Biomedical Applications of Chitosan

140

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Contents

Abstract

In the past few decades, extensive researches are being undertaken to develop innovative drug delivery systems and improve scaffolds for regenerative/alternative substances that are currently one of the most rapidly growing fields of modern science. Chitosan (CH) is a copolymer, composed of glucosamine and *N*-acetylglucosamine, and considered as a polycationic, biocompatible, and biodegradable polymer. It is one of the most explored and studied polymers. It bears several reactive functional groups, which make it capable of

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possessing strong biological properties with less toxicity, biodegradability, and environmental compatibility. Due to its low solubility, it has limitation for biomedical purposes. Chemical modification into composites or hydrogels provides versatile biomaterials because of their nontoxicity, low allergenicity, biocompatibility, and biodegradability. This chapter discusses the several biomedical applications of CH.

Introduction

The increasing interest of scientists has been emerging on the development and applications of renewable and eco-safe materials. Natural polymers, being biodegradable in nature, are preferably considered for an assortment of value-added functionalities. The most abundant biopolymer found in nature is cellulose, a polysaccharide. Chitin is the second most abundant polysaccharide in nature after cellulose and produced by a variety of exoskeletons of crustaceans and mollusks, insect cuticles, and fungi. Chitin is usually converted to CH by deacetylation process, obtaining a more soluble material, CH, in aqueous acid medium (Fig. 1). Crustacean members have decalcified cuticles that contain approximately 55–85% chitin. Chitin is secreted over the entire body of the animal by a single layer of cells of the epidermis, whereas the exocuticle does not contain chitin. The endocuticle is made of several chitin-containing layers, generally impregnated with mineral salts, such as carbonates and phosphates of calcium [1, 2]. Other constituents of the shell are proteins, lipids, and some pigments, like carotenoids. Table 1 depicts the various sources of chitin/CH in invertebrates. CH possesses nontoxicity, biodegradability, and biocompatibility with the environment and has found potential applications in pharmaceutical, textile, paper, and food industries, as well as in agriculture and medicine [1, 3, 4]. The physicochemical characteristics of CH such as crystallinity, molecular weight, and degree of deacetylation can be determined by using several methodologies summarized in Table 2. This chapter summarizes the current aspects in biomedical applications of CH and its derivatives.

Properties of CH

Solubility and Physicochemical Properties

In general, CH is found insoluble in most solvents but soluble in dilute organic acids, for example, acetic acid, formic acid, lactic acid, and malic acid. The utilization of CH is limited due to its characteristic physicochemical properties like insolubility in water, high viscosity, and tendency to coagulate with a number of proteins at high pH. In a simple way, CH contains the following chemical features [1–5]:

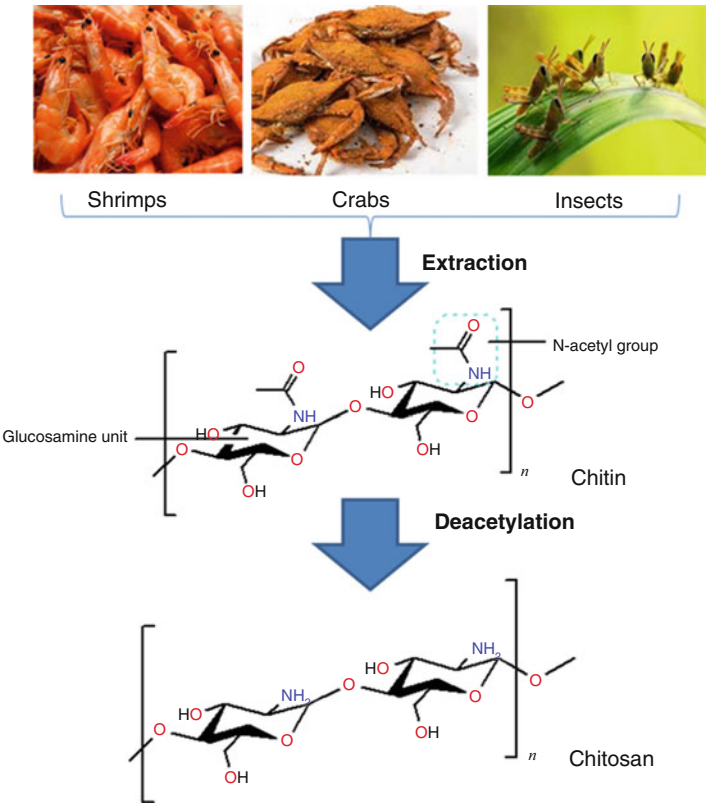

Fig. 1 Schematic representation of chitosan production

Table 1 Sources of chitin/chitosan in invertebrates (crustaceans/insects, etc.) [1, 3–7, 25]

