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REVIEW ARTICLE

Chitosan: A Promising Marine Polysaccharide for Biomedical Research

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ABSTRACT

Biomaterials created 50 years ago are still receiving considerable attention for their potential to support development in the biomedical field. Diverse naturally obtained polysaccharides supply a broad range of resources applicable in the biomedical field. Lately, chitosan, a marine polysaccharide derived from chitins—which are extracted from the shells of arthropods such as crab, shrimp, and lobster—is becoming the most wanted biopolymer for use toward therapeutic interventions. This is a general short review of chitosan, highlighting the history, properties, chemical structure, processing method, and factors influencing the usage of chitosan derivatives in the biomedical field.

Key words: Chitosan, history, processing, properties, structure

INTRODUCTION

Biomedical research is comprised of basic, applied, and translational research, normally conducted to support the development and growing body of new therapeutics in the medical field. Basically, biomedical research is a process that aids in the discovery of new medicines and therapies, which demands scientific experimentation, evaluation, and quantification by employing biotechnological techniques.[1] The term "biomaterial" was coined 50 years ago. The study of biomaterial is known as biomaterial science. In a new biological era, biomaterial science embraces the constituents of medicine, biology, chemistry, tissue engineering, and materials science. Based on the American National Institute of Health definition, biomaterials are said to refer to any substance or combination of substances, apart from drugs obtained naturally or modified synthetically, that can be used entirely or partially for the replacement of any tissue, organ, or function of the body. Biomaterials can be used as autograft, allograft, or xenograft transplant material. A successful biomaterial should possess a few important characteristics, such as: Being compatible with the body; being antimicrobial, nontoxic, and noncarcinogenic; promoting better drug delivery; and being inexpensive. Chitosan-derived biomaterials are found to be a very unique marine polysaccharide, and evidently they have variety of physiochemical and biological properties, leading to applicability in various biomedicine-related fields. Although recent discoveries of chitosan biomaterials have heightened the application of chitosan in modern medicine, the involved mechanism of chitosan in all the studies still remains unexplained by researchers. The present short review aims to highlight the history, properties, chemical structure, processing method, and factors influencing the usage of chitosan derivatives in the biomedical field.

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HISTORY OF CHITOSAN

Chitosan was first identified and observed in the mushrooms by French Professor Henri Braconnot in 1811. Subsequently, further researches has successfully been conducted by many scientists up to this 20th century. Table 1 clearly depicted the history of chitosan.

Chitosan properties

Chitin is a mucopolysaccharide, derived naturally and found to be produced abundantly (second to cellulose) through biosynthesis. Chitins are characterized as white, nonelastic, hard, nitrogenous polysaccharides that have been estimated to be synthesized in approximately one billion tons annually.[4,5] Chitosan is derived from the *N*-deacetylation form of chitin. Chitosan is composed of β (1→4)-linked 2-acetamido-2-deoxy-β-D-glucose (*N*-acetylglucosamine). Chitin is structurally identical to cellulose, but it has acetamide groups (-$NHCOCH_3$) at the C2-portion. On the other hand, chitosan is a linear polymer formed by α (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose and derived by *N*-deacetylation, characterized by the degree of deacetylation, which is the copolymer of *N*-acetylglucosamine and glucosamine. Chitosans are the major elements derived from the shells of arthropods such as crabs, shrimps, lobsters, and insects, also produced extracellularly by the cell walls of fungi and brown algae. Chitosan is rarely found in nature but does occur in dimorphic fungi, such as *Mucor rouxii*, by the action of the deacetylase enzyme on chitin.[6-8]

Chitosan is an aminopolysaccharide molecule with a strong positive electrical charge, which strongly attracts and bonds to negatively charged

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Table 1: Henry Braconnot[2,3]

