J Polym Environ (2017) 25:854–866
DOI 10.1007/s10924-016-0865-5

ORIGINAL PAPER

Chitin and Chitosan: Structure, Properties and Applications in Biomedical Engineering

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Published online: 18 October 2016

Abstract Chitin and its deacetylated derivative chitosan are natural polymers composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). Biopolymers like chitin and chitosan exhibit diverse properties that open up a wide-ranging of applications in various sectors especially in biomedical science. The latest advances in the biomedical research are important emerging trends that hold a great promise in wound-healing management products. Chitin and chitosan are considered as useful biocompatible materials to be used in a medical device to treat, augment or replace any tissue, organ, or function of the body. A body of recent studies suggests that chitosan and its derivatives are promising candidates for supporting materials in tissue engineering applications. This review article is mainly focused on the contemporary research on chitin and chitosan towards their applications in numerous biomedical fields namely tissue engineering, artificial kidney, skin, bone, cartilage, liver, nerve, tendon, wound-healing, burn treatment and some other useful purposes.

Keywords Chitin · Chitosan · Tissue engineering · Wound-healing · Burn treatment · Antimicrobial application

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Introduction

Chitin a naturally abundant mucopolysaccharide is white, hard, inelastic, and nitrogenous compound that is a by-product of the fishery industry and is considered as a regenerating raw material which is only second to cellulose in terms of abundance. Its natural abundance amounts to more than 1000 tons per year and about 70 % of which comes from marine species. Chitins is the main component in the shells of crustaceans such as shrimp, crab and lobster, and is also be found in exoskeletons of mollusks and insects as well as in the cell walls of some fungi [1, 2]. It is mainly produced commercially by deacetylation or removing acetyl groups from the chitin polymer by treatment with alkali [3].

The main driving force for the development of wider applications of chitin and chitosan lies in the fact that these polysaccharides are not only naturally abundant but also nontoxic and biodegradable. It was demonstrated by many researchers that chitosan had a great potential for a wide range of uses due to its versatile biological, chemical and physical properties [2]. The application of chitosan includes a variety of areas such as pharmaceutical and medical applications, paper production, textile wastewater treatment, biotechnology, cosmetics, food processing and agriculture [4]. But the biocompatibility, biodegradability, nontoxicity and antimicrobial activity of chitosan attract the attention of researchers in the recent years and garnered considerable interests in the field of biomedical engineering [5].

Investigation of chitosan for the biomedical application has been a long journey for scientific exploration and technological development. The journey is currently enriched with studies on the biological phenomena and typically carried out via chemical, biochemical, microbiological and medical assays of chitosan and its

derivatives. Various physical forms of chitosan in differing methods were applied by the researchers across the globe in studying biomedical applications of chitosan and its derivatives [6].

The aim of the present review is to concentrate on the current research on chitin and chitosan highlighting the relationship between the physicochemical properties of these two polymers and their biological activities as well as their applications in various biomedical fields namely tissue engineering, artificial kidney, skin, bone, cartilage, liver, nerve, tendon, wound-healing, burn treatment and some other purposes. The organization of this article is devoted to the preparation, structure, and analysis of biological properties of chitin and chitosan following various biomedical applications of both polymers and emphasizing the effects of the polymers' characteristics on these applications.

Chemical Structure of Chitin and Chitosan

The chemical structure of chitin and chitosan is very similar to that of cellulose which consists of several hundreds to more than thousand β-(1-4) linked D-glucose units [7] (Fig. 1). In chitin and chitosan structure hydroxyl at position C-2 of cellulose has replaced by an acetamide group. Chitosan, β-(1-4) linked 2-amino-2-deoxy-β-D-glucopyranose, is an N-deacetylated derivative of chitin obtained by transforming the acetamide groups into primary amino groups [8].

However, deacetylation of chitin is almost never complete and chitosan or deacetylated chitin still contains acetamide groups to some extent. Unlike cellulose, chitin and chitosan contain 5–8 % nitrogen, which in chitin is in form of acetylated amine groups and in chitosan in form of primary aliphatic amine groups, which makes chitin and chitosan suitable for typical reactions of amines [9]. However, chitosan is chemically more active than chitin due to the presence of primary and secondary hydroxyl groups on each repeat unit, and the amine group on each deacetylated unit (Fig. 2). These reactive groups are readily subject to chemical modification to alter mechanical and physical properties of chitosan.

The existence of amine groups in chitin and chitosan represents a great advantage because it enables distinctive

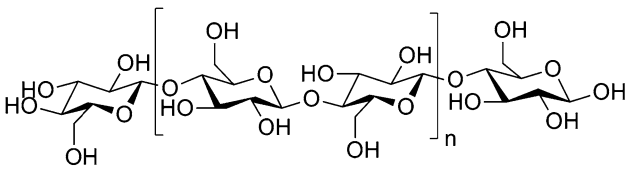

Fig. 1 Chemical structure of cellulose polymer

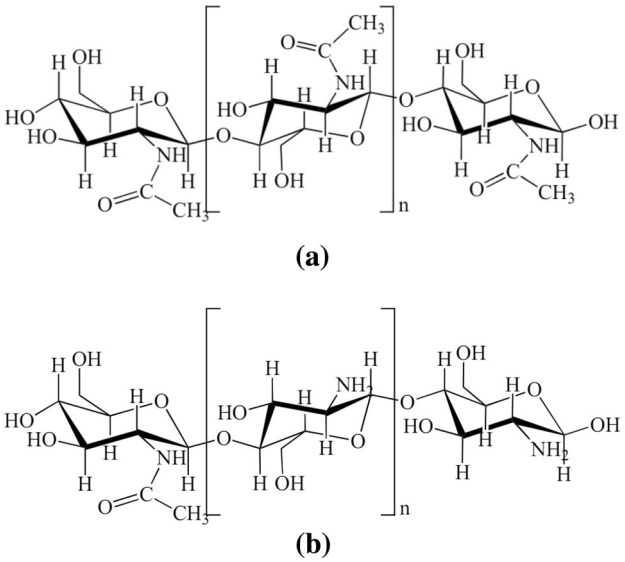

Fig. 2 Structural relationships between chitin (**a**) and chitosan (**b**)

biological functions as well as the application of modification reactions [10]. Excellent properties of these polysaccharides, such as biocompatibility, biodegradability, bioactivity, bioresorptivity, non-toxicity and good adsorption properties make these materials very suitable and essential biomaterials and draw a great deal of industrial attention as probable alternatives to synthetic polymers [11, 12].