Marine animals	Insects	Fungi/microorganisms
Crabs	Scorpions	β-type yeast
Shrimps	Spiders	Fungi (cell wall)
Prawn	Brachiopods	Green algae
Krill	Beetles	Green and brown algae
Annelids	Cockroaches	Spores
Mollusks	Ants	Chytridiaceae
Coelenterata		Blastocladiaceae
Lobsters		Ascomydes
Crustaceans		*Mycelia penicillium*

- Linear polyamine
- Presence of reactive amino groups
- Presence of reactive –OH groups
- Show chelating effect with abled transition metal ions

Table 2 Physiochemical characteristics of chitosan and determination methodologies

Physiochemical characteristics	Determination methodologies
Crystallinity	X-ray diffraction (XRD)
Molecular weight	Viscometry, gel permeation chromatography (GPC), matrix-assisted laser desorption/ionization-mass spectrometer (MLMS), light scattering (LS), high performance liquid chromatography (HPLC)
Degree of deacetylation	Infrared (IR) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, ultraviolet/visible (UV-Vis) spectroscopy, nuclear magnetic resonance (NMR, proton-H^1 and carbon-C^{13}) spectroscopy, conductometric titration, potentiometric titration, differential scanning calorimetry (DSC)

Biological Properties

The active primary amino and hydroxyl groups are available on the CH molecule being reactive and provide active sites for a variety of side group attachment employing mild reaction conditions. The attached side groups on CH provide it to be a versatile material with specific functionality and biological abilities. The following are the considerable biological properties [5–7] exhibited by CH:

- DNA binding ability (toward mammalian and microbial cells)
- Biocompatibility
- Biodegradability
- Eco-safe
- Hemostatic
- Fungistatic
- Spermicidal
- Antitumor
- Anticholesteremic
- Immunoadjuvant
- Depressant for central nervous system (CNS)
- Accelerating effect for bone formation

On account of several biological characteristics, CH based derivatives/scaffolds have been recently developed and investigated biomedical and pharmaceutical properties [4–8].

Biomedical Applications

CH is a linear, unbranched polysaccharide of glucosamine and N-acetylglucosamine. The primary amino ($-NH_2$) groups endow CH with many special properties, making it applicable in many areas and readily available for chemical reactions, for example, salt formation with acids. CH is positively charged, making it able to