Year	Important figures	Description
1811	Henri Bracannot (Director of the Botanical Garden in Nancy, France; Professor of Natural History)	Conducted research on mushrooms and extracted chitin Hypothesis: chitin did not dissolve in sulfuric acid
1823	Ojer	Named "chitin," based on Greek word "khiton" meaning "envelope"
1832	Opperman	Chitin was extracted from insects-similar substances as chitin can also be found in the structure of insects
1843	Lassaigne	Demonstrated the presence of nitrogen in chitin
1859	Rougeut	Discovered chitosan Observed that the substances in chitin could be manipulated through chemical and temperature treatments for it to become soluble Treated chitin with hydroxide potassium concentrated at higher temperature
1878	Ledderhose	Identified chitin as made of glucosamine and acetic acid
1894	Hoppe-Seyler (German scientist and physiologist)	Proposed the name of the chitosan
1930	Rammelburg	Identified more chitin sources apart from insects and fungi Chitosan can be extracted from marine arthropods. E.g., crab, shrimp, lobster Hydrolyzed chitin in several ways Detected that chitin is a polysaccharide of glucosamine
1950	Darmon and Rudall	Structure of chitosan discovered X-ray analysis advanced the study on the discoveries of chitin and chitosan X-ray, the most advanced technology at that period, recorded the existence of chitin and cellulose in the cell wall The absorption spectra of chitin, chitosan nitrate, and wood cellulose have been recorded in the region 3600-750 cm^{-1} using polarized radiation
1951	First book was published 140 years after the initial observation of Braconnot, which was then confirmations were done by many researchers on the discovery of chitosan biomaterials	
1960 Till present	Many researchers have conducted research using modified and unmodified chitosan derivatives in the biomedical field	

molecules. Chitosan-derived biomaterials have received considerable attention as an antimicrobial, functional, renewable, nontoxic, biocompatible, bioabsorbable, and biodegradable biopolymer agent.[9-11] Chitosan is insoluble in water and organic solvents; it is soluble once mixed with acetic, nitric, hydrochloric, perchloric, and phosphoric acids.[12-14] The solubility of chitosan derivatives can be observed especially in aqueous acidic solutions, which has a pH ratio lower than 6.5. At the same time, the solubility range also can be altered upon depolymerization, chemical modification of primary and secondary hydroxyl groups. Recently, carboxymethyl chitosan and oligochitosan are becoming widely studied groups due to their characteristics of promising synthesis and rich diversity of applications in biomedical and biopharmaceutical areas of study.[15]

Chemical structure and composition of chitosan

The amine groups in chitosan become protonated at acidic pH and transmit a positive charge to the chitosan chains. Most biological cell surfaces are anionic, and chitosan was thought to strongly adhere to the tissues at the site of a wound via electrostatic interactions due to its cationic characteristics. The solubilization of chitin to produce chitosan in the acidic environment is found to take place via the protonation of an $-NH_2$ function on the C2-position of the D-glucosamine repeat unit, whereby the polysaccharide is able to convert to a polyelectrolyte The chemical structure of chitosan clearly depicted in Figure 1.[16,17]

Generally, chitosan has three types of reactive functional groups. Its amino groups have both primary and secondary hydroxyl groups at the C2-, C3-, and C6- positions.[18] These are the groups that permit the modification of chitosan-like graft copolymerization for specific applications in the tissue engineering field. The degree of deacetylation (DDA), crystallinity, and molecular weight (MW) are the main aspects in which chitosan can be modified to obtain different physiomechanical properties. Chitosan consists of carbon (44.1%), hydrogen (6.84%), and nitrogen (7.97%), with an average MW of 5.3×10^5 Daltons.[19]