Preparation of Chitin and Chitosan

Chitin and chitosan are obtained from the shells of crustaceans such as crabs, prawns, lobsters and shrimps, the exoskeletons of insects, and the cell walls of fungi such as aspergillus and mucor. Crab and shrimp shell wastes are currently utilized as the major industrial source of biomass for the large-scale production of chitin and chitosan. Processing biowastes from marine food factories help recycle the wastes and make the derivatives or by-products useful in other fields. These crustacean shell wastes are composed of protein, inorganic salts, chitin and lipids as main structural components. Therefore, extraction of chitin and chitosan is mainly employed by stepwise chemical methods [13]. In the first stage, chitin production is associated with food industries such as shrimp canning. In the second stage, the production of chitosan is associated with fermentation processes, similar to those for the production of citric acid from aspergillus niger, mucor rouxii, and streptomyces, which involved alkali treatment yielding chitosan. Briefly, shells are ground to smaller sizes and minerals, mainly calcium carbonate, are removed by extraction (demineralization, decalcification) with dilute hydrochloric acid followed by stirring at ambient temperature.

The extraction of protein from the residual material is performed by treatment with dilute aqueous sodium hydroxide and thereby prevents contamination of chitin products from proteins. The resulting chitin is deacetylated in 40–45 % sodium hydroxide at 160 °C for 1–3 h (Fig. 3) in absence of oxygen followed by purification procedures to form chitosan with a cationic nature [14]. The deacetylation process involves the removal of acetyl group from chitin molecules and it determines the content of free amine group ($-NH_2$) in the chitosan. Repetition of the process can give deacetylation values up to 98 % but the complete deacetylation can never be achieved by this heterogeneous deacetylation process without modification [15]. The degree of deacetylation (DD) is proportional to the degree of transformation of the chitosan from chitin, which depends on NaOH concentration, the reaction temperature and time [16]. Depending on the production method and species used, the degree of deacetylation ranges from 56 to 99 %. But at least 85 % deacetylation is required for a good solubility of chitosan [17].

Biological Properties

Chitin and chitosan have some special properties that make them suitable for versatile applications. But the use of chitin and chitosan in medical and pharmaceutical sector has grown rapidly and currently received a great deal of interest from the researchers throughout the globe due to their interesting properties such as strong antibacterial effect, biocompatibility, biodegradability, non-toxicity and high humidity absorption [8]. Furthermore, other biological properties such as analgesic, antitumor, hemostatic, hypocholesterolemic, antimicrobian, and antioxidant properties have also been reported by various researchers in some recent studies [10, 18].

The majority of the biological properties are directly related to physicochemical characteristics of chitin and chitosan (Table 1) for biomedical application. The physicochemical characteristics of chitin and chitosan include molecular mass, the degree of deacetylation and the amount of moisture content [8]. The chitosan-mediated inhibition of bacterial and fungal growth largely relies on the molecular weight and functional groups of chitosan. Comparing to high molecular weight chitosan, a small oligomeric chitosan can easily penetrate the cell membrane of a microorganism and thereby prevents the growth of the cell by inhibiting RNA transcription [19]. Several other research studies [20, 21] also reported that the length of polymer chain and the distribution of acetyl groups also affect the biodegradation kinetics of chitin and chitosan.

In addition, the biological properties and antimicrobial activity of chitosan are also persuaded by the cationic behavior of chitosan. It is attributed to the polycationic nature of chitosan which most likely interacts with the predominantly anionic components resulting in changes in permeability that leads to the death of the cell by inducing leakage of intracellular components [22]. Moreover, chitosan could adsorb the electronegative substrate in the cell of microbe proteins and thereby disrupts the physiological activities of the microorganism leading to the death of cells [23]. A higher cationic charge density causes strong electrostatic interaction and the degree of deacetylation (DD) of chitosan and its derivatives greatly influences the positive charge density. It has also been observed that chitosan with a high DD (97.5 %) can lead to higher positive charge density which confers a stronger antibacterial activity than that of moderate DD (83.7 %) [6].

Biomedical Application of Chitin and Chitosan

Tissue Engineering

Tissue engineering is a branch of science that unifies materials engineering and biomedicine–deals with the production of materials that can substitute and/or induce advanced regenerative processes in damaged tissue. The growth and manipulation of laboratory-grown cells, tissues or organs that would substitute or support the function of defective or injured parts of the body is the main function of tissue engineering [3]. It involves the use of living cells, manipulated through their extra cellular environment or genetically, to develop biological substitutes for implantation into the body and to nurture remodeling of tissues in some active manner. The objective of tissue engineering is to repair, replace, maintain, or enhance the function of a particular tissue or organ [24]. The potential applications of

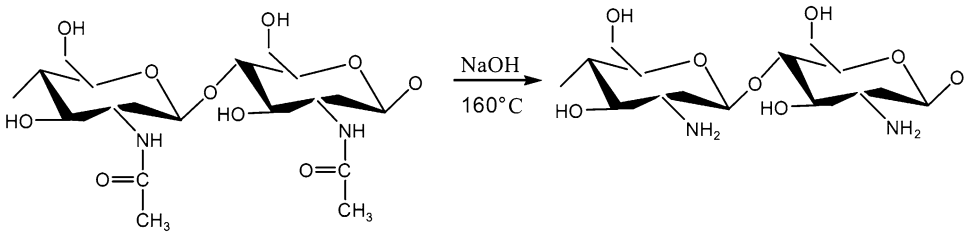

Fig. 3 Preparation of chitosan from chitin

Table 1 The biological properties of chitin and chitosan and the relationship with physicochemical characteristics [8]