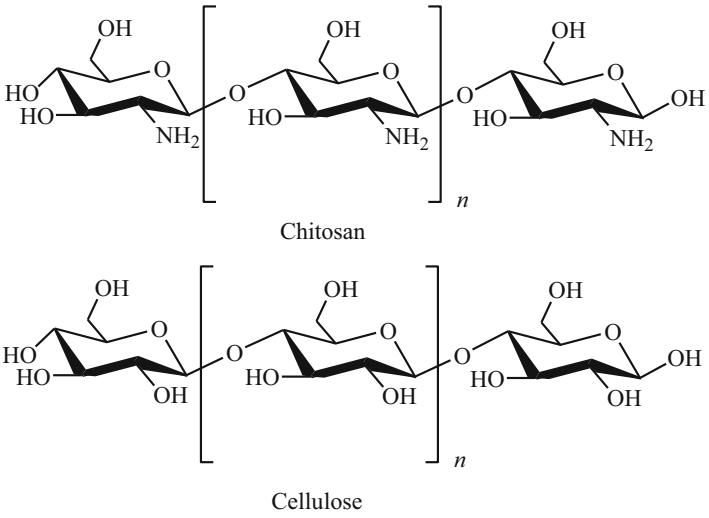

Fig. 2 Chemical structure of chitosan and cellulose

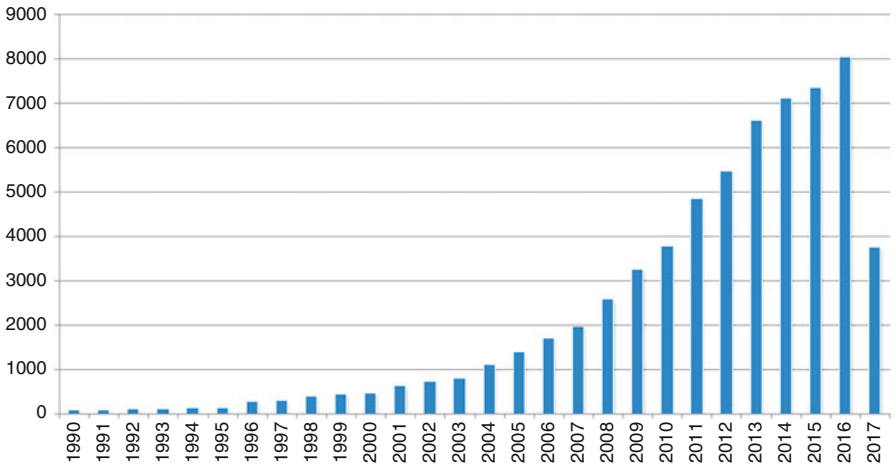

Fig. 3 Graphical representation for biomedical related publications on chitosan indexed in Scopus from January 1990 to April 2017

adhere to the negatively charged surface. It differs from cellulose at the C-2 carbon where an acetamide residue is available instead of a hydroxyl group (Fig. 2). Significant development has been achieved for the exploration of pharmaceutical as well as biomedical applications of CH and its derivatives. It can be reflected in the increasing number of related publications throughout the years. Figure 3 shows the

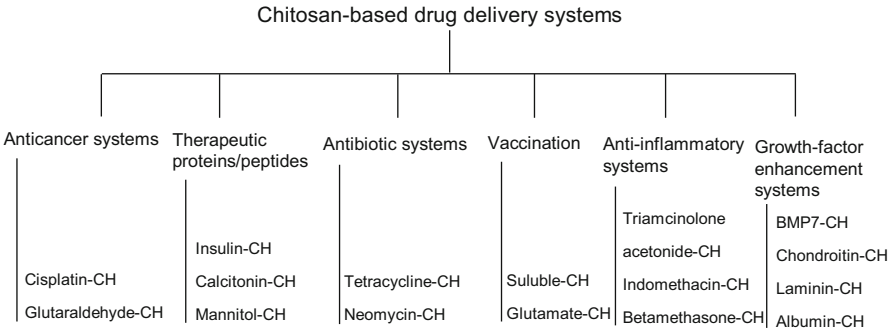

Fig. 4 Various types of chitosan based systems for drug delivery

number of Scopus indexed publications from January 1990 to April 2017 related to the biomedical potentialities of CH and its derivatives.

Interesting biomedical applications of CH and their scaffolds are summarized below:

Drug Delivery

CH has been investigated for drug delivery applications as a carrier for various active agents. Effectively, several forms are developed and utilized in drug delivery such as hydrogel system, drug conjugate, biodegradable release systems, microspheres, etc. (Fig. 4) [9–11]. Superior anticancer activity has been noticed when anticancer drugs were loaded with CH. Cisplatin-loaded and glutaraldehyde-loaded CH derivatives have found remarkable property in dogs against cancer cells of the liver. A considerable decrease in the number of arterioles in the liver, necrosis of nodules, and hepatic cell degeneration in the immobilized region was noticed using norcholesterol-CH hydrogels. Polyelectrolyte complexes of CH with therapeutic proteins or peptides have been studied for drug delivery applications. For example, insulin, calcitonin and tripolyphosphate (TPP) embedded CH were observed satisfactory drug release applications for the gastric cavity. The self-assembly and ionic interactions of CH and TPP were affected by reaction media; CH-based nanostructure could be obtained in adipic acid medium while nanoparticles were formed in acetic acid [12].

