capacity of the inhibit angiogenesis. In the work described by Pattavarakorn et al. [273], the authors found that the electroactive performance of the polythiophene/CS/carboxymethyl CS (PTh/CS/CMCS) as conductive hydrogel is dependent of the hydrogel composition, and the hydrogel prepared with 3:2 CS:CMCS ratio exhibited highest electric field response sensitivity. According to Tan et al. [275], CS and its derivatives are one of the most appropriate materials for enzyme immobilization because of a high specific surface area (high enzyme loading), nontoxicity, and biocompatibility, improving their stability and reusability.

Ulutürk and Alemdar [252] reported that the electroconductive hydrogel has great applicability as biosensor because this material can overcome some disadvantages of the inherently electroconductive polymers like toxicity and can also contribute to decreasing the release of the conductive polymer to the body. As mentioned before, hydrogels have been used in the biomedical area such as contact lens due to the possibility of increase up to 50% of drug bioavailability, which contributes to minimizing the collateral effect that this drug would provoke in the patient. However, the main disadvantage is that these carrier vehicles presented burst release in the first or couple of hours after application. In this way, Åhlén et al. [238] observed that contact lenses based on CS-poly(acrylic acid) nanoparticles and poly(vinyl alcohol) (PVA) hydrogels had greater potential for extended release

during 28 h. Postoperative peritoneal adhesion is one of the serious damages after surgeries, reaching until 67–93% after general surgical abdominal procedures, and as consequence, the patient may have various complications like chronic pain, female infertility, bowel obstruction, and others. *In vivo* studies realized by Song et al. [276] showed that the injectable N,O-carboxymethyl CS-aldehyde hyaluronic acid hydrogel had significant antiadhesion efficacy in a rat repeated-injury model. They observed that after 14 days, the peritoneum is completely recovered without adhesion aspect.

4. Future trends and perspectives

CT, CS, and CS derivatives have been used for wide technological applications, from metallic ions and dye absorbents in environmental to drug carriers in biomedical field. Another important application of CS derivative, for example, the trimethyled CS, is due to its antibacterial properties [277] and, at same time, no toxicity for human and animals. Most of the derivatives are soluble in the whole range of pH, for instance, the trimethyled CS, CS sulfate, and others. So, the drawback related to solubility of chitosan that is limited to acidic conditions (due to the pKa ca. 6.5) is overcome.

The use of CS and its derivatives as hydrogels is one very important issue. The fact of CS being easily cross-linked or doing complexes, forming chemical or physical 3D matrixes, induced plenty of researchers to target themself for producing new materials through different methodologies/strategies/formulations, aiming to intensify some desired properties improving new applications. This is also due mainly to the relatively low cost, abundance, renewability, and biodegradability, among other advantages for using CS and its derivatives. In the last two decades, important technologies have been developed mostly for chemically modifying CS enabling to the preparation of hydrogels with a wide range of desired properties. A lot of examples were given in this review, but a very large window in this issue remains opened [278]. Some highlights, among others, can be given as trends in this field:

- Studies show that functional properties of CS and its derivatives clearly depend on their molecular weight. So, many studies need to be performed to investigate this aspect, because the molecular weight of chitosan depends strongly on the methodology used for CS obtainment from CT or the one used for preparing the CS derivatives.

- Besides the chitosan and its derivatives are not toxic to human or animals, current matter of discussion is whether these biopolymers may have the potential to influence physiological functions or metabolism in the microorganisms [277]. So, huge enforces need to be done in this issue because the molecular weight of CS is dependent on the methodology used for CS obtainment from CT or for CS derivative preparation from raw CS.

- Another important issue is to evaluate if the mixture of chitosan and chitosan derivatives with other polymer (synthetic or natural) affects their low toxicities.

- The future of materials based on CS and CS derivatives is still more promising due to the lack of petroleum. In this way, eco-friendly extraction methods need to be developed. It was mentioned in this review that 1400 L of water is used for extracting and purifying 1 kg of chitosan. So, in the future, water will also suffer eminent lack.

Of course, the understanding of structure-properties-applications relationship in application of CS and CS derivatives as hydrogels will be expanded with more comprehensive studies. Certainly, this will increase the significance of these important soft materials, considering their application.

5. Conclusions

The objective of this review is to update and discuss important aspects related to chitin extraction from different sources and methods for obtaining and purifying chitosan (CS) and for chemically modifying chitosan to obtain CS derivatives with adequate properties. The particular position of the CS and its derivatives is due to the possibility of oil-based products replacement. Several chemical modification methods for CS have also been described in this review as well as for the preparation of hydrogels based on CS or CS derivatives are widely used in the last decades because of the multiple properties allowing many applications. The state of the art is the use of CS and its derivatives combined (or not) with synthetic or natural (sometimes nanostructured) moieties. Although patents and papers mentioning new structures and properties in materials derived from CS and CS derivatives appear in the literature almost every day, this review demonstrates that the window of opportunities in research and development is still opened. The influence of the molecular weight of the CS and CS derivative hydrogels mainly on biological properties can be pointed out as one of the future trends in this field. In addition, environmentally correct methods for extracting CS should be developed taking into account the fact that the water, extensively used in CS extraction/purification, will run out quickly. More comprehensive and in-depth studies will expand the understanding of the structure-properties-applications relationship of CS and CS derivative hydrogels, which will certainly further enhance the importance of this soft material class.

Author details

Kessily B. Rufato[1], Juliana P. Galdino[2], Kamila S. Ody[2], Antonio G.B. Pereira[3], Elisangela Corradini[2], Alessandro F. Martins[1,2,4], Alexandre T. Paulino[5], André R. Fajardo[6], Fauze A. Aouada[7], Felipe A. La Porta[2], Adley F. Rubira[1] and Edvani C. Muniz[1,2]*

1 Chemistry Department, State University of Maringa, Maringá, PR, Brazil

2 Federal Technological University of Paraná, PPGCEM, Londrina, PR, Brazil

3 Federal Technological University of Paraná, Dois Vizinhos, PR, Brazil

4 Federal Technological University of Paraná, Apucarana, PR, Brazil

5 Food and Chemical Engineering Department, Santa Catarina State University, Pinhalzinho, SC, Brazil

6 Federal University of de Pelotas, CCQFA, Pelotas, RS, Brazil

7 Grupo de Compósitos e Nanocompósitos Híbridos (GCNH), Department of Physics and Chemistry, Programa de Pós-Graduação em Ciência dos Materiais, São Paulo State University (UNESP), School of Engineering, Ilha Solteira, SP, Brazil

*Address all correspondence to: ecmuniz@uem.br

IntechOpen

References

[1] Guo J-H, Skinner G, Harcum W, Barnum P. Pharmaceutical applications of naturally occurring water-soluble polymers. Pharmaceutical Science & Technology Today. 1998;**1**:254-261. DOI: 10.1016/S1461-5347(98)00072-8

[2] Vasconcelos AG, de Araújo KV, Santana LDAB. Polissacarídeos extraídos de algas marinhas e suas aplicações biotecnológicas: Uma revisão. Revista Brasileira de Inovação Tecnológica em Saúde. 2015. DOI: 10.18816/r-bits.v5i3.5898. ISSN 2236-1103.5

[3] Pereira FV, De Paula EL, De Mesquita JP, De Almeida Lucas A, Mano V. Bionanocompósitos preparados por incorporação de nanocristais de celulose em polimeros biodegradáveis por meio de evaporação de solvente, automontagem ou eletrofiação. Quimica Nova. 2014;**37**:1209-1219. DOI: 10.5935/0100-4042.20140141

[4] Shibata M, Ozawa K, Teramoto N, Yosomiya R, Takeishi H. Biocomposites made from short abaca fiber and biodegradable polyesters. Macromolecular Materials and Engineering. 2003;**288**:35-43. DOI: 10.1002/mame.200290031

[5] Petersson L, Oksman K. Biopolymer based nanocomposites: Comparing layered silicates and microcrystalline cellulose as nanoreinforcement. Composites Science and Technology. 2006;**66**:2187-2196. DOI: 10.1016/j.compscitech.2005.12.010

[6] Silva HSRC, dos Santos KSCR, Ferreira EI. Chitosan: Hydrossoluble derivatives, pharmaceutical applications and recent advances. Quimica Nova. 2006;**29**:776-785. DOI: 10.1590/s0100-40422006000400026

[7] Shi C, Zhu Y, Ran X, Wang M, Su Y, Cheng T. Therapeutic potential of chitosan and its derivatives in regenerative medicine. The Journal of Surgical Research. 2006;**133**:185-192. DOI: 10.1016/j.jss.2005.12.013

[8] Croisier F, Jérôme C. Chitosan-based biomaterials for tissue engineering. European Polymer Journal. 2013;**49**:780-792. DOI: 10.1016/j.eurpolymj.2012.12.009

[9] Canella KMNdC, Garcia RB. Caracterização de quitosana por cromatografia de permeação em gel—Influência do metodo de preparacao e do solvente. Quimica Nova. 2001;**24**:13-17. DOI: 10.1590/S0100-40422001000100004

[10] Hong XY, Feng HB. Chemical modification of natural polymers in China. Journal of Macromolecular Science, Part A. Pure and Applied Chemistry. 1996;**33**:1459-1477. DOI: 10.1080/10601329608014920

[11] Tonhi E, De Guzzi Plepis AM. Obtenção e caracterização de blendas colágeno-quitosana. Quimica Nova. 2002;**25**:943-948. DOI: 10.1590/S0100-40422002000600011

[12] Kato N, Takizawa Y, Takahashi F. Magnetically driven chemomechanical device with poly(N-isopropylacrylamide) hydrogel containing γ-Fe_2O_3. Journal of Intelligent Material Systems and Structures. 1997;**8**:588-595. DOI: 10.1177/1045389X9700800703

[13] Carlos CW, Rodrigues FHA, Marcus MV, Da Silva LRD, Ricardo NMPS, Feitosa JPA, et al. Síntese e caracterização de hidrogéis compósitos a partir de copolímeros acrilamida-acrilato e caulim: Efeito da constituição de diferentes caulins do nordeste Brasileiro. Quimica

Nova. 2013;**36**:40-45. DOI: 10.1590/S0100-40422013000100008

[14] Rinaudo M. Chitin and chitosan: Properties and applications. Progress in Polymer Science. 2006;**31**:603-632. DOI: 10.1016/j.progpolymsci.2006.06.001

[15] Lee KY, Ha WS, Park WH. Blood compatibility and biodegradability of partially N-acylated chitosan derivatives. Biomaterials. 1995;**16**:1211-1216. DOI: 10.1016/0142-9612(95)98126-Y

