Chitosan as a Biomaterial

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Contributor: Rayssa Victor

Chitosan remarkable properties have aroused the interest of applying this material in several biomedical applications, such as tissue engineering, wound dressing, drug delivery, and cancer treatment, what has aroused the interest of this review to gather the state-of-the-art concerning this polysaccharide when used as a biomaterial, providing information about its characteristics, chemical modifications, and applications.

Keywords: chitosan ; biopolymer ; tissue engineering ; drug delivery system ; cancer treatment

1. Introduction

The current interest in green technology has attracted studies on biopolymers and bio-based polymers. They have excellent physical and biological properties and are biodegradable, unlike their equivalent synthetic polymers [1]. Chitosan is a good example, as it can be derived from partial deacetylation of chitin, a natural polymer found in various organisms [2].

Chitosan alone or in combination with other polymers or bioceramics has been used recently for tissue engineering, drug delivery systems, and cancer treatment applications [3]. In the form of a scaffold, chitosan acts as a physical support for cells and tissues, contributing to the structuring process of tissue, allowing countless cells to be seeded, migrate, and proliferate [4][5]. Moreover, it increases osteoblast activity, mineralization, collagen production, tissue regeneration, and hemostatic action, making chitosan one of the most prominent materials for tissue engineering applications.

Chitosan-based materials have been extensively studied in oral administration for drug delivery and in systems for topical delivery, colon-targeted drug delivery, gene delivery, and even carcinoma therapy due to their biocompatibility; abilities to serve as reaction sites with other bioactive compounds, and protect unstable drug molecules from strong gastric acids and blood flow responses; ability to adhere to mucosal tissues to improve the absorption of specific drugs; and convenience when combining them with anionic biomacromolecules such as DNA by electrostatic action [6]. Chitosan is soluble in dilute acidic solutions since it has primary amino groups (pKa = 6.3) [7], which makes this polymer a potential material for the oral administration of anticancer drugs.

Chitosan has inhibitory effects on tumor cell growth, tumor-induced angiogenesis, and tumor metastasis, thereby showing good anticancer activity. It is also possible to obtain a synergistic cytotoxic effect in cancer cells achieved by the co-administration of chitosan-based systems associated with therapeutic metal ions (TMIs), and drugs used in chemotherapy, improving the therapeutic potential and effectively reducing the tumor cell proliferation and contributing to accelerating the shrinkage and disappearance of tumors. Additionally, chitosan can induce innate immune responses by directly activating dendritic cells (DCs) (that can start responses of the innate immune system) and facilitating the cross-talk among DCs and natural killer (NK cells). Chitosan can promote the survival and improve the effector functions of human NK cells, implying in an in vivo antitumor activity [8][9].

2. Chitosan in Tissue Engineering

Chitosan has attracted attention in tissue engineering and regenerative medicine over the last few years. It has favorable properties, such as hydrophilicity, biocompatibility, biodegradability, and antimicrobial activities. Research on the development of scaffolds has emerged due to its functional character [10][11][12][13].

Chitosan porous scaffolds can be obtained by freeze-drying of CS solution or by processes such as an “internal bubbling process (IBP),” with the use of porogenic materials [14]. The highly porous structures with interconnected pores can improve in vitro and in vivo cell proliferation [15][16][17][18][19][20][21][22][23]. It is worth noting that, by modifying the freezing rate and the ice crystal size, the average pore size of this material can be controlled [24].

2.1. Bone Tissue Engineering

Chitosan-based scaffolds have been extensively utilized in bone tissue engineering due to their high cell adhesion and proliferation [25][26]. This behavior is associated with the scaffold's physical characteristics and electrostatic interactions (arising from the cationic nature of chitosan) [27] with many compounds, such as cytokines/growth factors. These compounds are responsible for improving cell colonization [24].