Hejazi and Amiji prepared the gastric residence time of tetracycline loaded CH microspheres by ionic cross-linking and precipitation method and investigated for antibiotic resistance as oral administration in gerbils [13]. CH-neomycin scaffold showed excellent drug delivery to the inner ear across the round window membrane of albino guinea pigs and was determined to be safe and effective carriers for inner ear therapy. Different CH loaded with a tracer drug, neomycin, was injected into the middle ear cavity of albino guinea pigs in this study [14]. Soluble CH has been effectively studied under clinical trials for parenteral and mucosal delivery of antigen

vaccines [15–18]. Nevertheless, human influenza vaccination with various antigens co-administered with CH produced both systemic and local immune responses. In a phase I clinical study, intranasal immunization with influenza vaccine formulated with soluble CH glutamate showed positive effects. A significant correlation was observed between the molecular weight of CH and dissolution rate constant or the mean absorption time and the area under the plasma concentration-time curve. In this regard, CH-based microspheres loaded with anti-inflammatory drugs were found to be effective as a transmucosal drug delivery system that responded to pH changes. For example, triamcinolone acetonide-CH, indomethacin-CH, and betamethasone-CH are much common under investigated operations [19, 20]. CH hydrogels coupled with bone morphogenetic protein (BMP)-7 have shown the ability to enhance lesion repair [5]. Additionally, chondroitin sulfate, found in cartilage, was immobilized in CH hydrogels, and marked enhancement in the cartilage formation was observed [21].

Tissue Engineering

The principles and methods of life sciences and engineering to utilize structural and functional relationships in normal and pathological tissue to develop biological substitutes to restore, maintain, or improve functioning are referred to as tissue engineering. It involves the in vitro seeding and proliferation of related cells within a scaffold support. Being biodegradable and nontoxic in nature, it can be utilized in a variety of forms including powders, gels, and films. It includes a wide range of applications such as repair or replacement of a part or whole tissues, for example, bone, blood vessels, cartilage, skin, muscle, bladder, etc. Recently, CH-based biomaterials have become a popular target in development for tissue engineering and significant progress has been developed [22–24]. It provides certain mechanical and structural properties for proper functioning of the repaired tissues being implied in the formulation of controlled delivery systems; a wide range of CH modifications can be made to improve the cell seedings. Many tissue analogues including have been prepared using engineering technology including:

- Bone tissue engineering
- Cartilage tissue engineering
- Nerve tissue engineering
- Hepatic or liver tissue engineering

Extensively, CH has been used in bone tissue engineering due to its cell growth-promoting ability and mineral-rich matrix deposition by osteoblasts cells in culture. The biocompatibility of CH also minimizes additional local inflammation, and it can be molded into porous structures to allow osteo-conduction phenomenon [25, 26]. CH was found as effective scaffolding material in particular cartilage engineering to play a pivotal role in modulating chondrocytes morphology, differentiation, and function. CH-β-tricalcium phosphate composite exhibited histocompatibility with beagle mesenchymal stem cells and was devoid of an effect on cellular growth and

proliferation. It manifested efficacy in enhancing osteogenesis and vascularization and repair of bone defects in conjunction with mesenchymal stem cells [27].

Antimicrobial Properties

The antimicrobial potential of chitin, CH, and their derivatives toward different groups of microorganisms such as algae, bacteria, yeast, and fungi has received great attention in the recent era, owing to its high biodegradability, nontoxicity, and antimicrobial properties. CH is widely used as an antimicrobial agent either alone or blended with other natural polymers [1, 4, 5]. The antimicrobial activities of CH are dependent on its physical characteristics, molecular weight, and degree of deacetylation. The microbial inhibition by CH particularly shows two main mechanisms. For type I mechanism, it is believed that the polycationic nature of CH interferes with bacterial metabolism by electrostatic stacking at the cell surface of bacteria. The type II mechanism involves the blocking of transcription of RNA from DNA by adsorption of penetrated CH to DNA molecules.

Soluble CH showed higher antibacterial activities than CH oligomers and markedly inhibited growth for bacteria tested, both Gram-negative and Gram-positive. Although, inhibitory effects differed with molecular weights of CH and the particular bacteria or fungi encountered/tested. CH generally showed stronger bactericidal effects with Gram-positive bacteria than Gram-negative bacteria in the presence of 0.1% CH. As a CH solvent, 1% acetic acid was effective in inhibiting the growth of most of the bacteria tested, except for lactic acid bacteria that were more effectively suppressed with 1% lactic or formic acids [28]. In general, the antimicrobial activity of CH was observed to be inversely affected by pH, particularly higher activity at lower pH value. In vitro studies demonstrated that CH has pivotal antibacterial as well as antifungal activity against several microbial isolates such as *F. acuminatum*, *Cylindrocladium floridanum*, *Saprolegnia parasitica*, etc. [29]. In addition, CH has been shown to be the fungicidal effect on various fungal species. Some typical examples are summarized in Table 3 showing the minimum inhibitory concentrations (MICs) for specific target organisms.