Property	Physicochemical characteristic
Biodegradability	Degree of deacetylation, distribution of acetyl groups, Molecular weight
Biocompatibility	Degree of deacetylation
Mucoadhesion	Degree of deacetylation, molecular weight
Hemostatic	Degree of deacetylation, molecular weight
Analgesic	Degree of deacetylation
Adsorption enhancer	Degree of deacetylation
Antimicrobian	Molecular weight
Anticholesterolemic	Degree of deacetylation, molecular weight, viscosity
Antioxidant	Degree of deacetylation, molecular weight

tissue engineering include the development or revolution of current technology in total heap, knee, cartilage, tendon and vascular replacement. The implantation of either an autologous or synthetic graft in place of the damaged area of the body is the contemporary practice of tissue engineering. Within the body the implant must satisfy requirements relative to biocompatibility as well as functional and mechanical stability [3]. Many materials can react compatibly with the body but unfortunately, they cannot meet the long-term mechanical, geometrical, and functional requirement of the body. Therefore, tissue engineering technology has developed to construct artificial tissues that can mimic the natural ones by combining with modulated cells with different scaffolding materials, including natural and synthetic polymers. Among these materials polylactide (PLA), polyglycolide (PGA) and their copolymer, polylactide-co-glycolide (PLGA) have received much attention as suitable candidates for tissue engineering because of their biodegradability and biocompatibility [25].

The designing of polymer scaffolds for tissue engineering must fulfill a few basic requirements that have been widely accepted by the researchers. A scaffold should possess a high porosity, with an appropriate pore size distribution and a high surface area. Biodegradability is yet another requirement, with the degradation rate matching the rate of neo-tissue formation. Moreover, the scaffold must possess the required structural integrity to prevent the pores of the scaffold from collapsing during neo-tissue formation, with the appropriate mechanical properties. Finally, the scaffold should be non-toxic to cells and be biocompatible, positively interacting with the cells to promote cell adhesion, proliferation, migration, and differentiated cell function [26].

A number of works are done to make chitosan suitable for tissue engineering. One of the most tried methods to prepare chitin or chitosan for cell seeding is to first make a precursor that is typically a gel followed by various lyophilization strategies. Chow et al. [27] made a series of porous chitin matrixes by producing chitin gels from chitin solutions followed by lyophilization to give porous chitin matrixes. Matrix pore sizes ranging from 100 to 500 mm were obtainable depending on the various pre-treatment procedures of chitin gels prior to lyophilization. Mouse and human fibroblast cell cultures exposed to these chitin matrixes were found to be growing and proliferating indicating the feasibility of using these porous chitin matrixes for cell transplantation applications to regenerate tissues. Similarly, Wang et al. [28] have demonstrated the preparation of chitin–plasma sprayed calcium HA matrixes, while Ma et al. [29] also established the utility of chitosan scaffolds to support cell growth and proliferation. In an attempt to extend the upper pore size limit of 500 mm for chitin matrixes obtained by lyophilization Chow and Khor [30] developed a novel method coined 'internal bubbling process' (IBP) to give open and large-pored chitin matrixes.

Chung et al. [31] prepared chitosan-based scaffolds by combining with alginate. In their work, chitosan was modified with lactobionic acid (LA) to produce galactosylated chitosan (GC) (Fig. 4) that was added to cross-linked alginate gel and this was followed by some pre-freezing treatments and was finally lyophilized. The scaffolds exhibited the usual pore configurations, the size depending on the freezing pre-treatments, the molecular weight of chitosan and amount of galactosylated chitosan.

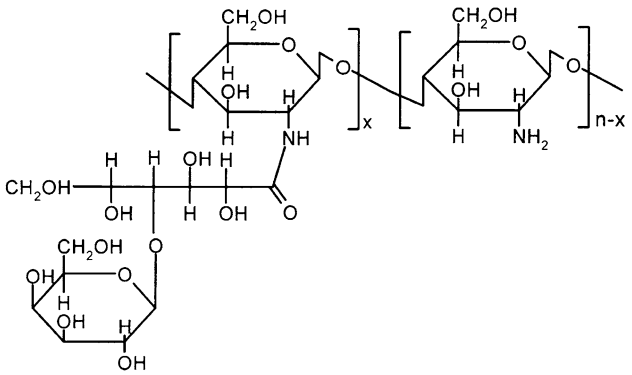

Fig. 4 Chemical structure of galactosylated chitosan (GC)

The amino acid–chitosan–PLA membrane demonstrated a good cytocompatibility to chondrocytes, behaving much like glycosaminoglycans (GAGs) found in tissue. Cai et al. [32] reported similar results using osteoblasts on chitosan-PLA membranes using a carbodiimide process to link chitosan onto the PLA surface.

Chung et al. [33] recently reported their study on growing human endothelial cells on chitosans that had cell adhesive peptides photochemically grafted onto their surfaces. Chitosan surfaces containing the grafted peptides were found to support the proliferation of human endothelial cells compared to chitosan only surfaces where no adherence was observed. Shalumon et al. [34] reported an electrospun water-soluble carboxymethyl chitin (CMC)/PVA blend for tissue engineering applications. The concentration of CMC (7 %) with PVA (8 %) was optimized, blended in different ratios (0–100 %) and electrospun to get nanofibers. Fibers were made water insoluble by cross-linking with glutaraldehyde vapors followed by thermal treatment. The prepared nanofibers were found to be bioactive and biocompatible.