Hybrid CS-based scaffolds have been developed by a combination of CS with nanoceramics, such as hydroxyapatite (HAp), silicon dioxide (SiO_2), titanium dioxide (TiO_2), bioactive glass-ceramic (BGC), and zirconium oxide (ZrO_2) [27]. Once implanted, chitosan induces a minimum foreign body reaction, furthering cell adhesion, proliferation, and differentiation. These hybrids combine each material's desirable characteristics, taking advantage of the organic part's flexibility and adequate molding capacity, and the inorganic part's properties, such as thermal stability and chemical resistance [28]. Besides, as evidenced by a mesenchymal stem cell (MSC) culture in a SiO_2–gelatin hybrid [29], SiO_2–collagen composites [30], and an osteosarcoma cell line SaOs-2 on a poly(γ-glutamic acid)/SiO_2 hybrid [31], the silica hybrid materials are not cytotoxic and favor cell attachment.

Hybrid nanofibers made of chitosan have been used to produce scaffolds. These hybrid nanofibers incorporated the benefits of different materials: they are cytocompatible, promoting the attachment and proliferation of cells, such as osteoblast-like 7F2-cells; and new adjustable properties can be incorporated when generating bioactive scaffolds for repairing of bone Toskas, et al. [32].

The use of calcium phosphates (CaP), such as hydroxyapatite (HA) and β-TCP, has been widely investigated in the production of chitosan–CaP hybrid scaffolds. However, the inorganic HA scaffolds' ideal morphology and porosity present unsatisfactory mechanical properties [33]. The properties expected for chitosan/calcium phosphate scaffolds are biocompatibility, biodegradation, osteoconductivity, antibacterial effects, osteoinduction, angiogenesis regulation, and mechanical strength [34].

Hydroxyapatite (HA)/gelatin–CS core-shell nanofiber composite scaffolds were developed by coaxial electrospinning in the work of Chen et al. [35]. Chitosan and gelatin are located inside the core-shell nanofibers and considerably favor cell adhesion and proliferation, which is further increased by the presence of HA deposited on the surfaces of nanofibers. Gelatin–CS core-shell structured nanofibers enhanced HA's mineralization efficiency and formed a homogeneous HA deposit when compared with CS, gelatin, and CS-gelatin nanofibers. The results evidence that HA deposited on the gelatin–CS core-shell nanofibers could further enhance osteoblast cell proliferation when the human osteoblast-like cell line (MG-63) is cultured on this material.

Chitosan-4-thio-butylamidine (CS-TBA), a water-soluble thiolate chitosan conjugate, was synthesized and utilized to prepare a CS-TBA/hydroxyapatite (HA)/disodium beta-glycerophosphate (-GP) thermosensitive hydrogel in the work of Liu et al. [36]. In vitro studies on the release behavior of cysteine terminated peptide 24 (P24) containing residues 73–92 of the BMP-2 knuckle epitope from CS-TBA based hydrogel showed a lower release rate and the maintenance of the release of this peptide for a longer time when compared to the unmodified chitosan system (CS/HA/-GP). This can be attributed to the thiol groups' reaction in the CS-TBA with the thiol groups in P24. The bioactivity of P24 was maintained during the release process, which may, in turn, have been associated with degradation of the chitosan network, since the covalent bound P24 was only released when the hydrogel broke down. The results indicate the potential for using this thermosensitive hydrogel as injectable support for bone tissue engineering.

Microporous methacrylated glycol-chitosan-montmorillonite nanocomposite hydrogel was developed in the work of Cui et al. [37]. The hydrogels formed an interconnected microporous network and promoted the proliferation, attachment, and differentiation of encapsulated mesenchymal stem cells in vitro. Additionally, CS-montmorillonite hydrogels were able to recruit native cells and promote calvarial healing without the delivery of additional therapeutic agents or stem cells, indicating a potential for tissue engineering.

Genipin (GP)-crosslinked and fucoidan (FD)-adsorbed nano-hydroxyapatite/hydroxypropyl chitosan composite scaffolds were developed in the work of Lu et al. [38]. Initially, the synthesized n-HA was incorporated into the HPCS solution, followed by crosslinking the n-HA/HPCS blends with GP and FD's adsorption to the n-HA/HPCS composite scaffolds. The genipin crosslinking of HPCS and the inclusion of n-HA in the scaffolds reduced the hydrophilic property, controlled the porous architecture, improved mechanical strength, and decreased degradation. Additionally, the incorporation of n-HA caused an open structure with interconnected pores and a rough morphology, and in addition to the adsorption of fucoidan, increased ALP activity in 7F2 osteoblast cells and promoted their mineralization.