Anticancer Activity

Of the most fatal diseases, cancer is also one of the most serious. However, despite great progress in a range of approaches to tumor eradication, including chemotherapy, using anticancer drugs relies on arresting the cell cycle and rapidly killing all proliferating cells [3, 5]. This killing includes noncancerous cells, such as bone marrow, gut epithelia, lymphatic system, red blood cells, and hair follicles. One of the main challenges in current chemotherapeutic treatments is drug toxicity to healthy organs due to the lack of selectivity. Additionally, these agents often introduce severe side effects also, thereby restricting effective treatment and patient's life [1, 29, 30]. So, suitable alternatives are being developed on the biopolymeric

Table 3 MIC of chitosan against some microbial species [1–5, 21–27]

Tested species		Minimum inhibitory concentration (MIC) μg/mL
Bacteria Gram-negative	*Escherichia coli* O157	9.0
	Pseudomonas aeruginosa	9.0
	Salmonella enteritidis	0.05
	Proteus mirabilis	0.15
	Enterobacter aerogenes	11.0
	Listeria monocytogenes	9.0
Gram-positive	*Staphylococcus aureus*	9.0
	S. epidermidis	5.0
	Enterococcus faecalis	15.0
	Bacillus cereus	9.0
	B. megaterium	9.0
Fungi	*Botrytis cinerea*	0.01
	Drechslera sorokiniana	0.01
	Micronectriella nivalis	0.01
	Fusarium oxysporum	0.1
	Rhizoctonia solani	1.0
	Trichophyton equinum	2.5
	Pyricularia oryzae	5.0
	Candida glabrata	20.0

routes. In this regard, a promising approach to addressing problems in anticancer drug solubility and selectivity is their conjugation with polymeric carriers to form polymer-based anticancer drugs [3, 26]. In vitro as well as in vivo, both models were examined for the effectiveness of CH and its derivatives as an antitumor agent, which exhibited remarked antitumor activity. In a study, the antitumor effect of CH derivatives was observed due to the increase in secretion of interleukin-1 and interleukin-2 which caused maturation and infiltration of cytolytic T lymphocytes. Furthermore, it has been concluded that CH polymeric micelle-paclitaxel conjugate was found superiorly active for elevated lymphokine production and proliferation of cytolytic T lymphocytes [31]. Other investigations showed that CH was involved in the direct killing of tumor cells by inducing apoptosis process and CH was shown to inhibit adhesion of primary melanoma A375 cells, proliferation of primary melanoma SKMEL28 cells, and cultured Schwann cells [32].

Treatment of Wound Healing

Biomaterials especially biopolymers have been successfully introduced to the emerging field of biomedical science to develop new anti-wound-healing agents

that should protect the wound from bacterial infection as well as promote healing effect. Recently, CH-based materials, produced in varying formulations, have been used in a number of wound-healing applications [33]. High molecular weight CH hydrogels were formed by UV-initiated cross-linking to sustain fibroblast growth factor-2 (FGF-2) residence at the wound site by Ishihara et al. [34]. Also, CH hydrogel scaffold impregnated with β-FGF-loaded microspheres was fabricated that accelerates wound closure in the treatment of chronic ulcers. CH-based films of CH were effectively found to have acceleration for the healing of incisional wounds in mice [35]. As a result, the wounds closed within 14 days, and mature epidermal architecture observed histologically with keratinized surface of normal thickness and a subsided inflammation in the dermis were found. Moreover, Sathiyaseelan et al. synthesized the porous structured and cell proliferative biodegradable fungal CH-based composites with potential antibacterial property using the plant extracts obtained from *Aloe vera* and *Cuscuta reflexa* [36]. They concluded from the study that as prepared composite is capable of pivotal antibacterial behavior. Also, the bioreductant from plant extracts used in the formation of nanocomposite reduces their toxic responses.

Conclusion

Today, significant signs of progress through modern science and technology in the development of regenerated alternative medicine are demanded due to the adverse responses of their synthetic counterparts, even though CH and its derivatives are considered as the versatile biomaterials having a wide range of biomedical applications such as drug delivery, tissue engineering, and anti-infectious properties. There is a window for the generation of bio-based materials to overcome the problems associated with synthetic target molecules for biomedical purposes to develop a green and successful system. Further research and development should result in new, additional applications of CH and its derivatives.

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