Several other studies [35, 36] developed chitin or chitosan/nBGC hybrid scaffolds by lyophilization technique. The composite scaffolds showed an adequate porosity when the nBGC (Bioactive glass ceramic nanoparticles) were homogenously distributed on the pore walls. The developed nanocomposite scaffolds showed an adequate swelling and degradation properties beside its ability to become bioactive. Scores of examples reported in the application of chitin and chitosan alone or in combination with other polymers have demonstrated the significant promise of these biopolymers for tissue engineering that can be fine-tuned to suit the increasing focus that this field requires.

Artificial Kidney Membrane

Artificial kidney membrane has been developed recently that makes possible repetitive hemodialysis and sustaining the life of patients with chronic kidney failure. Commercially regenerated cellulose or cuprophan is used as a semipermeable membrane of artificial kidney due to its good permeability and mechanical strength [37]. The development of better hemodialysis membranes require a greater selectivity and higher dialysis rates for medium and large size molecules such as uric acid and the like. To meet the requirement of dialysis applications, lots of works have been done for the modification of existing polymeric membranes or on synthesis of new polymeric membranes [38–41]. Chitosan holds promise for being used as an artificial kidney membrane due to its intrinsic high mechanical strength in addition to permeability to urea and creatinine [42, 43]. Moreover, these membranes are impermeable to serum proteins. The film-forming properties of chitosan were extensively studied by several workers and a variety of chitosan membranes have been proposed for reverse osmosis, ion exchange, metal ion uptake, diffusion of dyes and separation of water-alcohol mixture systems [37].

Chitosan can be modified by blending with water-soluble polymers and by graft copolymerization to develop dialysis properties [44]. Srinivasa et al. [45] reported that chitosan forms a clear homogenous blend with polyvinyl acetate (PVA) and the tensile strength of the resulting blend is greater than the component value. Uragami et al. [46] prepared a cross-linked chitosan/PVA blend with a fixed amount of cross-linking agent and studied the active transport of the halide ions through the chitosan/PVA membrane. Reinhart and Peppas [47] studied the diffusion of bovine serum albumin in highly cross-linked membrane. Other studies [48–52] investigated a variety of polymeric membranes from chitosan derivatives and reported on the transport of alkali metal ions and lower molecular weight solutes through these membranes. Hirano et al. [53, 54] prepared a series of membranes from chitosan and its derivatives and the membranes showed improved dialysis properties. Chitosan membranes were modified with vinyl monomers using ^{60}Co γ-ray irradiation that showed an improved blood compatibility and permeability [55–57].

Wound Healing/Wound Dressing

The healing of wound within a short time is desirable for every patient however, patients suffering from diabetes show an extremely slow rate of healing. The application of chitin and chitosan as possible wound-healing accelerators that has been investigated by many researchers for a long time [58–60]. Chitin and its derivatives can be applied safely to animals as well as the humans. Various forms of chitin-based products are available for medical applications, like finely divided powder, nonwoven fabrics, porous beads, lyophilized soft fleeces or gels, gauges, laminated sheets and transparent films [61].

A number of studies [26] have reported the use of chitosan scaffolds and membranes to treat patients with deep burns, wounds, etc. The direct use of ‘in situ’ chitin with fungal mycelia from the fungus Ganoderma tsugaue to produce wound healing sacchachitin membranes [62]. A non-woven mat obtained by first processing the mycelia to remove protein and pigment, followed by isolation of fibers in the 10–50 mm diameter range and a final consolidation into a freeze-dried membrane under aseptic conditions was used in a wound model study. The wound healing of this fungal-based non-woven mat as surmised from wound

contraction measurements on two different animal model studies turned out favorable [63]. No adverse responses were observed in vivo immunogenicity evaluations in vitro cell culture using rat fibroblasts [64].

Madhumathi et al. [65] developed novel α-chitin/nanosilver composite scaffolds for wound healing applications. These α-chitin/nanosilver composite scaffolds were found to possess excellent antibacterial activity against S. aureus and E. coli, combined with good blood clotting ability and could be used for wound healing applications. Kumar et al. [66] developed and characterized β-chitin/nanosilver composite scaffolds for wound healing applications using β-chitin hydrogel containing silver nanoparticles. These β-chitin/nanosilver composite scaffolds were found to be not only have anti-bactericidal against E. coli and S. aureus and good blood clotting ability but also have cell adhesion properties and this good cell attachment along with antibacterial activity is ideal for wound healing applications.

One of the best illustrations of the wound healing properties of chitin was reported at the 8th International Chitin and Chitosan Conference by Dung et al. [67]. Chitin membranes named Vinachitin TM was prepared by decrystallizing ricefield crabshells. Good results have been reported from 3 years clinical trial study that treated more than 300 patients for deep burns, orthopedic, trauma and ulcer conditions. Stone et al. [68] evaluated the healing at skin graft donor sites dressed with chitosan. A total of 20 patients requiring a split-skin graft during the 7-month period were entered into the study. The skin graft donor sites were dressed half with chitosan and half with a conventional dressing. Chitosan proved to be an easy dressing material to apply and maintain and was painless to remove. Histologically, skin occluded by the chitosan dressing showed marked differences to skin occluded by the conventional dressing at the newly healed time point. Chitosan biopsies showed a looser connective tissue stroma in the papillary dermis, which was richer in both glycosaminoglycan matrix and capillaries than control biopsies. Small dermal nerve fibers were also more numerous in chitosan biopsies and showed marked differences to skin occluded by the conventional dressing at the newly healed time point. In addition, digital color separation analysis of donor site scars demonstrated an earlier return to normal skin color at chitosan-treated areas.