[16] Muhammad Yusof NLB, Lim LY, Khor E. Preparation and characterization of chitin beads as a wound dressing precursor. Journal of Biomedical Materials Research. 2001;**54**:59-68. DOI: 10.1002/1097-4636(200101)54:1<59::AID-JBM7>3.0.CO;2-U

[17] Muzzarelli R, Baldassarre V, Conto F, Ferrara P, Biagini G, Gazzanelli G, et al. Biological activity of chitosan: Ultrastructural study. Biomaterials. 1988;**9**:247-252. DOI: 10.1016/0142-9612(88)90092-0

[18] Mima S, Miya M, Iwamoto R, Yoshikawa S. Highly deacetylated chitosan and its properties. Journal of Applied Polymer Science. 1983;**28**:1909-1917. DOI: 10.1002/app.1983.070280607

[19] Mohammadzadeh Pakdel P, Peighambardoust SJ. A review on acrylic based hydrogels and their applications in wastewater treatment. Journal of Environmental Management. 2018;**217**:123-143. DOI: 10.1016/j.jenvman.2018.03.076

[20] Aouada FA, De Moura MR, Fernandes PRG, Rubira AF, Muniz EC. Optical and morphological characterization of polyacrylamide hydrogel and liquid crystal systems. European Polymer Journal. 2005;**41**:2134-2141. DOI: 10.1016/j.eurpolymj.2005.03.020

[21] Aouada FA, Guilherme MR, Campese GM, Girotto EM, Rubira AF, Muniz EC. Electrochemical and mechanical properties of hydrogels based on conductive poly(3,4-ethylene dioxythiophene)/poly(styrenesulfonate) and PAAm. Polymer Testing. 2006;**25**:158-165. DOI: 10.1016/j.polymertesting.2005.11.005

[22] Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. European Journal of Pharmaceutics and Biopharmaceutics. 2004;**57**:19-34. DOI: 10.1016/S0939-6411(03)00161-9

[23] Figueroa-Pizano MD, Vélaz I, Peñas FJ, Zavala-Rivera P, Rosas-Durazo AJ, Maldonado-Arce AD, et al. Effect of freeze-thawing conditions for preparation of chitosan-poly(vinyl alcohol) hydrogels and drug release studies. Carbohydrate Polymers. 2018;**195**:476-485. DOI: 10.1016/j.carbpol.2018.05.004

[24] Lozinsky VI, Plieva FM. Poly(vinyl alcohol) cryogels employed as matrices for cell immobilization. 3. Overview of recent research and developments. Enzyme and Microbial Technology. 1998;**23**:227-242. DOI: 10.1016/S0141-0229(98)00036-2

[25] Peppas NA, Huang Y, Torres-Lugo M, Ward JH, Zhang J. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annual Review of Biomedical Engineering. 2000;**2**:9-29. DOI: 10.1146/annurev.bioeng.2.1.9

[26] Donati I, Holtan S, Mørch YA, Borgogna M, Dentini M, Skjåk-Bræk G. New hypothesis on the role of alternating sequences in calcium-alginate gels. Biomacromolecules. 2005;**6**:1031-1040. DOI: 10.1021/bm049306e

[27] Zheng H, Gao M, Ren Y, Lou R, Xie H, Yu W, et al. An improved pH-responsive carrier based on EDTA-Ca-alginate for oral delivery of Lactobacillus rhamnosus ATCC 53103. Carbohydrate Polymers. 2017;**155**:329-335. DOI: 10.1016/j.carbpol.2016.08.096

[28] Muniz E, Geuskens G. Influence of temperature on the permeability of polyacrylamide hydrogels and semi-IPNs with poly(N-isopropylacrylamide). Journal of Membrane Science. 2000;**172**:287-293. DOI: 10.1016/S0376-7388(00)00346-X

[29] Vedadghavami A, Minooei F, Mohammadi MH, Khetani S, Rezaei Kolahchi A, Mashayekhan S, et al. Manufacturing of hydrogel biomaterials with controlled mechanical properties for tissue engineering applications. Acta Biomaterialia. 2017;**62**:42-63. DOI: 10.1016/j.actbio.2017.07.028

[30] Singh B, Sharma V, Pal L. Formation of sterculia polysaccharide networks by gamma rays induced graft copolymerization for biomedical applications. Carbohydrate Polymers. 2011;**86**:1371-1380. DOI: 10.1016/j.carbpol.2011.06.041

[31] Wichterle O, Lím D. Hydrophilic gels for biological use. Nature. 1960;**185**:117-118. DOI: 10.1038/185117a0

[32] Kopeček J. Polymer chemistry: Swell gels. Nature. 2002;**417**:388-391. DOI: 10.1038/417388a

[33] Zhang N, Shen Y, Li X, Cai S, Liu M. Synthesis and characterization of thermo- and pH-sensitive poly(vinyl alcohol)/poly(N, N-diethylacrylamide-co-itaconic acid) semi-IPN hydrogels. Biomedical Materials. 2012;**7**:035014. DOI: 10.1088/1748-6041/7/3/035014

[34] Prabaharan M, Mano JF. Stimuli-responsive hydrogels based on polysaccharides incorporated with thermo-responsive polymers as novel biomaterials. Macromolecular Bioscience. 2006;**6**:991-1008. DOI: 10.1002/mabi.200600164

[35] Rubira AF, Muniz EC, Guilherme MR, Paulino AT, Tambourgi EB. Morfologia de hidrogéis-ipn termo-sensíveis e ph-responsivos para aplicação como biomaterial na cultura de células. Polímeros. 2009;**19**:105-110. DOI: 10.1590/S0104-14282009000200006

[36] Palacio DA, Urbano BF, Rivas BL. Hydrogels based on alkylated chitosan and polyelectrolyte copolymers. Journal of Applied Polymer Science. 2018;**135**:46556. DOI: 10.1002/app.46556

[37] Nilasaroya A, Poole-Warren LA, Whitelock JM, Jo Martens P. Structural and functional characterisation of poly(vinyl alcohol) and heparin hydrogels. Biomaterials. 2008;**29**:4658-4664. DOI: 10.1016/j.biomaterials.2008.08.011

[38] Yoshida R, Uchida K, Kaneko Y, Sakai K, Kikuchi A, Sakurai Y, et al. Comb-type grafted hydrogels with rapid deswelling response to temperature changes. Nature. 1995;**374**:240-242. DOI: 10.1038/374240a0

[39] Guilherme MR, Aouada FA, Fajardo AR, Martins AF, Paulino AT, Davi MFT, et al. Superabsorbent hydrogels based on polysaccharides for application in agriculture as soil conditioner and nutrient carrier: A review. European Polymer Journal. 2015;**72**. DOI: 10.1016/j.eurpolymj.2015.04.017

[40] Paulino AT, Guilherme MR, Reis AV, Campese GM, Muniz EC, Nozaki J. Removal of methylene blue dye from an aqueous media using superabsorbent hydrogel supported on modified polysaccharide. Journal of Colloid and Interface Science. 2006;**301**:55-62. DOI: 10.1016/j.jcis.2006.04.036.

[41] Zonatto F, Muniz EC, Tambourgi EB, Paulino AT. Adsorption and controlled release of potassium, phosphate and ammonia from modified Arabic gum-based hydrogel. International Journal of Biological Macromolecules. 2017;**105**:363-369. DOI: 10.1016/j.ijbiomac.2017.07.051

[42] Brazel CS, Peppas NA. Mechanisms of solute and drug transport in relaxing, swellable, hydrophilic glassy polymers. Polymer (Guildf). 1999;**40**:3383-3398. DOI: 10.1016/S0032-3861(98)00546-1

[43] Brazel CS, Peppas NA. Dimensionless analysis of swelling of hydrophilic glassy polymers with subsequent drug release from relaxing structures. Biomaterials. 1999;**20**:721-732. DOI: 10.1016/S0142-9612(98)00215-4

[44] Reis AV, Guilherme MR, Rubira AF, Muniz EC. Mathematical model for the prediction of the overall profile of in vitro solute release from polymer networks. Journal of Colloid and Interface Science. 2007;**310**:128-135. DOI: 10.1016/j.jcis.2006.12.058

[45] Guilherme MR, Reis AV, Takahashi SH, Rubira AF, Feitosa JPA, Muniz EC. Synthesis of a novel superabsorbent hydrogel by copolymerization of acrylamide and cashew gum modified with glycidyl methacrylate. Carbohydrate Polymers. 2005;**61**:464-471. DOI: 10.1016/j.carbpol.2005.06.017

[46] Spagnol C, Rodrigues FHA, Pereira AGB, Fajardo AR, Rubira AF, Muniz EC. Superabsorbent hydrogel composite made of cellulose nanofibrils and chitosan-graft-poly(acrylic acid). Carbohydrate Polymers. 2012;**87**:2038-2045. DOI: 10.1016/j.carbpol.2011.10.017

[47] Zu SZ, Han BH. Aqueous dispersion of graphene sheets stabilized by pluronic copolymers: Formation of supramolecular hydrogel. Journal of Physical Chemistry C. 2009;**113**:13651-13657. DOI: 10.1021/jp9035887

[48] Paulino AT, Fajardo AR, Junior AP, Muniz EC, Tambourgi EB. Two-step synthesis and properties of a magnetic-field-sensitive modified maltodextrin-based hydrogel. Polymer International. 2011;**60**:1324-1333. DOI: 10.1002/pi.3084

[49] Piai JF, Rubira AF, Muniz EC. Self-assembly of a swollen chitosan/chondroitin sulfate hydrogel by outward diffusion of the chondroitin sulfate chains. Acta Biomaterialia. 2009;**5**:2601-2609. DOI: 10.1016/j.actbio.2009.03.035

[50] Martins AF, de Oliveira DM, Pereira AGB, Rubira AF, Muniz EC. Chitosan/TPP microparticles obtained by microemulsion method applied in controlled release of heparin. International Journal of Biological Macromolecules. 2012;**51**:1127-1133. DOI: 10.1016/j.ijbiomac.2012.08.032

[51] Mansur HS, Sadahira CM, Souza AN, Mansur AAP. FTIR spectroscopy characterization of poly (vinyl alcohol) hydrogel with different hydrolysis degree and chemically crosslinked with glutaraldehyde. Materials Science and Engineering: C. 2008;**28**:539-548. DOI: 10.1016/j.msec.2007.10.088

[52] Senff H, Richtering W. Influence of cross-link density on rheological properties of temperature-sensitive microgel suspensions. Colloid & Polymer Science. 2000;**278**:830-840. DOI: 10.1007/s003960000329

[53] Milosavljević NB, Ristić MD, Perić-Grujić AA, Filipović JM, Štrbac SB, Rakočević ZL, et al. Hydrogel based on chitosan, itaconic acid and methacrylic acid as adsorbent of Cd2+ions from aqueous solution. Chemical Engineering Journal. 2010;**165**:554-562. DOI: 10.1016/j.cej.2010.09.072