2.2. Cartilage Tissue Engineering

Tissue engineering techniques have been considered a favorable option for repairing articular cartilage [39][40]. Cell-based therapies are an alternative to promote the generation of similar tissues and promote this repair by selecting an appropriate cell source and a biomimetic matrix to host the cells [41][42][43][44].

Hydrogels can be used to partially mimic the stromatolytic structures and anisotropic compositions of the cartilage matrix, promoting their repair [45][46][47]. Thus, the widespread use of chitosan-based hydrogels stands out for the regeneration of cartilaginous tissue, since this polymer has a structure similar to glycosaminoglycan (GAG), a key component in the cartilage matrix, in addition to biocompatibility, biodegradability, bioadhesion, cell affinity, and intrinsic antibacterial, chondro-conductive, and chondro-integrative properties [28][45][48].

Chitosan hydrogels have the potential for cell-based tissue repair since they efficiently support chondrogenic activity, maintain the round morphology of chondrocytes, and preserve their ability to synthesize the cell-specific extracellular matrix, allowing expression of ECM cartilage proteins by chondrocytes. Chitosan administered by intra-articular injection promotes thicker epiphyseal cartilage in the tibial and femoral joints, with the proliferation of chondrocytes. These characteristics make this polymer a potential material for use in cartilage tissue engineering to modulate chondrocyte morphology, modulate differentiation, and stimulate chondrogenesis [28][49][50][51][52][53][54][55].

However, unmodified chitosan-based hydrogels are generally inefficient for cartilage repair due to their low strength and elasticity, fast in vivo degradation, and limited capacity for tissue adhesion, which can be attributed to weak interactions with tissues without the formation of mutually intertwined chains between the two contact interfaces [45][48][56][57][58][59]. In this context, the conjugated catechol groups in the chitosan backbone promote covalent bonds between oxidized catechol groups and amine or thiol groups present in the proteoglycan structure, providing additional adhesion strength to tissue surfaces [60][61][62].

Thermosensitive and injectable adhesive hydrogels of catechol-conjugated chitosan and thiol-terminated Pluronic tissue were developed in the work of Ryu et al. [63]. The hydrogels showed excellent mechanical properties, in vitro and in vivo stability, strong adhesiveness to soft tissues and mucous layers, and superior hemostatic properties. After injection, they were able to immediately form adhesive gels to the tissue. Additionally, they presented arrest bleeding properties, which allows the use of this material in the administration of injectable drugs, tissue engineering hydrogels, tissue adhesives, and antibleeding materials.

A composite hydrogel derived from N-succinyl-chitosan (S-CS), and aldehyde hyaluronic acid (A-HA) was prepared in the work of Tan et al. [64]. The encapsulation of bovine articular chondrocytes demonstrated the potential of the compound hydrogel as an injectable scaffold within the compound hydrogel matrix in vitro. The results showed that the hydrogel compound promoted cell survival. The cells maintained the regular spherical chondrocytic morphology, supporting cell adhesion and indicating its potential application in cartilage tissue engineering.

Anionic salts, such as sodium glycerophosphate (GP), are excellent when trying to produce chitosan/GP gel with thermosensitive characteristics that can deliver different cells while supporting its growth [58]. Crosslinking of these hydrogels with the use of hydroxyethyl cellulose improved gelation, and their use has been demonstrated in the repair of articular cartilage defects (AC), the encapsulation of intervertebral disc (IVD) cells, and the accumulation of a functional extracellular matrix that mimics that of the nucleus pulposus (NP) [65]. All the formulations of the in vitro cell-seeded chitosan hydrogels evidenced that most of the proteoglycan produced by encapsulated NP cells was retained in the gel instead of being released into the culture medium, which indicates that chitosan may be a suitable scaffold for cell-based supplementation to assist in the restoration of the NP function during the early stages of IVD degeneration.