Artificial Skin

Individuals who have suffered extensive losses of skin, commonly fire induced, are actually ill and in danger of succumbing either to massive infection or to severe fluid loss. Patients must often cope with problems of rehabilitation arising from deep, disfiguring scars and crippling contractures. The analogous structural characteristics of chitosan to glycosaminoglycans could be a well-thought-out for developing a skin replacement. Yannas and Burke [69] proposed a design for artificial skin applicable to long-term chronic use, with the focus on a nonantigenic membrane that performs as a biodegradable template for neodermal tissue. Kifune et al. [70] recently developed a new wound dressing material, Beschitin W, a commercial product that is composed of chitin nonwoven fabric that has been found to be beneficial in clinical practice. Kim and Min [71] have developed a wound covering material from polyelectrolyte complexes of chitosan and sulfonated chitosan. Wound healing is accelerated by oligomers of degraded chitosan by tissue enzymes, and this material was found to be effective in regenerating the skin tissue of the wound area. A pharmaceutical company named Katakurachikarin in Hokkaido, Japan, manufactures an artificial skin by chitosan–collagen composite that seems to improve recovery from surgical wounds or burns.

Mao et al. [72] have studied a novel absorbable scaffold for artificial skin composed of chitosan and gelatin that was fabricated by freezing and lyophilizing methods. The chitosan–gelatin cross linked scaffolds was prepared and fibroblasts were co-cultured with keratinocyte construct an artificial bilayer skin. The artificial skin obtained was flexible and had good mechanical properties. It was also found that it did not elicit any adverse inflammatory reactions once implanted under the skin of rabbits. The scaffolds were biocompatible and biodegradable over the course of the implantation and there was no contraction observed in the in vitro cell culture test.

Bone Damage

Bone consist of mainly hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] along with some other materials including collagen, chondroitin sulfate, keratin sulfate and lipids. In recent years, substantial progress has been made to treat the loss or failure of bone tissue. The treatment of damage or broken bone is carried out by using biodegradable substitutes which act as a temporary skeleton inserted into the defective sites of skeleton or lost bone sites. This temporary skeleton support and stimulates bone tissue regeneration. After that they gradually degrade and are replaced by new bone tissue. Both bioactive ceramics and polymers have been developed and analyzed for use as tissue engineering scaffolds [73]. Bioactive ceramics are chemically similar to natural bone which allows osteogenesis to occur and can provide a bony contact or bonds with host bone [74]. But the main limitation of these bioceramics is its brittleness and low biodegradability. A number of natural

and synthetic polymers have been studied for overcoming these drawbacks of the bone substitutes. In particular, chitosan has been also extensively studied in bone tissue engineering since after observing its capacity to promote growth and mineral rich matrix deposition by osteoblasts in culture [75]. Recently, grafted chitosan natural polymer with carbon nanotubes has been incorporated to increase the mechanical strength of these composites. Carbon nanotubes are allotropes of carbon with a cylindrical nanostructure and constructed with length-to-diameter ratio of up to 28,000,000:1. These cylindrical carbon molecules have some novel properties, like high Young's modulus (1.0–1.8 TPa), high tensile strength (30–200 GPa) and high elongation at break (10–30 %), extremely small size (about 1–10 nm in diameter), high aspect ratio (>1000), high structural and chemical stability, and stiffness [76, 77] which make them potentially useful in many applications in nanotechnology, electronics, optics and materials sciences. It has been observed that the combination of carbon nanotube with chitosan leads to an enormous increase in the mechanical strength of the composite [78].

A number of studies have been focused on chitosan–calcium phosphates (CP) composites for this purpose in bone tissue engineering [79]. Beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) of CP bioceramics are excellent candidates for bone repair and regeneration because of their similar chemical composition with inorganic components of bone. Zhang et al. [80, 81] prepared CP bioceramic embedded with chitosan sponge which enhanced mechanical property of the ceramic phase via matrix reinforcement and preserving the osteoblast phenotype. Ge et al. [82] reported a hydroxyapatite (HA)–chitin material that was osteoinductive and exhibited rapid degradation and neovascularization in vivo during a 3-month period. Kawakami et al. [83] studied the in vivo effect of a chitosan–HA paste when applied on the surface of the tibia after periosteum removal. Formation of new bone was observed after 1 week and continued during a 20-week follow-up indicating suitability of this paste for further clinical studies as a bone filling material. The issue of mechanical resistance of chitosan based composites was addressed by Hu et al. [84], who reported a chitosan–HA multilayer nanocomposite with high strength and bending modulus rendering the material suitable for possible application for internal fixation of long bone fractures. All this research and development testify the chitosan composite as great possibilities for future bone tissue engineering.

Articular Cartilage

Articular cartilage is the highly specialized connective tissue of diarthrodial joints. Its principal function is to provide a smooth, lubricated surface for articulation and to facilitate the transmission of loads with a low frictional coefficient. Articular cartilage consists of the isolation of articular chondrocytes or their precursor cells that may be expanded in vitro and then seeded into a biocompatible matrix, or scaffold, for cultivation and subsequent implantation into the joint [85]. A selection of biomaterial is the most important factor for the successful repairing of cartilage [86]. The perfect cell-carrier substance should be similar in the natural environment of the articular cartilage matrix. It has been shown that cartilage-specific extracellular matrix (ECM) components like collagen and glycosaminoglycans play a critical role in regulating expression of the chondrocytic phenotype and in supporting chondrogenesis in vitro as well as in vivo [87, 88]. Chitosan has a similar structure with various glycosaminoglycans (Fig. 5) found in articular cartilage makes it an elite scaffolding material in articular cartilage engineering [85].

Lu et al. [89] demonstrated that the chitosan solution injected into the knee articular cavity of rats led to a significant increase in the density of chondrocytes in the knee articular cartilage, indicating that chitosan could be potentially beneficial to the wound healing of articular cartilage. Mattioli-Belmonte et al. [90] showed that the bone morphogenetic protein (BMP)-7, associated with *N,N*-dicarboxymethyl chitosan induces or facilitates the repair of artificial cartilage lesions in rabbit, hypothesizing a synergism of their corresponding biological outcome.