[54] De Vicente G, Lensen MC. Topographically and elastically

micropatterned PEG-based hydrogels to control cell adhesion and migration. European Polymer Journal. 2016;**78**:290-301. DOI: 10.1016/j.eurpolymj.2016.03.020

[55] Paulino AT, Simionato JI, Garcia JC, Nozaki J. Characterization of chitosan and chitin produced from silkworm crysalides. Carbohydrate Polymers. 2006;**64**:98-103. DOI: 10.1016/j.carbpol.2005.10.032

[56] Simionato JI, Paulino AT, Garcia JC, Nozaki J. Adsorption of aluminium from wastewater by chitin and chitosan produced from silkworm chrysalides. Polymer International. 2006;**55**:1243-1248. DOI: 10.1002/pi.2070

[57] Sveučilište u Zagrebu W. Prehrambeno-biotehnološki fakultet., L. Hrvatsko društvo za biotehnologiju., L. Saveza hrvatskih društava za unapređenje prehrane., A. Slovenskog mikrobiološkog društva. Food technology and biotechnology, Faculty of Food Technology and Biotechnology, University of Zagreb. 2013. https://hrcak.srce.hr/99743 [Accessed August 16, 2018]

[58] Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. Structure, properties and applications. Marine Drugs. 2015;**13**:1133-1174. DOI: 10.3390/md13031133

[59] Younes I, Ghorbel-Bellaaj O, Nasri R, Chaabouni M, Rinaudo M, Nasri M. Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization. Process Biochemistry. 2012;**47**:2032-2039. DOI: 10.1016/J.PROCBIO.2012.07.017

[60] Zhang M, Haga A, Sekiguchi H, Hirano S. Structure of insect chitin isolated from beetle larva cuticle and silkworm (*Bombyx mori*) pupa exuvia. International Journal of Biological

Macromolecules. 2000;**27**:99-105. DOI: 10.1016/S0141-8130(99)00123-3

[61] Ottøy MH, Vårum KM, Christensen BE, Anthonsen MW, Smidsrød O. Preparative and analytical size-exclusion chromatography of chitosans. Carbohydrate Polymers. 1996;**31**:253-261. DOI: 10.1016/S0144-8617(96)00096-3

[62] Chen RH, Hwa HD. Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane. Carbohydrate Polymers. 1996;**29**:353-358. DOI: 10.1016/S0144-8617(96)00007-0

[63] Chen RH, Lin JH, Yang MH. Relationships between the chain flexibilities of chitosan molecules and the physical properties of their casted films. Carbohydrate Polymers. 1994;**24**:41-46. DOI: 10.1016/0144-8617(94)90115-5

[64] le Dung P, Milas M, Rinaudo M, Desbrières J. Water soluble derivatives obtained by controlled chemical modifications of chitosan. Carbohydrate Polymers. 1994;**24**:209-214. DOI: 10.1016/0144-8617(94)90132-5

[65] Teng WL, Khor E, Tan TK, Lim LY, Tan SC. Concurrent production of chitin from shrimp shells and fungi. Carbohydrate Research. 2001;**332**:305-316. DOI: 10.1016/S0008-6215(01)00084-2

[66] Synowiecki J, Al-Khateeb NA. Production, properties, and some new applications of chitin and its derivatives. Critical Reviews in Food Science and Nutrition. 2003;**43**:145-171. DOI: 10.1080/10408690390826473

[67] Fajardo AR, Pereira AGB, Martins AF, Paulino AT, Muniz EC, Hsieh Y-L. Chitin and chitosan-based (NANO) composites. In: Handb. Compos. from

Renew. Mater. Hoboken, NJ, USA: John Wiley & Sons Inc; 2017. pp. 671-700. DOI: 10.1002/9781119441632.ch147

[68] Illum L. Chitosan and its use as a pharmaceutical excipient. Pharmaceutical Research. 1998;**15**:1326-1331. DOI: 10.1023/A:1011929016601

[69] Hirano S, Midorikawa T. Novel method for the preparation of N-acylchitosan fiber and N-acylchitosan-cellulose fiber. Biomaterials. 1998;**19**:293-297. DOI: 10.1016/S0142-9612(97)00216-0

[70] Paulino AT, Belfiore LA, Kubota LT, Muniz EC, Tambourgi EB. Efficiency of hydrogels based on natural polysaccharides in the removal of Cd2+ ions from aqueous solutions. Chemical Engineering Journal. 2011;**168**:68-76. DOI: 10.1016/j.cej.2010.12.037

[71] Facin BR, Moret B, Baretta D, Belfiore LA, Paulino AT. Immobilization and controlled release of β-galactosidase from chitosan-grafted hydrogels. Food Chemistry. 2015;**179**:44-51. DOI: 10.1016/j.foodchem.2015.01.088

[72] Yassue-Cordeiro PH, Zandonai CH, da Silva CF, Fernandes-Machado NRC. Desenvolvimento e caracterização de filmes compósitos de quitosana e zeólitas com prata. Polímeros. 2015;**25**:492-502. DOI: 10.1590/0104-1428.2059

[73] Pighinelli L, Kucharska M. Chitosan-hydroxyapatite composites. In: Carbohydrate Polymers. Oxon, England: Elsevier Sci. Ltd.; 2013. pp. 256-262. DOI: 10.1016/j. carbpol.2012.06.004

[74] Cojocariu A, Lenuta P, Cheaburu C, Cornelia V. Chitosan/montmorillonite composites as matrices for prolonged delivery of some novel nitric oxide donor compounds based on theophylline and paracetamol. Cellulose Chemistry and Technology.

2012;**46**:35-43 http://www.cellulosechemtechnol.ro/pdf/CCT1-2(2012)/p.35-43.pdf [Accessed August 21, 2018]

[75] Domard A. Determination of N-acetyl content in chitosan samples by c.d. measurements. International Journal of Biological Macromolecules. 1987;**9**:333-336. DOI: 10.1016/0141-8130(87)90004-3

[76] Li S, Xiao L, Deng H, Shi X, Cao Q. Remote controlled drug release from multi-functional Fe_3O_4/GO/Chitosan microspheres fabricated by an electrospray method. Colloids Surfaces B Biointerfaces. 2017;**151**:354-362. DOI: 10.1016/j.colsurfb.2016.12.029

[77] Benvenutti EV, Moro CC, Costa TMH, Gallas MR. Materiais híbridos à base de sílica obtidos pelo método sol-gel. Quimica Nova. 2009;**32**:1926-1933. DOI: 10.1590/S0100-40422009000700039

[78] Brysch CN, Wold E, Patterson M, Ordoñez Olivares R, Eberth JF, Robles Hernandez FC. Chitosan and chitosan composites reinforced with carbon nanostructures. Journal of Alloys and Compounds. 2014;**615**:S515-S521. DOI: 10.1016/j.jallcom.2014.01.049

[79] Lü T, Chen Y, Qi D, Cao Z, Zhang D, Zhao H. Treatment of emulsified oil wastewaters by using chitosan grafted magnetic nanoparticles. Journal of Alloys and Compounds. 2017;**696**:1205-1212. DOI: 10.1016/j.jallcom.2016.12.118

[80] Picasso G, Cruz R, Sun Kou M d R. Preparation by co-precipitation of Ce-Mn based catalysts for combustion of n-hexane. Materials Research Bulletin. 2015;**70**:621-632. DOI: 10.1016/j. materresbull.2015.05.039

[81] Rekik SB, Gassara S, Bouaziz J, Deratani A, Baklouti S. Development and characterization of porous

membranes based on kaolin/chitosan composite. Applied Clay Science. 2017;**143**:1-9. DOI: 10.1016/j.clay.2017.03.008

[82] Bazhenov VV, Wysokowski M, Petrenko I, Stawski D, Sapozhnikov P, Born R, et al. Preparation of monolithic silica-chitin composite under extreme biomimetic conditions. International Journal of Biological Macromolecules. 2015;**76**:33-38. DOI: 10.1016/j.ijbiomac.2015.02.012

[83] Madhumathi K, Binulal NS, Nagahama H, Tamura H, Shalumon KT, Selvamurugan N, et al. Preparation and characterization of novel β-chitin-hydroxyapatite composite membranes for tissue engineering applications. International Journal of Biological Macromolecules. 2009;**44**:1-5. DOI: 10.1016/j.ijbiomac.2008.09.013

[84] Cesano F, Fenoglio G, Carlos L, Nisticò R. One-step synthesis of magnetic chitosan polymer composite films. Applied Surface Science. 2015;**345**:175-181. DOI: 10.1016/j.apsusc.2015.03.154

[85] Rogina A, Ressler A, Matić I, Gallego Ferrer G, Marijanović I, Ivanković M, et al. Cellular hydrogels based on pH-responsive chitosan-hydroxyapatite system. Carbohydrate Polymers. 2017;**166**:173-182. DOI: 10.1016/j.carbpol.2017.02.105

[86] Ang WL, Mohammad AW, Teow YH, Benamor A, Hilal N. Hybrid chitosan/$FeCl_3$ coagulation-membrane processes: Performance evaluation and membrane fouling study in removing natural organic matter. Separation and Purification Technology. 2015;**152**:23-31. DOI: 10.1016/j.seppur.2015.07.053

[87] Carson L, Kelly-Brown C, Stewart M, Oki A, Regisford G, Luo Z, et al. Synthesis and characterization of chitosan-carbon nanotube composites.

