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Review

Recent Advancement of Molecular Structure and Biomaterial Function of Chitosan from Marine Organisms for Pharmaceutical and Nutraceutical Application

Bailei Li [1], Jeevithan Elango [1,*] and Wenhui Wu [1,2,3,*]

1 Department of marine bio-pharmacology, College of Food Science and Technology, Shanghai Ocean University, Shanghai 201306, China; libl1213@foxmail.com
2 Shanghai Engineering Research Center of Aquatic-Product Processing & Preservation, Shanghai 201306, China
3 National Experimental Teaching Demonstration Center for Food Science and Engineering, Shanghai Ocean University, Shanghai 201306, China
* Correspondence: srijeevithan@gmail.com (J.E.); whwu@shou.edu.cn (W.W.)

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Abstract: Chitosan is an innate cationic biological polysaccharide polymer, naturally obtained from chitin deacetylation, that possesses broad-spectrum properties such as antibacterial, biodegradability, biocompatibility, non-toxic, non-immunogenicity, and so on. Chitosan can be easily modified owing to its molecular chain that contains abundant active amino and hydroxyl groups, through various modifications. Not only does it possess excellent properties but it also greatly accelerates its solubility and endows it with additional special properties. It can be developed into bioactive materials with innovative properties, functions, and multiple uses, especially in the biomedical fields. In this paper, the unique properties and the relationship between the molecular structure of chitosan and its derivatives are emphasized, an overview of various excellent biomedical properties of chitosan and its current progress in the pharmaceutical and nutraceutical field have prospected, to provide the theoretical basis for better development and utilization of new biomedical materials of chitosan and its derivatives.

Keywords: chitosan; structure; modification; properties; pharmaceutical application

1. Introduction

Only by tracing back the history, we can look forward to the future. In 1811, chitin was first discovered in mushrooms and named fungi by French scholar Brano [1]. Chitin is the second largest natural biopolymer only after cellulose and exists extensively in marine organisms (Figure 1), such as the shells of shrimp and crab, bacterial and algal cell membranes, shells and skeletons of mollusks and cell walls of higher plants. It is a recyclable, renewable and inexhaustible resource, mainly distributed in coastal areas [2]. It is reported that there are about 10 billion tons of chitin biosynthesis each year, more than 150,000 tons of chitin are available for commercial purposes [3]. Chitin constitutes a major component of arthropod exoskeletons, tendons and the linings of their excretory, respiratory and digestive systems. It is also found in the eye iridophores and epidermis of cephalopods and molluscan arthropods and the cuticle of vertebrates [4], up to now, its commercial sources are mainly crabs, shrimp, krill shells, fungi, etc. The crustacean shell is composed of 30–40% protein, 30–50% calcium carbonate and calcium phosphate and 20–30% chitin. The ratio of chitin obtained from dried shrimp and crab processing waste was 14–27% and 13–15% respectively [5]. Due to the intractable molecular structure

of chitin, it is still the main underutilized resource despite its easy availability and huge annual output. It has vital research significance as a biomaterial with potential activity in different fields.