Liver

Patients suffering from liver problem have been increasing day by day and the treatment of these patients is much more problematic. One of the most acute problems is the lacking of donor organs for orthotopic liver transplantation. This factor has now amplified the necessity for new therapies for severe and long-lasting liver disease [91]. The treatment of fulminant hepatic failure (FHF) by bioartificial liver (BAL) is one of the promising applications of the tissue engineering [92]. The principal goal is to develop a bioartificial liver device in which patient plasma is

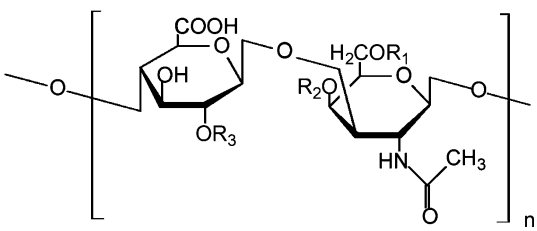

Fig. 5 Chemical structure of glycosaminoglycans (chondroitin sulfate)

circulated extra corporeally through a bioreactor that houses metabolically active liver cells. One of the important issues for bioartificial liver devices is the proper choice of cell sources, such as primary hepatocytes, hepatic cell lines and liver stem cells. Many researchers are attempting to develop bioartificial liver devices in which hepatocytes are optimally maintained so that they carry out many activities as possible [93]. Bioartificial liver devices require a suitable extracellular matrix (ECM) for hepatocyte culture because hepatocytes are anchorage-dependent cells and are highly sensitive to the extracellular matrix milieu for the maintenance of their viability and differentiated functions [94–96]. Chitosan being a favorable biomaterial can be applied in liver tissue engineering. One of the reasons for selecting chitosan as a scaffold for hepatocytes culture is that its structure is analogous to glycosaminoglycans which are components of the liver extracellular matrix [97–99].

Chupa et al. [100] reported that chitosan and chitosan complexes with glycosaminoglycans had an important potential for the design of a novel biologically active biomaterial which can modulate the activities of vascular endothelial and smooth muscle cells in vitro and in vivo. Other studies [98, 99] revealed that the micro-structure of porous scaffolds provide a large surface area for cells to adhere and facilitate nutrient and oxygen transportation. Wang et al [101]. prepared chitosan collagen matrix (CCM) by cross-linking agent EDC in NHS buffer system. The EDC cross-linked CCM imparted a moderate mechanical strength, good hepatocyte compatibility as well as excellent blood compatibility. The implantable bioartificial liver can restore, maintain or improve liver functions or offer the possibility of permanent liver replacement.

Nerve

Nerve injuries are one of the most complicated physical injuries because mature neurons have little capacity for undergoing cell division. Once the nervous system is impaired, its recovery is difficult and failure of other parts of the body occurs [102]. The repair of nerve lesions has been attempted in many different ways which have in common the goal of directing the regenerating nerve fibers into the proper endoneurial tubes. A wide variety of materials have been suggested for the production of artificial tubes for nerve repair, including biocompatible, non-degradable and degradable materials [103]. Thus, artificial tubes to bridge large defects in nerve repair should contain a biodegradable matrix which can provide an optimal structural, cellular, and molecular framework. Chitosan has been considered as a potential material for nerve regeneration due to its unique properties like antitumor, antibacterial activity, biodegradability and biocompatibility [104]. Haipeng et al. [105] reported that neurons cultured on the chitosan membrane can grow well and that chitosan tube can greatly promote the repair of the peripheral nervous system. Several other studies [106, 107] also suggested that chitosan fibers reinforced the adhesion, migration and proliferation of schwann cells which provide a similar guide for regenerating axons to bungner bands in the nervous system.

Artificial Tendon

Fracture or damage in tendon is another type of difficulties that can be solved by the use of chitin and its derivatives. Chitin-NWF (a chitin based biomaterials) is used for calves with flexure deformity of the fetlock joint [108]. In case of the fetlock joint deformity, chitin-NWF is used as a tendon substitute in the elongation technique. When the superficial and deep digital flexor tendons are cut off and the fetlock joint is elongated to the normal position, the cut parts are separated proportionally to the degree of contraction of the two flexor tendons. Flexor deformities of the fetlock joint are completely cured in all cases without complications.

Burn Treatment

The treatment of skin after burning can be performed by using chitosan. Chitosan forms tough, water absorbent, biocompatible films directly on the burn by the application of an aqueous solution of chitosan acetate [109]. Another benefit of this type of chitosan treatment is that it allows an excellent oxygen permeability that is essential to avert oxygen deprivation of the injured tissues. In addition, chitosan film has the ability to absorb water and is naturally degraded by body enzymes [110]. This fact means that the chitosan needs not to be removed from the burning area of the body. In most injuries especially in case of burning, the removal of wound dressing can cause damage to the injury site [3]. Moreover, the development of Beschitin W which is the nonwoven fabric of polymeric *N*-acetyl-D-glucosamine (chitin) and acetate chitosan can elicit the analgesic effects on burn injuries in human beings [111].

Blood Anticoagulation

One of the most widely used blood anticoagulants is heparin but its cost is very high. In order to reduce the cost, lots of work has been done to prepare synthetic coagulant but none are found as nontoxic as heparin. It has been reported that cellulose and starch sulfuric acid esters are

toxic, whereas chitin disulfuric acid is less toxic. It has also been reported that the protein moiety of chitin is responsible for the inflammatory response when material containing chitin is injected into tissues of higher animals.