An injectable and adhesive chitosan-based cell-delivery vehicle was developed in the work of Hoemann et al. [66]. The developed cytocompatible chitosan solution is space-filling and adheres to cartilage in situ, suggesting a potential for use as an arthroscopically injectable vehicle for cell-assisted cartilage repair. The chitosan gel was cultured in vitro, with and without chondrocytes, and injected subcutaneously in nude mice to form subcutaneous dorsal implants. The histochemical, biochemical, and mechanical properties of the resulting tissue constructions are comparable to those observed in vitro for primary chondrocytes cultured in 2% agarose. After being injected, the gel was retained for 1 day in vivo in a chondral defect of the full thickness of a rabbit, and for up to 1 week in osteochondral defects of rabbits, indicating the capacity of the gelling chitosan solution—while persisting in osteochondral defects for at least 1 week in vivo—supports the in vitro and in vivo accumulation of the cartilage matrix by primary chondrocytes.

A thermoresponsive chitosan-g-poly(N-isopropylacrylamide) (CS-g-PNIPAAm) copolymer was synthesized in the work of Mellati et al. [44] as a carrier of mesenchymal stem cells (MSCs) to provide support for their proliferation and differentiation. CS-g-PNIPAAm hydrogels were loaded with 3D microengineered cells with different microstrip widths to control cell alignment and elongation better to mimic the superficial zone of natural cartilage better. After 28 days of incubation in a chondrogenic medium, the MSCs encapsulated in the synthesized hydrogel showed 6 and 7 fold increases in glycosaminoglycans' (GAGs) secretion and total collagen, according to the biochemical tests.

Hydroxybutyl CS (HBC)/oxidized chondroitin sulfate (OCS) hydrogels were fabricated in the work of Li et al. [67] by 3D bioprinting technique. The synergistic association of two biopolymers enabled good injectability of the composite hydrogel. The favorable biocompatibility allowed the 3D in vitro culturing of human adipose-derived mesenchymal stem cells (HAMSCs) with high viability. Additionally, HBC/OCS hydrogels provoked low inflammatory gene expression of macrophages in vitro, and inadequate inflammatory responses in vivo, inhibiting acute immune responses in 7 days.

2.3. Neural Tissue Engineering

Peripheral nerve damage is a global clinical problem with a high incidence in today's society that impairs quality of life for thousands of patients [68][69][70]. Chitosan has attracted significant attention among the various biomaterials proposed for the formation of nerve conduits due to its biocompatibility, biodegradability, low toxicity, and antibacterial effects—its potential for nerve regeneration was demonstrated in several in vitro and in vivo studies [71][72][73][74][75][76][77].

A freeze-dried chitosan gel sponge was examined in the work of Ishikawa et al. [76] as a scaffold for nerve regeneration in rats. A gap of 8 mm was made with the removal of a segment of the sciatic nerve, and a chitosan gel sponge sandwiched the distal and proximal stumps. Four days after the operation it was possible to observe the regenerating axons, and 14 days later, nerves extended the distal stump. Several macrophages seemed to phagocytize chitosan, implying in the formation of a dense cell layer. The regenerating axons did not touch the chitosan and extended across the space surrounded by this polymer stacked by macrophages. It was also observed that after 2 months of surgery, the regenerating nerves were well myelinated, and their diameters after 2 and 4 months of surgery were, on average, 2.45 and 2.75 mm, respectively. The results presented in this work indicate the possibility of using the chitosan gel sponge sandwich as a graft for peripheral nerve regeneration.

A laser-activated adhesive sheet based on chitosan, indocyanine green, acetic acid, and water was used to perform the in vivo anastomosis without suturing the rats' tibial nerves. Strips were bonded to the sciatic nerves of rats and sheep's intestines by laser activation with low fluence (50 J/cm^2) to test the adhesive strength in vitro. Good adhesion to the tissue was observed with a tensile strength of 12.5 ± 2.6 kPa, which was suitable to maintain the in vivo continuity of the anastomosed nerves 3 days after surgery. Additionally, when compared to intact nerves, the number and morphology of myelinated axons were typical (ca. 96%) [78].