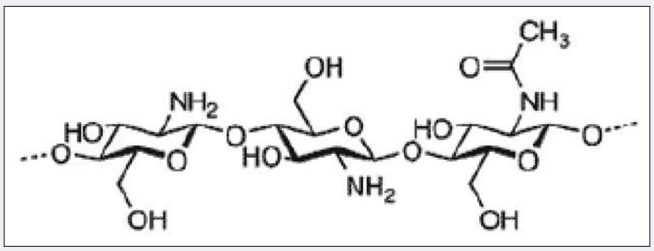

Figure 1: The chemical structure of chitosan

Chitosan chemical properties are insoluble in most solvents but slightly soluble in diluted organic acids such as acetic, lactic, malic, formic, and succinic acids.[20] The usage and benefits of chitosan are limited due to its insolubility in water, high viscosity, and aggregation of the protein molecules at the higher pH levels. Pyrolysis gas chromatography, gel permeation chromatography and ultraviolet spectrophotometry, titration, and separation spectrometry and near-infrared spectroscopy are the specific methods to detect the DDA of chitosan.[21] Commercialized chitosan biomaterials possess DDA greater than 70% and with MW ranging from 1×10^5 Daltons to 1.2×10^6 Daltons.[22] Chitosan derivatives with higher MW are potentially capable of providing better surface and film-forming properties due to its internal hydrogen bonding. Then again, it was reported that chitosan, with higher level of MW, possibly slows drug release.[23] At the same time, chitosan is contains nitrogen in comparison to cellulose and this property highly beneficial for metal chelation and polyoxysalt and film formations compared to cellulose. However, chitosan derivatives also potentially chelate metal ions such as iron, magnesium, and cadmium. The DDA of a chitosan biomaterial is the actual molarity of the glucosamine residue in the polymer chain to indicate the cationic charge on the molecule once diluted in acid

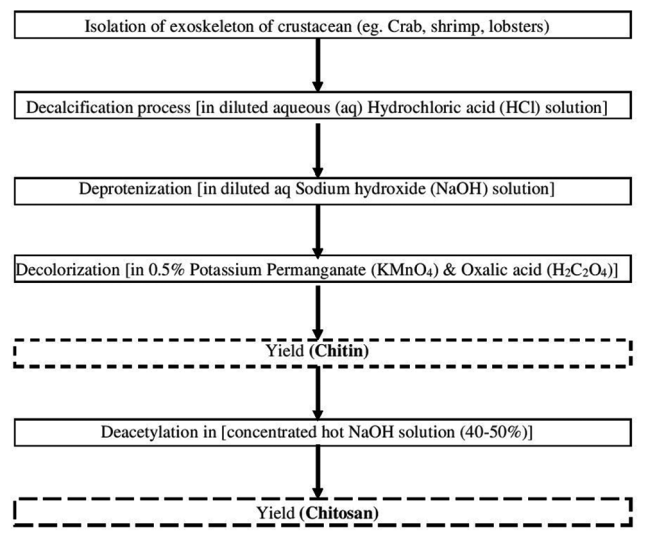

Figure 2: Stages of chitosan processing

solution. This is clearly evident from the proportion of free amino groups in the chitosan biopolymer.[24]

Chitosan processing

The following are some important processes in the production of chitosan biomaterials [Figure 2].

Extraction process of chitosan. Chitin is mainly derived from arthropods such as crab and shrimp. Chitin is extracted by acidic treatments to dissolve calcium carbonate, followed by alkali extraction to solubilize proteins. Exoskeleton of arthropods must be (i) decalcified in HCl, (ii) deprotonated in NaOH, and (iii) decolorized in $KMnO_4$ and $H_2C_2O_4$ to yield chitin. Processed chitin need to be deacetylated in hot, concentrated NaOH to produce chitosan[12,25]

FACTORS INFLUENCING CHITOSAN DERIVATIVES

Many factors and qualities of chitosan derivatives lead them to be recognized as a most significant marine polysaccharide in the biomedical field, such as the following: Biocompatibility, biodegradability, antibacterial, renewability, immunoadjuvant, promoting absorption, bioadhesivity, antithrombogenic, nontoxic, polycationic substance, film-forming, nonallergenic, antifungal, hydrating agent and anticholesteremic agent. Testing the factors-influencing the chitosan biomaterials are the useful experiments to describe or depict the hidden toxic effects of leachable materials or their derivatives, such as residual monomers, catalysts, polymer erosion related properties, chemical compositions, MW, polydispersity, and the degradation ability.[26] Effective alteration by the different level of DDA, crosslinking, MW, polyethylene glycol, wheat germ agglutination therapy, graphene support, viscosity, regularity, nature of bonds, degree of crystallinity, rigorous heat intervention, and oxygenated plasma treatments are noted to play a significant role to address chitosan as a biocompatible and biodegradable biomaterial. The capability and assessment of materials and devices to be used for human biological responses in a specific/necessary situation that does not cause toxic and injurious effects are defined as biocompatible. Researchers demonstrated that the biocompatibility of chitosan scaffolds showed healthy cell morphology and proliferation.[17]