Dutkiewicz et al. [112] have reported that chitosan renders both hemostatic effect as well as anticoagulant properties. Chitin is a suitable starting material for the production of heparinlike blood anticoagulants. Sulfonation of chitosan has been one of the most attractive modifications owing to the possibility of preparing anticoagulant polysaccharides in view of the structural similarity with heparin. For sulfonation, various reagents have been used by many researchers [113–115]. Sulfonated derivatives of chitosan possess blood anticoagulant activity. Conversion of position 6 into a carboxyl group in N-sulfonated chitosan gives a product with 23 % of the activity of heparin [116]. It has been reported that as the content of sulfur increases in chitosan, the anticoagulant activity of sulfonated chitosan increases. N-Carboxymethyl chitosan 3,6-disulfonate of low molecular weight exhibited an anticoagulant activity similar to that of heparin and showed no adverse effects on the cellular structures when added to blood [117].

Blood Vessel

Vascular disease is a common illness that causes the death of thousands of people in recent time. Coronary artery and peripheral vascular disease are one of the highest causes of mortality. Vascular transplantation is the main treatment for vascular disease. Polyethylene terephthalate (PET, Dacron) and expanded polytetrafluoroethylene (ePTFE) have been regarded as the standard biomaterials for prosthetic vascular grafts [118]. But both PET and ePTFE grafts have been shown to perform well at diameters of N6 mm, but neither material has been suitable for any small-diameter (b4 mm) applications.

Chupa et al. [100] have made an effort to overcome both incomplete endothelialization and smooth muscle cell hyperplasia, which are the two main problems contribute to the poor performance of the existing small-diameter (b4 mm) vascular grafts through complexation of glycosaminoglycans with porous chitosan scaffolds. Glycosaminoglycans-based materials hold promise for vascular grafts because of their growth inhibitory effects on vascular smooth muscle cells and their anti-coagulant activity. Madihally and Matthew [119] fabricated a family of chitosan scaffolds including heparin-modified porous tubes which had the potential for application in blood vessel tissue engineering. Furthermore, chitosan induced accelerating effects of wound healing in small animals such as rat and dog was investigated for a hemostatic agent for vascular grafts [120, 121].

Application for Hernia

Chitin is used in the treatment of umbilical hernia. The treatment is carried out by applying chitin-NWF (a chitin based biomaterials) which has a sheet form and as prosthesis is buried in the subcutaneous portion for the stitches of the hernia ring and the peripheral portion of the sheet is fixed by an interrupted suture. The curing of umbilical hernia is outstanding without any complications [108]. Chitin-NWF was also applied for the reduction of a perineal hernia in a dog, utilizing the effect of rapid organization of the subcutaneous fat promoted by chitin. This chitin-NWF technique requires a shorter operating time of less than 20 min in comparison with conventional techniques, and dyschezia is gone on the first post-operative day with no indication of reappearance [109].

Absorbable Sutures

Suture is a stitch or row of stitches holding together the edges of a wound or surgical incision. Commercially available suture materials such as catgut, chromic catgut, polyglycolic acid and polylactic acid are used for various types of surgical operations but are not ideal as their degradation properties in various biological conditions do not live up to expectations [122]. It is reported that chitin is a suitable material for absorbable and flexible sutures tobe used in contact with bile, urine and pancreatic juices [123]. The sutures developed from chitin with sufficient strength and flexibility were absorbed in about 4 months in rat muscles without any adverse effects [123]. Again preparation of chitin filament from formic acid solution and embedding of wounds with chitin filaments in rats results in an increase in the wound strength without changing the hydroxyproline content of the skin collagen [37]. These explanations are related to the design of surgical sutures.

Antimicrobial Applications

Chitin and its derivatives have strong antimicrobial activities that give protection against bacteria and fungi. The serum of warm-blooded animals containing antibodies developed by chitin is useful for making a person or animal immune to infection against parasitic attack and the associated diseases [42]. A number of recent studies [124–126] also show the antibacterial activity of chitosan against many microorganisms. Evan and Kent [127] were able to show that chitosan is capable of agglutinating a wide variety of microbial cell types. They investigated microbial cell-binding properties of chitosan against several microorganisms. The study revealed that there is a significant reduction

in the growth of microorganisms on exposure to chitosan. It has been observed that bacterial growth of S. aureus and K. pneumoniae was depressed due to the presence of chitosan in the textile fiber [125]. Other materials examined include some commercially available chitosan derivatives, like chitosan lactate and glutamate show antagonistic effects against E. coli, S. aureus, and S. cerevisia. Chitosan can also be used for the preservation of foods [128]. Chitosan with a low degree of polymerization (DP) and chito-oligosacchride ranks prominently among derivatives with reported preservative functions. Several chitosan salts have also been used as additives for food preservation [129].

Drug Delivery Systems

The discovery and development of drug in the clinical phase is highly challenging and expensive process because most of the drugs fail to achieve favorable clinical effects due to their inability to reach the target site of action. A considerable amount of the administrated drug is disseminated over the normal tissues or organs that are not involved in the pathological process, often leading to severe side effects [130]. To overcome this critical matter, an efficient approach is the development of targeted drug delivery systems that release the drugs or bioactive agents at the desired site of action.

In this regard, the cationic polysaccharide chitin and chitosan, has drawn an increasing attention in pharmaceutical and biomedical sectors, owing to its abundant availability, inherent pharmacological properties, and other favorable biological properties such as biocompatibility, biodegradability, non toxic profile and low-immunogenicity that lead to potential application in the design of carriers for controlled release of drug delivery [5, 37]. In addition, the degraded products of chitosan do not cause any side effects in the body. Hence, it can be a suitable matrix, available in different forms, for a sustained release of various drug formulations. A range of drugs may be well incorporated into chitosan matrix in a variety of forms such as beads, films, microcapsules, coated tablets etc. for controlled-release therapies. Chitosan with a low degree of polymerization like 60 % deacetylated chitin or hydroxypropyl chitosan is being considered as a major development of per-oral sustained release tablets. Drugs incorporated into chitosan, in different forms, are listed in Table 2.