Materials Letters. 2009;**63**:617-620. DOI: 10.1016/j.matlet.2008.11.060

[88] Zhang Q, Zhai M, Peng J, Hao Y, Li J. Radiation-induced synthesis and characterization of ruthenium/carboxymethylated-chitosan nanocomposites. In: Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms. North-Holland, Amsterdam, The Netherlands: Elsevier Science BV; 2012. pp. 334-340. DOI: 10.1016/j.nimb.2012.01.005

[89] Kim H-C, Chung YS. Preparation and radiopaque properties of chitosan/$BaSO_4$ composite fibers. Fibers and Polymers. 2013;**14**:292-297. DOI: 10.1007/s12221-013-0292-3

[90] Xiong X, Wang Y, Zou W, Duan J, Chen Y. Preparation and characterization of magnetic chitosan microcapsules. Journal of Chemistry. 2013;**2013**:1-8. DOI: 10.1155/2013/585613

[91] Retuert J, Quijada R, Arias V, Yazdani-Pedram M. Porous silica derived from chitosan-containing hybrid composites. Journal of Materials Research. 2003;**18**:487-494. DOI: 10.1557/JMR.2003.0062

[92] Kousalya GN, Rajiv Gandhi M, Meenakshi S. Removal of toxic Cr(VI) Ions from aqueous solution using nano-hydroxyapatite-based chitin and chitosan hybrid composites. Adsorption Science and Technology. 2010;**28**:49-64. DOI: 10.1260/0263-6174.28.1.49

[93] Yan C, Liu R, Cao Y, Zhang C, Zhang D. Synthesis of zirconium carbide powders using chitosan as carbon source. Ceramics International. 2013;**39**:3409-3412. DOI: 10.1016/j.ceramint.2012.09.032

[94] Amin A, Kandil H, Awad HM, Ismail MN. Preparation and characterization of chitosan–hydroxyapatite–glycopolymer/cloisite

30 B nanocomposite for biomedical applications. Polymer Bulletin. 2015;**72**:1497-1513. DOI: 10.1007/s00289-015-1351-2

[95] Chen XG, Park HJ. Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions. Carbohydrate Polymers. 2003;**53**:355-359. DOI: 10.1016/S0144-8617(03)00051-1

[96] Liu YF, Huang KL, Peng DM, Ding P, Li GY. Preparation and characterization of glutaraldehyde cross-linked O-carboxymethylchitosan microspheres for controlled delivery of pazufloxacin mesilate. International Journal of Biological Macromolecules. 2007;**41**:87-93. DOI: 10.1016/j.ijbiomac.2007.01.003

[97] Wu J, Wei W, Wang LY, Su ZG, Ma GH. A thermosensitive hydrogel based on quaternized chitosan and poly(ethylene glycol) for nasal drug delivery system. Biomaterials. 2007;**28**:2220-2232. DOI: 10.1016/j.biomaterials.2006.12.024

[98] Kim S, Cui ZK, Kim PJ, Jung LY, Lee M. Design of hydrogels to stabilize and enhance bone morphogenetic protein activity by heparin mimetics. Acta Biomaterialia. 2018;**72**:45-54. DOI: 10.1016/j.actbio.2018.03.034

[99] Cao L, Werkmeister JA, Wang J, Glattauer V, McLean KM, Liu C. Bone regeneration using photocrosslinked hydrogel incorporating rhBMP-2 loaded 2-N, 6-O-sulfated chitosan nanoparticles. Biomaterials. 2014;**35**:2730-2742. DOI: 10.1016/j.biomaterials.2013.12.028

[100] Gomes S, Rodrigues G, Martins G, Henriques C, Silva JC. Evaluation of nanofibrous scaffolds obtained from blends of chitosan, gelatin and polycaprolactone for skin tissue engineering. International Journal of Biological Macromolecules.

2017;**102**:1174-1185. DOI: 10.1016/j.ijbiomac.2017.05.004

[101] Martins AF, Facchi SP, Follmann HDM, Pereira AGB, Rubira AF, Muniz EC. Antimicrobial activity of chitosan derivatives containing N-quaternized moieties in its backbone: A review. International Journal of Molecular Sciences. 2014;**15**:20800-20832. DOI: 10.3390/ijms151120800

[102] Martins AF, Facchi SP, Follmann HDM, Gerola AP, Rubira AF, Muniz EC. Shielding effect of "surface ion pairs" on physicochemical and bactericidal properties of N,N,N-trimethyl chitosan salts. Carbohydrate Research. 2015;**402**:252-260. DOI: 10.1016/j.carres.2014.10.002

[103] Follmann HDM, Martins AF, Nobre TM, Bresolin JD, Cellet TSP, Valderrama P, et al. Extent of shielding by counterions determines the bactericidal activity of N,N,N-trimethyl chitosan salts. Carbohydrate Polymers. 2016;**137**:418-425. DOI: 10.1016/j.carbpol.2015.10.083

[104] Santander-Ortega MJ, Peula-García JM, Goycoolea FM, Ortega-Vinuesa JL. Chitosan nanocapsules: Effect of chitosan molecular weight and acetylation degree on electrokinetic behaviour and colloidal stability. Colloids Surfaces B Biointerfaces. 2011;**82**:571-580. DOI: 10.1016/j.colsurfb.2010.10.019

[105] Silva DS, Delezuk JAM, La Porta FA, Longo E, Campana-Filho SP. Comparison of experimental and theoretical data on the structural and electronic characterization of chitin and chitosan. Current Physical Chemistry. 2016;**5**:206-213. DOI: 10.2174/187794680503160223163623

[106] Sreekumar S, Goycoolea FM, Moerschbacher BM, Rivera-Rodriguez GR. Parameters influencing the size of chitosan-TPP nano- and microparticles.

Scientific Reports. 2018;**8**:4695. DOI: 10.1038/s41598-018-23064-4

[107] Zhang H, Oh M, Allen C, Kumacheva E. Monodisperse chitosan nanoparticles for mucosal drug delivery. Biomacromolecules. 2004;**5**:2461-2468. DOI: 10.1021/bm0496211

[108] Berkland C, King M, Cox A, Kim K, Pack DW. Precise control of PLG microsphere size provides enhanced control of drug release rate. Journal of Controlled Release. 2002;**82**:137-147. DOI: 10.1016/S0168-3659(02)00136-0.

[109] Almeida A, Silva D, Gonçalves V, Sarmento B. Synthesis and characterization of chitosan-grafted-polycaprolactone micelles for modulate intestinal paclitaxel delivery. Drug Delivery and Translational Research. 2018;**8**:387-397. DOI: 10.1007/s13346-017-0357-8

[110] Xu Y, Du Y. Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. International Journal of Pharmaceutics. 2003;**250**:215-226. DOI: 10.1016/S0378-5173(02)00548-3

[111] Wei W, Wang LY, Yuan L, Wei Q, Da Yang X, Su ZG, et al. Preparation and application of novel microspheres possessing autofluorescent properties. Advanced Functional Materials. 2007;**17**:3153-3158. DOI: 10.1002/adfm.200700274

[112] Gulrez SKH, Phillips GO, Al-Assaf S. Hydrogels: Methods of preparation, characterisation and applications. London: IntechOpen; 2011. DOI: 10.5772/24553

[113] Akhtar MF, Hanif M, Ranjha NM. Methods of synthesis of hydrogels ... A review. Saudi Pharmaceutical Journal. 2016;**24**:554-559. DOI: 10.1016/j.jsps.2015.03.022

[114] Pellá MCG, Lima-Tenório MK, Tenório-Neto ET, Guilherme MR, Muniz EC, Rubira AF. Chitosan-based hydrogels: From preparation to biomedical applications. Carbohydrate Polymers. 2018;**196**:233-245. DOI: 10.1016/j.carbpol.2018.05.033

[115] Berger J, Reist M, Mayer JM, Felt O, Gurny R. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. European Journal of Pharmaceutics and Biopharmaceutics. 2004;**57**:35-52. DOI: 10.1016/S0939-6411(03)00160-7

[116] Klouda L. Thermoresponsive hydrogels in biomedical applications: A seven-year update. European Journal of Pharmaceutics and Biopharmaceutics. 2015;**97**:338-349. DOI: 10.1016/j.ejpb.2015.05.017

[117] Ahmadi F, Oveisi Z, Samani M, Amoozgar Z. Chitosan based hydrogels: Characteristics and pharmaceutical applications. Research in Pharmaceutical Sciences. 2015;**10**:1-16. http://www.ncbi.nlm.nih.gov/pubmed/26430453 [Accessed August 18, 2018]

[118] De Jong SJ, Van Eerdenbrugh B, Van Nostrum CF, Kettenes-van den Bosch JJ, Hennink WE. Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: Degradation and protein release behavior. Journal of Controlled Release. 2001;**71**:261-275. DOI: 10.1016/S0168-3659(01)00228-0

[119] Racine L, Texier I, Auzély-Velty R. Chitosan-based hydrogels: Recent design concepts to tailor properties and functions. Polymer International. 2017;**66**:981-998. DOI: 10.1002/pi.5331

[120] Mohamed RR, Elella MHA, Sabaa MW. Cytotoxicity and metal ions removal using antibacterial biodegradable hydrogels based on

N-quaternized chitosan/poly(acrylic acid). International Journal of Biological Macromolecules. 2017;**98**:302-313. DOI: 10.1016/j.ijbiomac.2017.01.107

[121] Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. Pharmaceutical Research. 2007;**24**:2198-2206. DOI: 10.1007/s11095-007-9367-4

[122] Kim KH, Kim J, Jo WH. Preparation of hydrogel nanoparticles by atom transfer radical polymerization of N-isopropylacrylamide in aqueous media using PEG macro-initiator. Polymer (Guildf). 2005;**46**:2836-2840. DOI: 10.1016/j.polymer.2005.02.009

[123] Kufelt O, El-Tamer A, Sehring C, Meißner M, Schlie-Wolter S, Chichkov BN. Water-soluble photopolymerizable chitosan hydrogels for biofabrication via two-photon polymerization. Acta Biomaterialia. 2015;**18**:186-195. DOI: 10.1016/j.actbio.2015.02.025

[124] Kizilel S, Sawardecker E, Teymour F, Pérez-Luna VH. Sequential formation of covalently bonded hydrogel multilayers through surface initiated photopolymerization. Biomaterials. 2006;**27**:1209-1215. DOI: 10.1016/j.biomaterials.2005.08.025

[125] Gao X, Zhou Y, Ma G, Shi S, Yang D, Lu F, et al. A water-soluble photocrosslinkable chitosan derivative prepared by Michael-addition reaction as a precursor for injectable hydrogel. Carbohydrate Polymers. 2010;**79**:507-512. DOI: 10.1016/j.carbpol.2009.08.033

[126] Wong RSH, Ashton M, Dodou K. Effect of crosslinking agent concentration on the properties of unmedicated hydrogels. Pharmaceutics. 2015;**7**:305-319. DOI: 10.3390/pharmaceutics7030305

[127] Wu M, Bao B, Yoshii F, Makuuchi K. Irradiation of crosslinked, poly(vinyl alcohol) blended hydrogel for wound dressing. Journal of Radioanalytical and Nuclear Chemistry. 2001;**250**:391-395. DOI: 10.1023/A:1017988822121

[128] Zhao L, Mitomo H, Zhai M, Yoshii F, Nagasawa N, Kume T. Synthesis of antibacterial PVA/CM-chitosan blend hydrogels with electron beam irradiation. Carbohydrate Polymers. 2003;**53**:439-446. DOI: 10.1016/S0144-8617(03)00103-6

[129] Zhai M, Yoshii F, Kume T, Hashim K. Syntheses of PVA/starch grafted hydrogels by irradiation. Carbohydrate Polymers. 2002;**50**:295-303. DOI: 10.1016/S0144-8617(02)00031-0

[130] Wei W, Yuan L, Hu G, Wang LY, Wu J, Hu X, et al. Monodisperse chitosan microspheres with interesting structures for protein drug delivery. Advanced Materials. 2008;**20**:2292-2296. DOI: 10.1002/adma.200702663

[131] Balagangadharan K, Dhivya S, Selvamurugan N. Chitosan based nanofibers in bone tissue engineering. International Journal of Biological Macromolecules. 2017;**104**:1372-1382. DOI: 10.1016/j.ijbiomac.2016.12.046

[132] Husain S, Al-Samadani KH, Najeeb S, Zafar MS, Khurshid Z, Zohaib S, et al. Chitosan biomaterials for current and potential dental applications. Materials (Basel). 2017;**10**:602. DOI: 10.3390/ma10060602

[133] Logithkumar R, Keshavnarayan A, Dhivya S, Chawla A, Saravanan S, Selvamurugan N. A review of chitosan and its derivatives in bone tissue engineering. Carbohydrate Polymers. 2016;**151**:172-188. DOI: 10.1016/j.carbpol.2016.05.049

[134] Elviri L, Bianchera A, Bergonzi C, Bettini R. Controlled local drug delivery strategies from chitosan hydrogels for wound healing. Expert Opinion on

Drug Delivery. 2017;**14**:897-908. DOI: 10.1080/17425247.2017.1247803

[135] Zhao D, Yu S, Sun B, Gao S, Guo S, Zhao K. Biomedical applications of chitosan and its derivative nanoparticles. Polymers (Basel). 2018;**10**:462. DOI: 10.3390/polym10040462.