Biodegradation plays a significant role in the metabolic fate of chitosan in the body and it is essential with respect to all the polymers utilized in a drug delivery system and scaffolds in tissue engineering. Due to its systemic absorptions and hydrophilic properties, chitosan is known as a biodegradable biomaterial. Chitosan biomaterials are capable of degrading enzymatically by hydrolyzing glucosamine–glucosamine and *N*-acetyl-glucosamine–*N*-acetylglucosamine linkages.[27] Depolymerization via oxidation-reduction reaction and free-radical degradation contribute toward *in vivo* degradation.[28,29] All of these important properties make the surface-modified chitosan biomaterial an excellent biopolymer that can be readily applied clinically.

As chitosan is a well-known nontoxic biopolymer with antibacterial properties, many studies have been conducted with a different focus in order to highlight the hemocompatibility of chitosan-derived biomaterials. As a result, they were proved to serve as a good hemostatic agent, and chitosan-induced blood coagulum is also generally well accepted. Even though chitosan-based hemostatic agents have been fabricated by blending them with other improved substances under various preparation conditions, to the best of our understanding many research groups still have not completely elucidated the standardized mechanical pathway of chitosan that affects the coagulation cascade.[30-32] There are a few important properties involved in determining protein response at the chitosan/biomaterial interfaces, such as membrane surface, topography, hydrophobicity, and charge density. Strong chemical bonds formed between chitosan and the protein will increase its affinity for the surface.[33-36]

CONCLUSION AND RECOMMENDATION

Chitosan derivatives have been discovered ever since 1859 upon the chemical modification of chitin. Various types and forms of chitosan biomaterials are used in diverse biomedical fields, such as hydrogel, powder, paste, sheet, porous scaffold, solution, sponge, beads, film, fiber, and nanoparticles using their respective methods of processing. Although lately much attention has been paid to these naturally obtained chitosan biomaterials, the mechanical pathways influencing the properties of these chitosans remain undetermined. In the future, more advanced clinical studies on animals and *in vitro* studies are needed to establish and elucidate the capability of chitosan derivatives. This can rectify the quality of testing and usage of chitosan in human clinical trials.

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Conflicts of interest

The authors declare that no competing interest exists.

REFERENCES

1. The University of New Mexico. New Jersey Association for Biomedical Research. 2014. Available from: http://biology.unm.edu/MARC/what-is-biomedical-research.html. [Last accessed on 2014 Nov 29].
2. What is Chitosan: Origins of Chitosan. 2014. Available from: http://www.fitnesstipsforlife.com/what-is-chitosan.html. [Last accessed on 2014 Nov 29].
3. Darmon SE, Rudall KM. Infra-red and x-ray studies of chitin. Disc Faraday Soc 1950;9: 251-60.
4. Muzzarelli RA. Chitin. Oxford, UK: Pergamon Press; 1977..
5. Muzzarelli RA, Muzzarelli C. Chitosan chemistry: Relevance to the biomedical sciences. Adv Polym Sci 2005;186:151-209.
6. Aranaz I, Mengibar M, Harris R, Paños I, Miralles B, Acosta N, *et al.* Functional characterization of chitin and chitosan. Curr Chem Biol 2009;3:203-30.
7. Koide SS. Chitin-Chitosan: Properties, benefits and risks. Nutr Res 1998;18: 1091-101.
8. Kumar MV, Hudson SM. Chitosan. In: Wnek GE, Bowlin GL, editors. Encyclopedia of Biomaterials and Biomedical Engineering. New York: Marcel Dekker; 2004. p. 310-23.
9. Seda Tiğli R, Karakeçili A, Gümüşderelioğlu M. *In vitro* characterization of chitosan

