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 produced abundantly (second to cellulose) through biosynthesis.

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] 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 (NHCOCH₂) 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, 7]

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

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

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

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.[24] 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

| Year | Important figures | Description |
|------|------------------|-------------|
| 1811 | Henri Braconnot (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 | Discovered chitosan |
| 1859 | Rougeut | Observed that the substances in chitin could be manipulated through chemical and temperature treatments for it to become soluble |
| 1878 | Ledderhose | Treated chitin with hydroxide potassium concentrated at higher temperature |
| 1894 | Hoppe-Seyler (German scientist and physiologist) | Identified chitin as made of glucosamine and acetic acid |
| 1930 | Rammelburg | Proposed the name of the chitosan |
| 1950 | Darmon and Rudall | Identified more chitin sources apart from insects and fungi |
| 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 |

Figure 1: The chemical structure of chitosan
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 KMnO₄ and H₂C₂O₄ to yield chitin. Processed chitin need to be deacetylated in hot, concentrated NaOH to produce chitosan\[11,23\]

**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.\[25\]

Effectual 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.

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