[136] Francis Suh J-K, Matthew HW. Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: A review. Biomaterials. 2000;**21**:2589-2598. DOI: 10.1016/S0142-9612(00)00126-5

[137] Casettari L, Vllasaliu D, Castagnino E, Stolnik S, Howdle S, Illum L. PEGylated chitosan derivatives: Synthesis, characterizations and pharmaceutical applications. Progress in Polymer Science. 2012;**37**:659-685. DOI: 10.1016/j.progpolymsci.2011.10.001

[138] Sung HW, Sonaje K, Liao ZX, Hsu LW, Chuang EY. PH-responsive nanoparticles shelled with chitosan for oral delivery of insulin: From mechanism to therapeutic applications. Accounts of Chemical Research. 2012;**45**:619-629. DOI: 10.1021/ar200234q

[139] Sogias IA, Williams AC, Khutoryanskiy VV. Why is chitosan mucoadhesive? Biomacromolecules. 2008;**9**:1837-1842. DOI: 10.1021/bm800276d

[140] Grenha A, Al-Qadi S, Seijo B, Remuñán-López C. The potential of chitosan for pulmonary drug delivery. Journal of Drug Delivery Science and Technology. 2010;**20**:33-43. DOI: 10.1016/S1773-2247(10)50004-2

[141] Xing L, Du L, Luo C-Q, Zhou T-J, Zhu Y, Gong J-H, et al. Chitosan and its derivatives as chemical drug delivery carriers. Current Organic Chemistry. 2018;**22**:690-707. DOI: 10.2174/1385272821666170818160236

[142] Hong SC, Yoo SY, Kim H, Lee J. Chitosan-based multifunctional platforms for local delivery of therapeutics. Marine Drugs. 2017;**15**:60. DOI: 10.3390/md15030060

[143] Fajardo AR, Fávaro SL, Rubira AF, Muniz EC. Dual-network hydrogels based on chemically and physically crosslinked chitosan/chondroitin sulfate. Reactive and Functional Polymers. 2013;**73**:1662-1671. DOI: 10.1016/j.reactfunctpolym.2013.10.003

[144] Luckanagul JA, Pitakchatwong C, Ratnatilaka Na Bhuket P, Muangnoi C, Rojsitthisak P, Chirachanchai S, et al. Chitosan-based polymer hybrids for thermo-responsive nanogel delivery of curcumin. Carbohydrate Polymers. 2018;**181**:1119-1127. DOI: 10.1016/j.carbpol.2017.11.027

[145] Chaterji S, Kwon IK, Park K. Smart polymeric gels: Redefining the limits of biomedical devices. Progress in Polymer Science. 2007;**32**:1083-1122. DOI: 10.1016/j.progpolymsci.2007.05.018

[146] Rizwan M, Yahya R, Hassan A, Yar M, Azzahari AD, Selvanathan V, et al. pH sensitive hydrogels in drug delivery: Brief history, properties, swelling, and release mechanism, material selection and applications. Polymers (Basel). 2017;**9**:137. DOI: 10.3390/polym9040137

[147] Che Y, Li D, Liu Y, Ma Q, Tan Y, Yue Q, et al. Physically cross-linked pH-responsive chitosan-based hydrogels with enhanced mechanical performance for controlled drug delivery. RSC Advances. 2016;**6**:106035-106045. DOI: 10.1039/c6ra16746b

[148] Quadrado RFN, Fajardo AR. Fast decolorization of azo methyl orange via heterogeneous Fenton and Fenton-like reactions using alginate-Fe^{2+}/Fe^{3+} films as catalysts. Carbohydrate Polymers. 2017;**177**:443-450. DOI: 10.1016/j.carbpol.2017.08.083

[149] Yuan D, Jacquier JC, O'Riordan ED. Entrapment of protein in chitosan-tripolyphosphate beads and its release in an in vitro digestive model. Food Chemistry. 2017;**229**:495-501. DOI: 10.1016/j.foodchem.2017.02.107

[150] Bin Chen W, Wang LF, Chen JS, Fan SY. Characterization of polyelectrolyte complexes between chondroitin sulfate and chitosan in the solid state. Journal of Biomedical Materials Research Part A. 2005;**75**:128-137. DOI: 10.1002/jbm.a.30393

[151] Gao W, Zhang Y, Zhang Q, Zhang L. Nanoparticle-hydrogel: A hybrid biomaterial system for localized drug delivery. Annals of Biomedical Engineering. 2016;**44**:2049-2061. DOI: 10.1007/s10439-016-1583-9

[152] Huang B, Liu M, Zhou C. Chitosan composite hydrogels reinforced with natural clay nanotubes. Carbohydrate Polymers. 2017;**175**:689-698. DOI: 10.1016/j.carbpol.2017.08.039

[153] Zhao X, Zou X, Ye L. Controlled pH- and glucose-responsive drug release behavior of cationic chitosan based nano-composite hydrogels by using graphene oxide as drug nanocarrier. Journal of Industrial and Engineering Chemistry. 2017;**49**:36-45. DOI: 10.1016/j.jiec.2016.12.023

[154] Rinaudo M. Main properties and current applications of some polysaccharides as biomaterials. Polymer International. 2008;**57**:397-430. DOI: 10.1002/pi.2378

[155] Prabaharan M. Review paper: Chitosan derivatives as promising materials for controlled drug delivery. Journal of Biomaterials Applications. 2008;**23**:5-36. DOI: 10.1177/0885328208091562

[156] Hsiao MH, Chiou SH, Larsson M, Hung KH, Wang YL, Liu CJL, et al. A temperature-induced and shear-reversible assembly of latanoprost-loaded amphiphilic chitosan colloids: Characterization and in vivo glaucoma treatment. Acta Biomaterialia. 2014;**10**:3188-3196. DOI: 10.1016/j.actbio.2014.03.016

[157] Ways TMM, Lau WM, Khutoryanskiy VV. Chitosan and its derivatives for application in mucoadhesive drug delivery systems. Polymers (Basel). 2018;**10**:267. DOI: 10.3390/polym10030267

[158] Seong JS, Yun ME, Park SN. Surfactant-stable and pH-sensitive liposomes coated with N-succinyl-chitosan and chitooligosaccharide for delivery of quercetin. Carbohydrate Polymers. 2018;**181**:659-667. DOI: 10.1016/j.carbpol.2017.11.098

[159] Huang J, Jiang X. Injectable and degradable pH-responsive hydrogels via spontaneous amino-yne click reaction. ACS Applied Materials & Interfaces. 2018;**10**:361-370. DOI: 10.1021/acsami.7b18141

[160] Botelho da Silva S, Krolicka M, van den Broek LAM, Frissen AE, Boeriu CG. Water-soluble chitosan derivatives and pH-responsive hydrogels by selective C-6 oxidation mediated by TEMPO-laccase redox system. Carbohydrate Polymers. 2018;**186**:299-309. DOI: 10.1016/j.carbpol.2018.01.050

[161] Hoque J, Prakash RG, Paramanandham K, Shome BR, Haldar J. Biocompatible injectable hydrogel with potent wound healing and antibacterial properties. Molecular Pharmaceutics. 2017;**14**:1218-1230. DOI: 10.1021/acs.molpharmaceut.6b01104

[162] Ghadi R, Jain A, Khan W, Domb AJ. Microparticulate polymers and hydrogels for wound healing. In: Wound Heal. Biomater. Amsterdam, The Netherlands: Elsevier;

2016. pp. 203-225. DOI: 10.1016/B978-1-78242-456-7.00010-6

[163] Muncaster D. The physiology of wound healing and wound assessment. British Journal of Perioperative Nursing. 2001;**11**:362-370. DOI: 10.1016/j.mpsur.2014.06.010

[164] Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H. Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnology Advances. 2011;**29**:322-337. DOI: 10.1016/j.biotechadv.2011.01.005

[165] Aranaz I, Mengibar M, Harris R, Panos I, Miralles B, Acosta N, et al. Functional characterization of chitin and chitosan. Current Chemical Biology. 2009;**3**:203-230. DOI: 10.2174/187231309788166415

[166] Dai T, Tanaka M, Huang YY, Hamblin MR. Chitosan preparations for wounds and burns: Antimicrobial and wound-healing effects. Expert Review of Anti-Infective Therapy. 2011;**9**:857-879. DOI: 10.1586/eri.11.59

[167] Bhattarai N, Gunn J, Zhang M. Chitosan-based hydrogels for controlled, localized drug delivery. Advanced Drug Delivery Reviews. 2010;**62**:83-99. DOI: 10.1016/j.addr.2009.07.019

[168] Hamman JH. Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Marine Drugs. 2010;**8**:1305-1322. DOI: 10.3390/md8041305

[169] Luo Y, Wang Q. Recent development of chitosan-based polyelectrolyte complexes with natural polysaccharides for drug delivery. International Journal of Biological Macromolecules. 2014;**64**:353-367. DOI: 10.1016/j.ijbiomac.2013.12.017

[170] Zhang L, Ma Y, Pan X, Chen S, Zhuang H, Wang S. A composite hydrogel of chitosan/heparin/poly(γ-glutamic acid) loaded with superoxide dismutase for wound healing. Carbohydrate Polymers. 2018;**180**:168-174. DOI: 10.1016/j.carbpol.2017.10.036