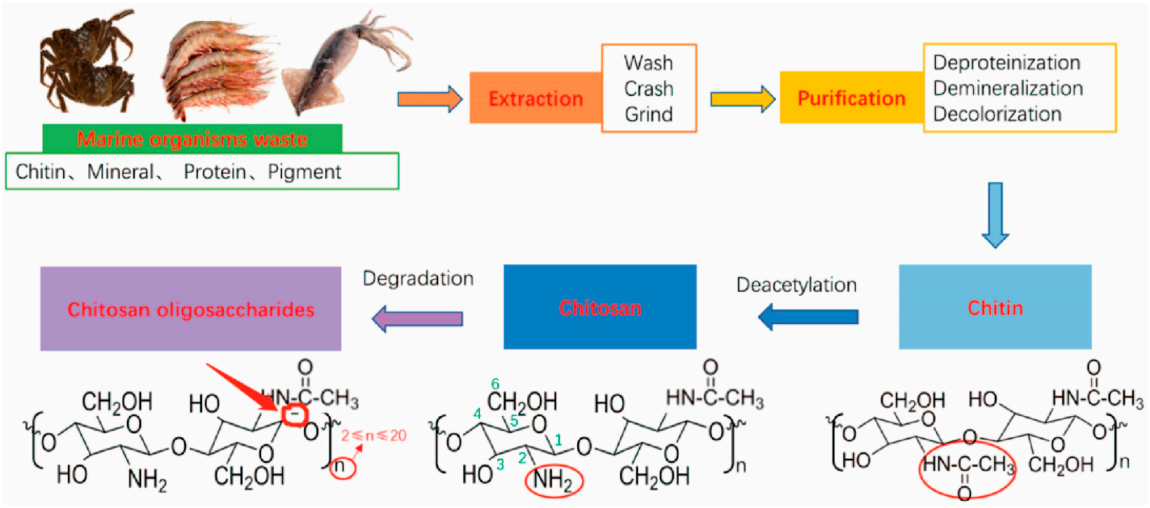

Figure 1. Overview of production and structure of chitosan.

In 1894, the German scientist Hopper Seyler [6] used potassium hydroxide solution to boil chitin for modification and obtained deacetylated chitin, which was named chitosan. Chitosan is a high molecular weight compound with a deacetylation degree higher than 55% of chitin, the deacetylation degree or degree of acetylation (DA) is derived from the amount of the acetamido-2-deoxy-d-glucopyranose monomeric unit that exists in the polymer chain. The deacetylated chitin (chitosan) is the only natural basic polysaccharide, soluble in aqueous solutions of inorganic or organic acids, with more than 90% glucosamine content, which is found in large quantities in the biological world.

Chitosan is structurally similar to mucopolysaccharide, which is widely distributed in tissues, and is one of the organic components of cell membranes. It possesses excellent biological activities, such as biocompatibility, biodegradability, film-forming, bacteriostaticity and non-toxicity, and can resist inflammation and bacteria, promote wound healing and has acid resistance, is anti-ulcer, reduces lipids and reduces the cholesterol effect [7,8]. Furthermore, chitosan demonstrates anticancer activity via activating the immune system and promotes it when applied to combine with existing anticancer drugs. It has become a research hotspot in the field of biomaterials in recent years owing to containing safe and reliable natural bioactive activities [9–11].

Since 1970, the use of this natural polymer has been accelerated in many countries. Several international symposiums have been held and several academic monographs on chitosan have been published. Besides, the number of papers and patents published each year also shows a significant growth trend. This fully reflects that people are more and more interested in the application value of chitosan with abundant resources. Biomaterials are natural or synthetic special functional materials used to contact and interact with living systems to diagnose, repair, replace or induce regeneration of cells, tissues and organs. It usually consists of living cells or biological tissues combined with inanimate materials to form a single, composite or hybrid material. Biomaterials interact with biological organisms, and the direct combination is a characteristic of their therapeutic method [12]. Compared with non-pharmaceutical natural polymers, the biocompatibility and biodegradability of synthetic polymers are limited. Nevertheless, the reactivity and capacity for further application of extensive materials found in nature are limited. Due to the similarity between natural polymers and biomolecules, natural polymers are easy to be recognized by the biological environment and thus easy to be metabolized into non-toxic residues for natural elimination, which has attracted wide attention [13]. In this regard, aments of scientific literature showed that natural and abundant chitosan with biocompatibility, biodegradability and non-immunogenic properties could be the smart polymers

for numerous biomedical purposes such as drugs and gene delivery vehicles, permeable membranes, tissue engineering scaffolds and so on [14].

Chitosan can be biodegraded in the human body, so it is not accumulated in the body, and there is no toxicity, irritation and antigen immunity. It is generally considered to have good biocompatibility and plays a very important role in human physiological activities. Although chitosan has superior biological activity, biocompatibility, biodegradability, antibacterial, antiseptic, hemostatic and wound healing and other special functions, its application is limited to a certain extent by its poor water solubility and mechanical properties [15]. In a practical application, due to the molecular chain of chitosan containing a large amount of amino and hydroxyl groups, the chemical modification of chitosan, such as acylation, carboxylation and etherification, especially graft copolymerization and blending modification, can change the molecular structure of chitosan, generating a series of chitosan derivatives, which can improve its water solubility, biological activity and mechanical properties, and endow on it some special properties, extending the application of chitosan in various fields.