scaffolds: Influence of composition and deacetylation degree. J Mater Sci Mater Med 2007;18:1665-74.
10. Biagini B, Muzzarelli RA, Giardino R, Castaldini C. Biological materials for wound healing. In: Brine CJ, Sandford PA, Zikakis JP, editors, Advances in Chitin and Chitosan. London, New York: Elsevier; 1992. p. 16-24.
11. Zhang J, Xia W, Liu P, Cheng Q, Tahirou T, Gu W, *et al.* Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs 2010;8: 1962-87.
12. Rinaudo M. Chitin and chitosan: Properties and applications. Prog Polym Sci 2006;31:603-32.
13. Sankararamakrishnan N, Sanghi R. Preparation and characterization of a novel xanthated chitosan. Carbohydr Polym 2006;66:160-7.
14. Kurita K. Chitin and chitosan: Functional biopolymers from marine crustaceans. Mar Biotechnol (NY) 2006;8:203-26.
15. Mourya VK, Inamdar NN, Tiwari A. Carboxymethyl chitosan and its applications. Adv Mat Lett 2010;1:11-33.
16. Dash M, Chiellini F, Ottenbrite RM, Chiellini E. Chitosan - A versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 2011;36:981-1014.
17. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci 2007;32:762-98.
18. Xia WS. Physiological activities of chitosan and its application in functional foods. J Chin Inst Food Sci Technol 2003;3:77-81.
19. Soutter W. Chitosan Nanoparticles-Properties and Applications. 2014.
20. Sannan T, Kurita K, Iwakura Y. Studies on Chitin. V. Kinetics of deacetylation reaction. Polym J 1977;9:649-51.
21. Kumar. A review of chitin and chitosan applications. Reactiv and functi Polym 2000;46:1-27.
22. Li Q, Lunn ET, Grandmason EW, Goosen MF. Applications and Properties of Chitosan. Lancaster: Technomic Publishing Co. Inc.; 1997. p. 3.
23. Cervera MF, Heinämäki J, Krogars K, Jörgensen AC, Karjalainen M, Colarte AI, *et al.* Solid-state and mechanical properties of aqueous chitosan-amylose starch films plasticized with polyols. AAPS Pharm Sci Tech 2004;5:E15.
24. Shigemasa Y, Matsuura H, Sashiwa H, Saimoto H. Evaluation of different absorbance ratios from infrared spectroscopy for analyzing the degree of deacetylation in chitin. Int J Biol Macromol 1996;18:237-42.
25. Roberts GA. Chitin Chemistry. 1st ed. London: MacMillan; 1992.
26. Keong LC, Halim AS. In vitro models in biocompatibility assessment for biomedical-grade chitosan derivatives in wound management. Int J Mol Sci 2009;10:1300-13.
27. Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev 2009;62:3-11.
28. Hsu SC, Don TM, Chiu WY. Free radical degradation of chitosan with potassium persulfate. Polym Degrad Stab 2002;75:73-83.
29. Zoldners J, Kiseleva T, Kaiminsh I. Influence of ascorbic acid on the stability of chitosan solutions. Carbohydr Polym 2005;60:215-8.
30. Okamoto Y, Yano R, Miyatake K, Tomohiro I, Shigemasa Y, Minami S. Effects of chitin and chitosan on blood coagulation. Carbohydr Polym 2003;53:337-42.
31. Lord MS, Cheng B, McCarthy SJ, Jung M, Whitelock JM. The modulation of platelet adhesion and activation by chitosan through plasma and extracellular matrix proteins. Biomaterials 2011;32:6655-62.
32. Yamazaki M. The Chemical Modification of Chitosan Films for Improved Hemostatic and Bioadhesive Properties. Raleigh, NC: North Carolina State University; 2007.
33. Corum LE. Evaluating Surface Induced Platelet Adhesion and Activation with Surface Patterning and Protein Immobilization Techniques. Utah, USA: University of Utah; 2011.
34. Gorbet MB, Sefton MV. Biomaterial-associated thrombosis: Roles of coagulation factors, complement, platelets and leukocytes. Biomaterials 2004;25:5681-703.
35. Schmidt DR, Waldeck H, Kao WJ. Protein adsorption to biomaterials. In: Bizios R, Puleo D, editors. Biological Interactions on Materials Surfaces: Understanding and Controlling Protein, Cell, and Tissue. New York: Springer; 2009. p. 1-18.
36. Andrade JD, Hlady V. Plasma protein adsorption: The big twelve. Ann N Y Acad Sci 1987;516:158-72.


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