Chitosan derived from crab tendons is promising for creating hollow tubular structures, useful in nerve regeneration. However, chitosan tubes have two problems that need to be solved: the low mechanical strength presented by the tubes for lateral pressure and the swelling of the tube walls that reduces the tubes' internal space in vivo, limiting their effective use for nerve regeneration. To solve these problems, apatite was incorporated into the chitosan tubes to increase the mechanical strength of the tube walls in the work of Yamaguchi et al. [74]. The formation of apatite crystals on the walls of the chitosan tubes was identified by transmission electron microscopy, and a good alignment of the crystals' c-axis was observed in parallel with the chitosan molecules, indicating that the growth of these crystals occurs from the nucleation sites of chitosan molecules, probably by forming complexes with amino groups of chitosan and calcium ions. Animal tests using male Sprague–Dawley (SD) rats showed that the chitosan tubes effectively induced nerve tissue regeneration, while being gradually degraded and absorbed in vivo.

The mechanical and biological properties of chitosan can be improved by mixing it with collagen or gel polymers, such as gelatin (Gel), which presents free carboxyl groups that can interact with the cationic groups of chitosan, resulting in the formation of a network by hydrogen bonding [79]. These chitosan–gelatin mixtures give rise to a structural scaffold for embryonic stem cells or bone marrow mesenchymal stem cells-based tissue engineering [80].

Chitosan (Cs)/gelatin (Gel) porous scaffolds containing hyaluronic acid (HA) and heparan sulfate (HS) were fabricated via freeze-drying in the work of Guan et al. [81]. Cs/Gel/HA/HS composite scaffolds presented very homogeneous and interconnected pores with porosity above 96% and a controllable degradation rate. Cell culture studies showed that the presence of HA and HS in the Cs/Gel/HA/HS scaffolds promoted relevant initial NS/PCs adhesion and supported the long term growth in a 3D environment, and compared to the Cs/scaffolds Gel, NS/PCs in the Cs/Gel/HA/HS also maintained multilinear differentiation potentials with increased neuronal differentiation.

2.4. Chitosan in Wound Healing

Wound healing may be defined as a dynamic process that involves various molecules and cells, such as mediators, natural extracellular matrix (ECM), blood, and parenchymal cells [82]. Among the most frequently used natural polymers for the development of hydrogels applied in skin regeneration, chitosan (CS) stands out because it has characteristics such as antimicrobial action and reduced wound healing time [83]. Throughout the various stages of wound healing, a positive role is played by chitosan-based hydrogels. CS advances surface-induced thrombosis and blood clotting, and quickens coagulation in vivo. Besides, it influences platelet activation, the most significant component in blood clotting, and releases cytokines to improve the healing process [84]. The four stages for wound healing can be described as follows:

(1)Immediately after the injury, coagulation and hemostasis occur in the wound, which can avoid exsanguination and arrange a matrix for the invasion of cells necessary in the subsequent stages of healing [85].

(2)Soon after, the inflammatory phase of wound healing begins [86], controlled by inflammatory reactions moderated by cytokines, chemokines, growth factors, and their actions on cell receptors. The activation of intracellular signaling cascades contributes to cell proliferation, migration, and differentiation. Additionally, distinct types of cells (such as granulocytes and macrophages) are recruited by chemoattractant factors to initiate repair at the wound site [87]. In this process, an appropriate inflammatory microenvironment conducive to healing is formed by regulating cell activity and factors released by chitosan (CS)-based hydrogels.

(3)In 2 to 10 days after the injury, proliferation begins, featuring proliferation and migration in distinct cell types. The proliferative phase involves neoangiogenesis, development of granulation tissue and ECM, and re-epithelialization [88]. CS provides a non-protein matrix for 3D tissue growth and the activation of macrophages for tumoricidal activity. CS-based hydrogels can promote fibroblast proliferation, angiogenesis, and regular collagen deposition; enhance the natural hyaluronic acid (HA) level at the wound site; accelerate wound healing; and act in scar avoidance [4][28][89].