[171] Xie Y, Liao X, Zhang J, Yang F, Fan Z. Novel chitosan hydrogels reinforced by silver nanoparticles with ultrahigh mechanical and high antibacterial properties for accelerating wound healing. International Journal of Biological Macromolecules. 2018;**119**:402-412. DOI: 10.1016/j.ijbiomac.2018.07.060

[172] Mukherjee D, Azamthulla M, Santhosh S, Dath G, Ghosh A, Natholia R, et al. Development and characterization of chitosan-based hydrogels as wound dressing materials. Journal of Drug Delivery Science and Technology. 2018;**46**:498-510. DOI: 10.1016/j.jddst.2018.06.008

[173] Yoo Y, Hyun H, Yoon SJ, Kim SY, Lee DW, Um S, et al. Visible light-cured glycol chitosan hydrogel dressing containing endothelial growth factor and basic fibroblast growth factor accelerates wound healing in vivo. Journal of Industrial and Engineering Chemistry. 2018;**67**. DOI: 10.1016/j.jiec.2018.07.009

[174] Koehler J, Brandl FP, Goepferich AM. Hydrogel wound dressings for bioactive treatment of acute and chronic wounds. European Polymer Journal. 2018;**100**:1-11. DOI: 10.1016/j.eurpolymj.2017.12.046

[175] Mao X, Sun X, Cheng L, Cheng R, Zhang Y, Cui W. Hydrogel fibrous scaffolds for accelerated wound healing. In: Electrofluidodynamic Technol. Biomater. Med. Devices Princ. Adv. Amsterdam, The Netherlands: Elsevier; 2018. pp. 251-274. DOI: 10.1016/B978-0-08-101745-6.00013-X

[176] George M, Abraham TE. Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan—A review. Journal of Controlled Release. 2006;**114**:1-14. DOI: 10.1016/j.jconrel.2006.04.017

[177] Yang J, Bai R, Suo Z. Topological adhesion of wet materials. Advanced Materials. 2018;**30**:1800671. DOI: 10.1002/adma.201800671

[178] Chen SC, Wu YC, Mi FL, Lin YH, Yu LC, Sung HW. A novel pH-sensitive hydrogel composed of N,O-carboxymethyl chitosan and alginate cross-linked by genipin for protein drug delivery. Journal of Controlled Release. 2004;**96**:285-300. DOI: 10.1016/j.jconrel.2004.02.002

[179] Xu Y, Zhan C, Fan L, Wang L, Zheng H. Preparation of dual crosslinked alginate-chitosan blend gel beads and in vitro controlled release in oral site-specific drug delivery system. International Journal of Pharmaceutics. 2007;**336**:329-337. DOI: 10.1016/j.ijpharm.2006.12.019

[180] Yu CY, Yin BC, Zhang W, Cheng SX, Zhang XZ, Zhuo RX. Composite microparticle drug delivery systems based on chitosan, alginate and pectin with improved pH-sensitive drug release property. Colloids Surfaces B Biointerfaces. 2009;**68**:245-249. DOI: 10.1016/j.colsurfb.2008.10.013

[181] Shamloo A, Sarmadi M, Aghababaie Z, Vossoughi M. Accelerated full-thickness wound healing via sustained bFGF delivery based on a PVA/chitosan/gelatin hydrogel incorporating PCL microspheres. International Journal of Pharmaceutics. 2018;**537**:278-289. DOI: 10.1016/j.ijpharm.2017.12.045

[182] Grenha A, Gomes ME, Rodrigues M, Santo VE, Mano JF, Neves NM, et al. Development of new chitosan/carrageenan nanoparticles for drug delivery applications. Journal of Biomedical Materials Research Part A. 2010;**92**:1265-1272. DOI: 10.1002/jbm.a.32466

[183] Vermonden T, Censi R, Hennink WE. Hydrogels for protein delivery. Chemical Reviews. 2012;**112**:2853-2888. DOI: 10.1021/cr200157d

[184] Salama A. Chitosan based hydrogel assisted spongelike calcium phosphate mineralization for in-vitro BSA release. International Journal of Biological Macromolecules. 2018;**108**:471-476. DOI: 10.1016/j.ijbiomac.2017.12.035

[185] Zhang J, Liang X, Zhang Y, Shang Q. Fabrication and evaluation of a novel polymeric hydrogel of carboxymethyl chitosan-g-polyacrylic acid (CMC-g-PAA) for oral insulin delivery. RSC Advances. 2016;**6**:52858-52867. DOI: 10.1039/C6RA05078F

[186] Huang J, Deng Y, Ren J, Chen G, Wang G, Wang F, et al. Novel in situ forming hydrogel based on xanthan and chitosan re-gelifying in liquids for local drug delivery. Carbohydrate Polymers. 2018;**186**:54-63. DOI: 10.1016/j.carbpol.2018.01.025

[187] Kotzé AF, Lueßen HL, De Leeuw BJ, De Boer BG, Verhoef JC, Junginger HE. N-Trimethyl chitosan chloride as a potential absorption enhancer across mucosal surfaces: In vitro evaluation in intestinal epithelial cells (Caco-2). Pharmaceutical Research. 1997;**14**:1197-1202. DOI: 10.1023/A:1012106907708

[188] Follmann HDM, Martins AF, Gerola AP, Burgo TAL, Nakamura CV, Rubira AF, et al. Antiadhesive and antibacterial multilayer films via layer-by-layer assembly of TMC/heparin complexes. Biomacromolecules. 2012;**13**:3711-3722. DOI: 10.1021/bm3011962

[189] Kotze AF, Thanou MM, Luessen HL, De Boer ABG, Verhoef JC, Junginger HE. Effect of the degree of quaternization of N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2). European Journal of Pharmaceutics and Biopharmaceutics. 1999;**47**:269-274. DOI: 10.1016/S0939-6411(99)00006-5

[190] Jia Z, Shen D, Xu W. Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydrate Research. 2001;**333**:1-6. DOI: 10.1016/S0008-6215(01)00112-4

[191] Xu X, Jha AK, Duncan RL, Jia X. Heparin-decorated, hyaluronic acid-based hydrogel particles for the controlled release of bone morphogenetic protein 2. Acta Biomaterialia. 2011;**7**:3050-3059. DOI: 10.1016/j.actbio.2011.04.018

[192] Zhou H, Qian J, Wang J, Yao W, Liu C, Chen J, et al. Enhanced bioactivity of bone morphogenetic protein-2 with low dose of 2-N, 6-O-sulfated chitosan in vitro and in vivo. Biomaterials. 2009;**30**:1715-1724. DOI: 10.1016/j.biomaterials.2008.12.016

[193] Leitner VM, Walker GF, Bernkop-Schnürch A. Thiolated polymers: Evidence for the formation of disulphide bonds with mucus glycoproteins. European Journal of Pharmaceutics and Biopharmaceutics. 2003;**56**:207-214. DOI: 10.1016/S0939-6411(03)00061-4

[194] Liu X, Chen Y, Huang Q, He W, Feng Q, Yu B. A novel thermo-sensitive hydrogel based on thiolated chitosan/hydroxyapatite/beta-glycerophosphate. Carbohydrate Polymers. 2014;**110**:62-69. DOI: 10.1016/j.carbpol.2014.03.065

[195] Sajeesh S, Sharma CP. Novel pH responsive polymethacrylic acid-chitosan-polyethylene glycol nanoparticles for oral peptide delivery. Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2006;**76**:298-305. DOI: 10.1002/jbm.b.30372

[196] Zhang M, Li XH, Gong YD, Zhao NM, Zhang XF. Properties and biocompatibility of chitosan films modified by blending with PEG. Biomaterials. 2002;**23**:2641-2648. DOI: 10.1016/S0142-9612(01)00403-3

[197] Farahani BV, Ghasemzaheh H, Afraz S. Intelligent semi-IPN chitosan-PEG-PAAm hydrogel for closed-loop insulin delivery and kinetic modeling. RSC Advances. 2016;**6**:26590-26598. DOI: 10.1039/c5ra28188a

[198] Sultana N. Biodegradable PHBV polymer-based scaffolds for bone tissue engineering, In: SpringerBriefs in Applied Sciences and Technology. Switzerland AG: Springer Nature; 2013: pp. 43-60. doi:10.1007/978-3-642-34802-0_3

[199] Abreu E. Review of Scaffolding in Tissue Engineering. In: Ma PX, Elisseeff J, editors. BioMed Central. USA: CRC Press, Taylor and Francis Group; 2006. DOI: 10.1186/1475-925X-5-52

[200] Ahmed S, Annu AA, Sheikh J. A review on chitosan centred scaffolds and their applications in tissue engineering. International Journal of Biological Macromolecules. 2018;**116**:849-862. DOI: 10.1016/j.ijbiomac.2018.04.176

[201] Martins AF, Facchi SP, Da Câmara PCF, Camargo SEA, Camargo CHR, Popat KC, et al. Novel poly(e-caprolactone)/amino-functionalized tannin electrospun membranes as scaffolds for tissue engineering. Journal of Colloid and Interface Science. 2018;**525**:21-30. DOI: 10.1016/j.jcis.2018.04.060

[202] Martins JG, Camargo SEA, Bishop TT, Popat KC, Kipper MJ, Martins AF. Pectin-chitosan membrane scaffold

imparts controlled stem cell adhesion and proliferation. Carbohydrate Polymers. 2018;**197**. DOI: 10.1016/j.carbpol.2018.05.062

[203] Kim IY, Seo SJ, Moon HS, Yoo MK, Park IY, Kim BC, et al. Chitosan and its derivatives for tissue engineering applications. Biotechnology Advances. 2008;**26**:1-21. DOI: 10.1016/j.biotechadv.2007.07.009

[204] Tentor FR, de Oliveira JH, Scariot DB, Lazarin-Bidóia D, Bonafé EG, Nakamura CV, et al. Scaffolds based on chitosan/pectin thermosensitive hydrogels containing gold nanoparticles. International Journal of Biological Macromolecules. 2017;**102**:1186-1194. DOI: 10.1016/j.ijbiomac.2017.04.106

[205] Villalobos LF, Neelakanda P, Karunakaran M, Cha D, Peinemann KV. Poly-thiosemicarbazide/gold nanoparticles catalytic membrane: In-situ growth of well-dispersed, uniform and stable gold nanoparticles in a polymeric membrane. Catalysis Today. 2014;**236**:92-97. DOI: 10.1016/j.cattod.2013.10.067