Table 2 Chitosan based drug delivery systems [37]

Drug	Dosage form
Chlorpheniramine maleate (CPM)	Tablet
Aspirin	Wet granulation formulation
Prednisolone	Granules
Pullulan	Film
Oxyphenbutazone	Coated tablet
Dapsone	Gel

Cancer Treatment

Chitosan and its derivatives have demonstrated their promising application for the treatment of cancer [131]. The targeted anticancer drug delivery as well as tracking the path of the drug carrier with a bio-friendly heavy metal free quantum dot is a great contribution to cancer therapy [26]. The quantum dot (FA-CMCS-ZnS:Mn) nanoparticles were developed by using a novel folic acid (FA) conjugated carboxymethyl chitosan (CMCS) coordinated to manganese doped zinc sulphide (ZnS:Mn) [132]. This system can be used for targeting, controlled drug delivery and also for imaging of cancer cells.

Gene therapy treatment where genes are used to treat illnesses shows potentiality to treat cancer. Due to physical size of therapeutic plasmids and because of their circulation half-life which normally in minute a carrier is required [133, 134]. This carrier should be biocompatible, biodegradable, non-immunogenic, non-toxic and able to carry a variety of types of molecular agents without changing its own or their chemical constitution. Moreover, it has ability to release the ferried agent in a sustained (controlled) manner, be relatively easy and inexpensive to formulate, not require biohazardous chemicals or unsafe formulation procedures for manufacture and be formulated from abundant natural raw materials and one such material is chitosan.

Chitosan-coated polyisohexylcyanoacrylate (PIHCA) nanoparticles have been developed for intravenous delivery of small interfering Ribonucleic acid (siRNA) and have been shown to possess efficacy against the Ras homolog gene family, member A (RhoA) cancer target gene [135]. In 2001, it was shown that chitosan can cause apoptotic death of bladder tumour cells via caspase-3 activation [136]. Intratumoural administration of a chitosan gel promoted reduction of metastatic breast cancer progression in animals [137]. Chitosan also stimulates macrophages to mature into cytotoxic macrophages and suppresses tumour growth in mice [138]. Elevated secretion of IL-1 and IL-2 was believed to cause the anti-tumour effect through maturation and infiltration of cytolytic T-lymphocytes [139]. Chitosan directly inhibits tumour cell proliferation by inducing apoptosis [140]. In addition, chitosan inhibits Ehrlich ascites tumour growth by reducing glycolysis, thereby decreasing glucose uptake and ATP level in the tumour cells [141]. Recently, chitosan nanoparticles have been shown to cause necrotic death of liver cancer cells via

neutralization of cell surface charge, decrease in mitochondrial membrane potential and induction of lipid peroxidation [142]. More importantly, when administered orally at a dose of 1 mg kg^{-1} in mice, tumour growth was inhibited by up to 62 %, with no evident toxicity to the liver. This study demonstrates that it possesses potent activity against cancer.

Catheter

Catheter is a thin tube made as a medical device that can be inserted in the body to treat diseases or perform a surgical procedure. This flexible tube inserted through a narrow opening into a body cavity, particularly the bladder for removing fluid. The use of heparin coated chitosan in catheters draw much attention of the researchers in the field of biomedical engineering due to its great clinical performance and physical compatibility [143]. Chitosan–heparin cyanoborohydride surface is very promising as catheter compared to other biomaterials such as cationic tridodecyl methylammonium chloride–heparin surfacethat exhibits poor compatible interfacial properties with surrounding tissues and fluids [144]. Chitosan–heparin coated polymers also display excellent thromboresistance properties. The lifetime of the thromboresistance can be extended by covalently binding the heparin to chitosan with the aid of sodium cyanoborohydride. This surface treatment is useful for biomedical applications requiring blood compatibility for periods as long as 4 days [145].

Ophthalmology

Ophthalmology is the branch of medicine that deals with the anatomy, physiology and diseases of the eye. The ophthalmological applications of chitin and chitosan attract the attention of many researchers in recent times due to their distinguishing features which have replaced the use of synthetic polymers in the field of ophthalmology. The optical clarity, mechanical stability and sufficient optical correction, gas permeability-partially towards oxygen, wettability and immunologically compatibility of chitosan can fulfill the requisite for a perfect contact lens [146]. Contact lenses are made from partially depolymerized and purified squid pen chitosan by spin casting technology. These contact lenses are clear, tough and possess other required physical properties such as modulus, tensile strength, tear strength, elongation, water contact and oxygen permeability [147]. In addition, excellent antimicrobial and wound healing properties along with outstanding film forming capability make chitosan appropriate for the progression of ocular bandage lens [148].

Conclusion

Chitin and chitosan represent a great variety of properties due to high charge density, reactive hydroxyl and amino groups as well as extensive hydrogen bonding capacity and unique chemical structure as a linear polycation. The combination of versatile physicochemical and biological characteristics, allowing them to have a wide range of biomedical applications. Over the past decades, a considerable interests and attention have been focused on chitin and chitosan and attracted a great scientific and industrial interest across the globe contributing to its potential applications in biomedical engineering. The potentiality of chitin and its derivatives in biomedical applications is mainly focused in this review article after compiling all the available information regarding chemistry and biological properties of chitin and chitosan. In view of these properties along with their very safe toxicity profile, chitin and chitosan are posed to be exciting and interesting excipient in the biomedical industry for the present and future applications in tissue engineering, wound dressing and cancer diagnosis. The myriad uses of chitin and chitosan based materials even includes cancer targeting and drug delivery system. Chitosan has potential applications in artificial kidney, bone, liver, tendon, blood vessel, blood anticoagulation, nerve, and burn treatment. All these explorations indicate that biomedical products based on chitin and chitosan will be miracles of the world market in the near future.

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