(4)In this phase, remodeling occurs, in which, by many enzymes and stress actions, the content and disposition of collagen fibers in the scar tissue are adapted to adjust to the physiological work, and this ends in the development of normal epithelium and maturation of scar tissue. A vital component of the dermal tissue present in chitosan is N-acetyl glucosamine (NAG), which is essential for repairing scar tissue [90].

Due to the antimicrobial characteristics and the capacity to transport extrinsic antimicrobial agents, chitosan is used to avoid or treat wounds and burn infections. Additionally, to improve wound healing, chitosan can be utilized as a slow-release drug-delivery vehicle for growth factors [91]. Some researchers have also reported that chitosan induces analgesia [92][93]. Allan and co-workers [93] verified that chitosan provided a refreshing, pleasant, and soothing topical sensation to open wounds. Okamoto, et al. [94] and Shigemasa and Minami [95] noticed that when animal wounds were covered with chitosan, accelerated wound healing was observed, along with a reduction in the treatment frequency, protection for the wound surface, and a decrease in or complete absence of pain.Morphine, a commonly recommended drug for cancer pain relief, is regularly administered orally. Episodic and temporary pain is short-lived (40 min), frequently unpredictable, and can rapidly worsen, and due to slow pain control, treatment with oral opioid administration is not ideal. Parenteral administration provides faster control of pain relief but is not always an available, convenient, or preferred option. The nasal administration of painkillers offers faster pain relief. Therefore, it is worthwhile to adopt other non-oral routes of administration, such as transmucosal, nasal, or pulmonary, which can supply quickly pain alleviation [96]. The most convenient alternative method for releasing analgesic drugs seems to be the nasal route of administration. Morphine, however, being hydrophilic, is poorly absorbed via the nasal route. This problem can be solved by combining morphine with chitosan, a bioadhesive material that delays mucociliary clearance of morphine, permitting an extensive absorption time [97][98]. This morphine–chitosan release system can be of particular benefit to home-care patients [96].Chitosan (CS) can act as a vehicle and enhance the action of some drugs used against pain. It has also been reported to enhance the bioavailability and dissolution properties of drugs with low solubility [99]. Maestrelli, et al. [100] evidenced CS's efficiency in enhancing naproxen dissolution, a weakly water-soluble non-steroidal anti-inflammatory drug. Zerrouk, et al. [101] found a significant increase in naproxen analgesic activity after oral administration in rats using chitosan matrices. This formulation allowed a decrease in the dose required to obtain the analgesic effect, reducing the incidence of adverse effects.

3. Conclusions

The remarkable properties presented by chitosan have aroused the interest of applying this material in several biomedical applications, such as tissue engineering, wound dressing, drug delivery, and cancer treatment.

Chitosan's biocompatibility and biodegradability explain the use of this polymer in the pharmaceutical field, and its chemical groups support many modifications, which made this polymer an option for use as a controlled drug release agent. Chitosan can act as a vehicle and improve the action of some drugs used against pain, increasing the bioavailability and dissolution properties of low solubility drugs. This polymer can also be combined with other polymers or inorganic materials to modulate its mechanical and chemical degradation properties, a trend observed in the studies over the last two decades.

Chitosan is also applied in tissue regeneration systems, with the ability to form extremely porous scaffolds structures with interconnected pores and intrinsic antibacterial activity that allows cells to be seeded, migrate into the inside, multiply, and be provided by enough nutrients. Chitosan polar groups provide a microenvironment with favorable physicochemical properties for cell adhesion and proliferation.

Chitosan scaffolds provide support for the fixation and multiplication of osteoblast cells, and also the generation of mineralized bone matrix. Chitosan-based hydrogels stand out for the regeneration of cartilaginous tissue, efficiently supporting chondrogenic activity. Chitosan-based materials have also been shown to promote adhesion, survival, and neurite outgrowths of neurons; chitosan's potential in nerve regeneration has been demonstrated in vitro and in vivo.

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