[206] Abdelrasoul GN, Farkas B, Romano I, Diaspro A, Beke S. Nanocomposite scaffold fabrication by incorporating gold nanoparticles into biodegradable polymer matrix: Synthesis, characterization, and photothermal effect. Materials Science and Engineering: C. 2015;**56**:305-310. DOI: 10.1016/j.msec.2015.06.037

[207] Huang J, Best SM, Bonfield W, Brooks RA, Rushton N, Jayasinghe SN, et al. In vitro assessment of the biological response to nano-sized hydroxyapatite. Journal of Materials Science. Materials in Medicine. 2004:441-445. DOI: 10.1023/B:JMSM.0000021117.67205.cf

[208] Nishida Y, Domura R, Sakai R, Okamoto M, Arakawa S, Ishiki R, et al. Fabrication of PLLA/HA composite scaffolds modified by DNA. Polymer (United Kingdom). 2015;**56**:73-81. DOI: 10.1016/j.polymer.2014.09.063

[209] Yoshikawa M, Tsuji N, Toda T, Ohgushi H. Osteogenic effect of hyaluronic acid sodium salt in the pores of a hydroxyapatite scaffold. Materials Science and Engineering: C. 2007;**27**:220-226. DOI: 10.1016/j.msec.2006.05.014

[210] Huang Y, Zhang X, Wu A, Xu H. An injectable nano-hydroxyapatite (n-HA)/glycol chitosan (G-CS)/hyaluronic acid (HyA) composite hydrogel for bone tissue engineering. RSC Advances. 2016;**6**:33529-33536. DOI: 10.1039/c5ra26160k

[211] Baei P, Jalili-Firoozinezhad S, Rajabi-Zeleti S, Tafazzoli-Shadpour M, Baharvand H, Aghdami N. Electrically conductive gold nanoparticle-chitosan thermosensitive hydrogels for cardiac tissue engineering. Materials Science and Engineering: C. 2016;**63**:131-141. DOI: 10.1016/j.msec.2016.02.056

[212] Huang Y, Onyeri S, Siewe M, Moshfeghian A, Madihally SV. In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. Biomaterials. 2005;**26**:7616-7627. DOI: 10.1016/j.biomaterials.2005.05.036

[213] Deng C, Zhang P, Vulesevic B, Kuraitis D, Li F, Yang AF, et al. A collagen–chitosan hydrogel for endothelial differentiation and angiogenesis. Tissue Engineering. Part A. 2010;**16**:3099-3109. DOI: 10.1089/ten.tea.2009.0504

[214] Deng B, Shen L, Wu Y, Shen Y, Ding X, Lu S, et al. Delivery of alginate-chitosan hydrogel promotes endogenous repair and preserves cardiac function in rats with myocardial infarction. Journal of Biomedical Materials Research Part A. 2015;**103**:907-918. DOI: 10.1002/jbm.a.35232

[215] El-Sherbiny IM, Yacoub MH. Hydrogel scaffolds for tissue engineering: Progress and challenges. Global Cardiology Science and Practice. 2013;**2013**:38. DOI: 10.5339/gcsp.2013.38

[216] Pok S, Myers JD, Madihally SV, Jacot JG. A multilayered scaffold of a chitosan and gelatin hydrogel supported by a PCL core for cardiac tissue engineering. Acta Biomaterialia. 2013;**9**:5630-5642. DOI: 10.1016/j.actbio.2012.10.032

[217] Martins AF, Bueno PVA, Almeida EAMS, Rodrigues FHA, Rubira AF, Muniz EC. Characterization of N-trimethyl chitosan/alginate complexes and curcumin release. International Journal of Biological Macromolecules. 2013;**57**:174-184. DOI: 10.1016/j.ijbiomac.2013.03.029

[218] Zhao X, Li P, Guo B, Ma PX. Antibacterial and conductive injectable hydrogels based on quaternized chitosan-graft-polyaniline/oxidized dextran for tissue engineering. Acta Biomaterialia. 2015;**26**:236-248. DOI: 10.1016/j.actbio.2015.08.006

[219] Goldring MB. Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. Therapeutic Advances in Musculoskeletal Disease. 2012;**4**:269-285. DOI: 10.1177/1759720X12448454

[220] Lash JW, Rosene K, Minor RR, Daniel JC, Kosher RA. Environmental enhancement of in vitro chondrogenesis. III. The influence of external potassium Ions and chondrogenic differentiation. Developmental Biology. 1973;**35**:370-375. DOI: 10.1016/0012-1606(73)90032-8

[221] Chandy T, Sharma CP. Chitosan—As a biomaterial. Biomaterials, Artificial Cells, and Artificial Organs. 1990;**18**:1-24. DOI: 10.3109/10731199009117286

[222] TIğlI RS, Gumüşderelioğlu M. Evaluation of alginate-chitosan semi IPNs as cartilage scaffolds. Journal of Materials Science. Materials in Medicine. 2009;**20**:699-709. DOI: 10.1007/s10856-008-3624-x

[223] Kaczmarek B, Sionkowska A, Osyczka AM. Scaffolds based on chitosan and collagen with glycosaminoglycans cross-linked by tannic acid. Polymer Testing. 2018;**65**:163-168. DOI: 10.1016/j.polymertesting.2017.11.026

[224] Hong Y, Gong Y, Gao C, Shen J. Collagen-coated polylactide microcarriers/chitosan hydrogel composite: Injectable scaffold for cartilage regeneration. Journal of Biomedical Materials Research Part A. 2008;**85**:628-637. DOI: 10.1002/jbm.a.31603

[225] Liang X, Wang X, Xu Q, Lu Y, Zhang Y, Xia H, et al. Rubbery chitosan/carrageenan hydrogels constructed through an electroneutrality system and their potential application as cartilage scaffolds. Biomacromolecules. 2018;**19**:340-352. DOI: 10.1021/acs.biomac.7b01456

[226] Kato Y, Onishi H, Machida Y. Biological characteristics of lactosaminated N-succinyl-chitosan as a liver-specific drug carrier in mice. Journal of Controlled Release. 2001;**70**:295-307. DOI: 10.1016/S0168-3659(00)00356-4

[227] Kamoun EA. N-succinyl chitosan-dialdehyde starch hybrid hydrogels for biomedical applications. Journal of Advanced Research. 2016;**7**:69-77. DOI: 10.1016/j.jare.2015.02.002

[228] Pandey A. Biological and synthetic scaffold: An extra cellular matrix for constructive tissue engineering. International Journal of Medical Research & Review. 2016;**4**:1882-1896

[229] Chung LY, Schmidt RJ, Hamlyn PF, Sagar BF, Andrew AM, Turner TD. Biocompatibility of potential wound management products: Fungal mycelia as a source of chitin/chitosan and their effect on the proliferation of human F1000 fibroblasts in culture. Journal of Biomedical Materials Research. 1994;**28**:463-469. DOI: 10.1002/jbm.820280409

[230] Chen KY, Liao WJ, Kuo SM, Tsai FJ, Chen YS, Huang CY, et al. Asymmetric chitosan membrane containing collagen I nanospheres for skin tissue engineering. Biomacromolecules. 2009;**10**:1642-1649. DOI: 10.1021/bm900238b

[231] Rhoades J, Roller S. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Applied and Environmental Microbiology. 2000;**66**:80-86. DOI: 10.1128/AEM.66.1.80-86.2000

[232] Hattori H, Amano Y, Nogami Y, Takase B, Ishihara M. Hemostasis for severe hemorrhage with photocrosslinkable chitosan hydrogel and calcium alginate. Annals of Biomedical Engineering. 2010;**38**:3724-3732. DOI: 10.1007/s10439-010-0121-4

[233] Cho YW, Cho YN, Chung SH, Yoo G, Ko SW. Water-soluble chitin as a wound healing accelerator. Biomaterials. 1999;**20**:2139-2145. DOI: 10.1016/S0142-9612(99)00116-7

[234] Franco RA, Nguyen TH, Lee BT. Preparation and characterization of electrospun PCL/PLGA membranes and chitosan/gelatin hydrogels for skin bioengineering applications. Journal of Materials Science. Materials in Medicine. 2011;**22**:2207-2218. DOI: 10.1007/s10856-011-4402-8

[235] Upadhyaya L, Singh J, Agarwal V, Tewari RP. The implications of recent advances in carboxymethyl chitosan

based targeted drug delivery and tissue engineering applications. Journal of Controlled Release. 2014;**186**:54-87. DOI: 10.1016/j.jconrel.2014.04.043

[236] Jaikumar D, Sajesh KM, Soumya S, Nimal TR, Chennazhi KP, Nair SV, et al. Injectable alginate-O-carboxymethyl chitosan/nano fibrin composite hydrogels for adipose tissue engineering. International Journal of Biological Macromolecules. 2015;**74**:318-326. DOI: 10.1016/j.ijbiomac.2014.12.037

[237] Ullah F, Othman MBH, Javed F, Ahmad Z, Akil HM. Classification, processing and application of hydrogels: A review. Materials Science and Engineering: C. 2015;**57**:414-433. DOI: 10.1016/j.msec.2015.07.053

[238] Åhlén M, Tummala GK, Mihranyan A. Nanoparticle-loaded hydrogels as a pathway for enzyme-triggered drug release in ophthalmic applications. International Journal of Pharmaceutics. 2018;**536**:73-81. DOI: 10.1016/j.ijpharm.2017.11.053

[239] Feng Y, Borrelli M, Reichl S, Schrader S, Geerling G. Review of alternative carrier materials for ocular surface reconstruction. Current Eye Research. 2014;**39**:541-552. DOI: 10.3109/02713683.2013.853803

[240] Pereira RM, Santos G, Andrade S, De Castro HF, Gabriela M, Campos N, et al. Performance of chitosan/glycerol phosphate hydrogel as a support for lipase immobilization. Materials Research. 2017;**20**:190-201. DOI: 10.1590/1980-5373-mr-2017-0091

[241] Charoenwongpaiboon T, Wangpaiboon K, Pichyangkura R, Prousoontorn MH. Highly porous core–shell chitosan beads with superb immobilization efficiency for Lactobacillus reuteri 121 inulosucrase and production of inulin-type fructooligosaccharides. RSC Advances.

2018;**8**:17008-17016. DOI: 10.1039/C8RA02241K

[242] Kim HJ, Jin JN, Kan E, Kim KJ, Lee SH. Bacterial cellulose-chitosan composite hydrogel beads for enzyme immobilization. Biotechnology and Bioprocess Engineering. 2017;**22**:89-94. DOI: 10.1007/s12257-016-0381-4

[243] Wen H, Gao T, Fu Z, Liu X, Xu J, He Y, et al. Enhancement of membrane stability on magnetic responsive hydrogel microcapsules for potential on-demand cell separation. Carbohydrate Polymers. 2017;**157**:1451-1460. DOI: 10.1016/j.carbpol.2016.11.022

[244] Li H, Ji Q, Chen X, Sun Y, Xu Q, Deng P, et al. Accelerated bony defect healing based on chitosan thermosensitive hydrogel scaffolds embedded with chitosan nanoparticles for the delivery of BMP2 plasmid DNA. Journal of Biomedical Materials Research Part A. 2017;**105**:265-273. DOI: 10.1002/jbm.a.35900

[245] Hoemann CD, Sun J, Légaré A, McKee MD, Buschmann MD. Tissue engineering of cartilage using an injectable and adhesive chitosan-based cell-delivery vehicle. Osteoarthritis and Cartilage. 2005;**13**:318-329. DOI: 10.1016/j.joca.2004.12.001

[246] Kashi M, Baghbani F, Moztarzadeh F, Mobasheri H, Kowsari E. Green synthesis of degradable conductive thermosensitive oligopyrrole/chitosan hydrogel intended for cartilage tissue engineering. International Journal of Biological Macromolecules. 2018;**107**:1567-1575. DOI: 10.1016/j.ijbiomac.2017.10.015

[247] Liu M, Zeng X, Ma C, Yi H, Ali Z, Mou X, et al. Injectable hydrogels for cartilage and bone tissue engineering. Bone Research. 2017;**5**:17014. DOI: 10.1038/boneres.2017.14

[248] T. Pelegrino M, B. Seabra A. Chitosan-based nanomaterials for skin regeneration. AIMS Medical Science. 2017;**4**:352-381. DOI: 10.3934/medsci.2017.3.352

[249] Valencia-Gómez LE, Martel-Estrada SA, Vargas-Requena C, Rivera-Armenta JL, Alba-Baena N, Rodríguez-González C, et al. Chitosan/mimosa tenuiflora films as potential cellular patch for skin regeneration. International Journal of Biological Macromolecules. 2016;**93 (**1217-1225. DOI: 10.1016/j.ijbiomac.2016.09.083

[250] Burrs SL, Vanegas DC, Bhargava M, Mechulan N, Hendershot P, Yamaguchi H, et al. A comparative study of graphene-hydrogel hybrid bionanocomposites for biosensing. The Analyst. 2015;**140**:1466-1476. DOI: 10.1039/c4an01788a

[251] Gao G, Fang D, Yu Y, Wu L, Wang Y, Zhi J. A double-mediator based whole cell electrochemical biosensor for acute biotoxicity assessment of wastewater. Talanta. 2017;**167**:208-216. DOI: 10.1016/j.talanta.2017.01.081

[252] Ulutürk C, Alemdar N. Electroconductive 3D polymeric network production by using polyaniline/chitosan-based hydrogel. Carbohydrate Polymers. 2018;**193**:307-315. DOI: 10.1016/j.carbpol.2018.03.099

[253] Hattori H, Ishihara M. Development of mucoadhesive chitosan derivatives for use as submucosal injections. Polymers (Basel). 2018;**10**:410. DOI: 10.3390/polym10040410

[254] Hattori H, Tsujimoto H, Hase K, Ishihara M. Characterization of a water-soluble chitosan derivative and its potential for submucosal injection in endoscopic techniques. Carbohydrate Polymers. 2017;**175**:592-600. DOI: 10.1016/j.carbpol.2017.08.028

[255] Ishihara M, Kumano I, Hattori H, Nakamura S. Application of hydrogels as submucosal fluid cushions for endoscopic mucosal resection and submucosal dissection. Journal of Artificial Organs. 2015;**18**:191-198. DOI: 10.1007/s10047-015-0843-z

[256] Ishizuka T, Hayashi T, Ishihara M, Yoshizumi Y, Aiko S, Nakamura S, et al. Submucosal injection, for endoscopic mucosal resection, of photocrosslinkable chitosan hydrogel in DMEM/F12 medium. Endoscopy. 2007;**39**:428-433. DOI: 10.1055/s-2007-966393

[257] Kumano I, Ishihara M, Nakamura S, Kishimoto S, Fujita M, Hattori H, et al. Endoscopic submucosal dissection for pig esophagus by using photocrosslinkable chitosan hydrogel as submucosal fluid cushion. Gastrointestinal Endoscopy. 2012;**75**:841-848. DOI: 10.1016/j.gie.2011.10.035

[258] Xu C, Guan S, Wang S, Gong W, Liu T, Ma X, et al. Biodegradable and electroconductive poly(3,4-ethylenedioxythiophene)/carboxymethyl chitosan hydrogels for neural tissue engineering. Materials Science and Engineering: C. 2017;**84**:32-43. DOI: 10.1016/j.msec.2017.11.032

[259] Lu M, Liu Y, Huang YC, Huang CJ, Tsai WB. Fabrication of photo-crosslinkable glycol chitosan hydrogel as a tissue adhesive. Carbohydrate Polymers. 2018;**181**:668-674. DOI: 10.1016/j.carbpol.2017.11.097

[260] Reakasame S, Boccaccini AR. Oxidized alginate-based hydrogels for tissue engineering applications: A review. Biomacromolecules. 2018;**19**:3-21. DOI: 10.1021/acs.biomac.7b01331

[261] Yang Y, Liu X, Li Y, Wang Y, Bao C, Chen Y, et al. A postoperative anti-adhesion barrier based on photoinduced imine-crosslinking hydrogel with tissue-adhesive ability. Acta Biomaterialia. 2017;**62**:199-209. DOI: 10.1016/j.actbio.2017.08.047

[262] Dang Q, Liu K, Zhang Z, Liu C, Liu X, Xin Y, et al. Fabrication and evaluation of thermosensitive chitosan/collagen/α, β-glycerophosphate hydrogels for tissue regeneration. Carbohydrate Polymers. 2017;**167**:145-157. DOI: 10.1016/j.carbpol.2017.03.053

[263] Lastra ML, Molinuevo MS, Cortizo AM, Cortizo MS. Fumarate copolymer–chitosan cross-linked scaffold directed to osteochondrogenic tissue engineering. Macromolecular Bioscience. 2017;**17**. Article ID: 1600219. DOI: 10.1002/mabi.201600219

[264] Konovalova MV, Markov PA, Popova GY, Nikitina IR, Shumikhin KV, Kurek DV, et al. Prevention of postoperative adhesions by biodegradable cryogels from pectin and chitosan polysaccharides. Journal of Bioactive and Compatible Polymers. 2017;**32**:487-502. DOI: 10.1177/0883911517690758

[265] Lin LX, Luo JW, Yuan F, Zhang HH, Ye CQ, Zhang P, et al. In situ cross-linking carbodiimide-modified chitosan hydrogel for postoperative adhesion prevention in a rat model. Materials Science and Engineering: C. 2017;**81**:380-385. DOI: 10.1016/j.msec.2017.07.024

[266] Chen CH, Chen SH, Mao SH, Tsai MJ, Chou PY, Liao CH, et al. Injectable thermosensitive hydrogel containing hyaluronic acid and chitosan as a barrier for prevention of postoperative peritoneal adhesion. Carbohydrate Polymers. 2017;**173**:721-731. DOI: 10.1016/j.carbpol.2017.06.019

[267] Qu J, Zhao X, Ma PX, Guo B. pH-responsive self-healing injectable hydrogel based on N-carboxyethyl chitosan for hepatocellular carcinoma therapy. Acta Biomaterialia.

2017;**58**:168-180. DOI: 10.1016/j. actbio.2017.06.001

[268] Mahdavinia GR, Mosallanezhad A, Soleymani M, Sabzi M. Magnetic- and pH-responsive κ-carrageenan/ chitosan complexes for controlled release of methotrexate anticancer drug. International Journal of Biological Macromolecules. 2017;**97**:209-217. DOI: 10.1016/j.ijbiomac.2017.01.012

[269] Catalano E, Di Benedetto A. Characterization of physicochemical and colloidal properties of hydrogel chitosan-coated iron-oxide nanoparticles for cancer therapy. Journal of Physics Conference Series. 2017:012010. DOI: 10.1088/1742-6596/841/1/012010

[270] Liu J, Qi C, Tao K, Zhang J, Zhang J, Xu L, et al. Sericin/dextran injectable hydrogel as an optically trackable drug delivery system for malignant melanoma treatment. ACS Applied Materials & Interfaces. 2016;**8**:6411-6422. DOI: 10.1021/acsami.6b00959

[271] Jain SA, Shetty G, Mullaji A, Bahadur D, Srivastava R. Development of a herbal thermoresponsive hydrogel for orthopedic applications. Advanced Science Letters. 2016;**22**:83-88. DOI: 10.1166/asl.2016.6816

[272] Fan M, Guo QF, Luo JC, Luo F, Xie P, Tang XH, et al. Preparation and in vitro characterization of dexamethasone-loaded poly(D,L-lactic acid) microspheres embedded in poly(ethylene glycol)-poly(ε-caprolactone)-poly(ethylene glycol) hydrogel for orthopedic tissue engineering. Journal of Biomaterials Applications. 2013;**28**:288-297. DOI: 10.1177/0885328212446097

[273] Pattavarakorn D, Youngta P, Jaesrichai S, Thongbor S, Chaimongkol P. Electroactive performances of conductive polythiophene/hydrogel hybrid artificial muscle. Energy Procedia. 2013;**34**:673-681. DOI: 10.1016/j.egypro.2013.06.799

[274] Jamal A, Shahzadi L, Ahtzaz S, Zahid S, Chaudhry AA, ur Rehman I, et al. Identification of anti-cancer potential of doxazocin: Loading into chitosan based biodegradable hydrogels for on-site delivery to treat cervical cancer. Materials Science and Engineering: C. 2018;**82**:102-109. DOI: 10.1016/j.msec.2017.08.054

[275] Tan Y, Ma S, Liu C, Yu W, Han F. Enhancing the stability and antibiofilm activity of DspB by immobilization on carboxymethyl chitosan nanoparticles. Microbiological Research. 2015;**178**:35-41. DOI: 10.1016/j.micres.2015.06.001

[276] Song L, Li L, He T, Wang N, Yang S, Yang X, et al. Peritoneal adhesion prevention with a biodegradable and injectable N,O-carboxymethyl chitosan-aldehyde hyaluronic acid hydrogel in a rat repeated-injury model. Scientific Reports. 2016;**6**:37600. DOI: 10.1038/srep37600

[277] Rabea EI, Badawy ME-T, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules. 2003;**4**:1457-1465. DOI: 10.1021/bm034130m

[278] Singh A, Tripathi K. Chitin, chitosan and their pharmacological activities: A review. International Journal ofPharmaceutical Sciences and Research. 2018;**9**:2626-2635. DOI: 10.13040/IJPSR.0975-8232.9(7).2626-35