A variety of polymer materials can be grafted on the primary, secondary hydroxyl and amino groups of chitosan. The graft copolymer of chitosan not only has the original biocompatibility and degradability of chitosan but also improves its solubility and endows it with other special properties. However, due to the small reactivity difference between the three functional groups, it is difficult to introduce a side chain at a fixed point and quantitatively, and it is difficult to separate and purify the intermediate product or the final product of the reaction, which limits the study on the chitosan grafting reaction to some extent. The research focuses on finding the appropriate reaction reagent, mild experimental conditions and efficient separation methods. At present, the application of the chitosan graft copolymer in medicine is still under continuous exploration. With the development of the research, the application of the chitosan graft copolymer in pharmacy will become more extensive. Blending modification is a common method of polymer modification. It is easy to give full play to the advantages of two or more kinds of polymers and effectively expand the application range of polymer materials. When the compatibility of components is good, a thermodynamic stable system can be formed to achieve the synergetic effect. Poor compatibility results in the separation of components. To avoid this disadvantage, a proper amount of cross-linking agents can be added to make the components cross-linked or the blends cross-linked through a hydrogen bond. The blend of chitosan with other natural or synthetic polymer materials can synthesize the excellent properties of each component to produce functional materials suitable for various fields.

A large number of studies have proved that the unique biomedical characteristics of chitosan and its derivatives are closely related to their structure. In this paper, the molecular structure and properties of chitosan are reviewed, and the modification of chitosan and its application as biomedical materials in recent years are summarized.

2. Production

Shell wastes of crab and shrimp are the main sources of commercial manufacturing of chitosan. The seafood waste production is reprocessed to achieve environmentally friendly biomaterial stability. A crustacean shell contains mainly chitin, proteins, minerals and lipids. In industrial processing, it is dissolved in calcium carbonate by acid treatment and then dissolved by alkali extraction, after deproteinization, decolorization and deacetylation, colorless products are obtained, and the process is shown in Figure 1. Firstly, shells are crushed to tiny sized minerals, mainly extracted with dilute hydrochloric acid to remove calcium carbonate followed by calcium chloride precipitation through stirring at ambient temperature. The next step is deproteinization, proteins are dissolved with dilute sodium hydroxide, and the *N*-acetyl backbone of the polymer is hydrolyzed randomly in this process. The following decolorization step aims to remove color. Chitosan was deacetylated in 40–45% sodium hydroxide without oxygen at 120 °C for 1–3 h. The degree of deacetylation is determined by three parameters: alkali concentration, time and temperature. To get 1 kg of 70% of deacetylated

chitosan, shrimp shells should be treated with 1.8 kg of NaOH and 6.3 kg of HCl along with 1.4 tons of nitrogen and water [16].

At present, chitosan mainly comes from the shrimp and crab shell, which is rich in resources, high in yield and low in price. However, there are two disadvantages of this method: first, limited in the collection by seasons and high in production cost and second, the quality and quantity of chitosan are not stable due to different types of raw materials, production areas and production processes. The method of producing chitosan by fermentation has been studied since the 1990s. For example, the technology of producing chitosan by fermentation was successfully developed by the Beijing University of Chemical Technology in 2000 [17]. This method can greatly reduce the production cost of chitosan and make it possible to replace the production method with the shrimp shell or crab shell as the raw material. The continuing increase in global marine biological resources associated with the production of processing wastes and byproducts makes it necessary to find new ways of utilization. The viable economical production of chitosan can promote its utilization effectively in numerous applications.

3. Structure of Chitosan

Chitin is a partially crystalline mono-polymer and consists of more than 5000 β-(1,4)-linked *N*-acetyl-D-glucosamine with over 106 molecular weight (MW). Chitosan is a partially or totally deacetylated product of chitin comprising of β-(1→4)-2-amino-D-glucose and β-(1→4)-2-acetamido-D-glucose units with the latter typically above 80% [18]. The basic components of chitosan are glucosamine and chitobiose and it owns a double helix structure comprising of six sugar residues that form a spiral plane with a pitch of 0.515 nm. The structure of chitosan is depicted schematically in Figure 1, where "n" specifies the degree of polymerization of glucosamine and *N*-acetylglucosamine units.

The secondary structure of chitosan is formed by a hydroxyl group, amino group and *N*-acetyl group on the molecular chain, which is involved in the formation of intra-molecular and inter-molecular hydrogen bonds of chitosan [19]. In the chair-like structure of chitosan glucosamine residues, there are two intramolecular hydrogen bonds, one of which forms C3-OH with the glycoside group on another adjacent chitosan molecular chain, and the other forms C3-OH with the oxygen atom on the adjacent chitosan furan ring. The C2-NH2, C3-OH and C6-OH functional groups of chitosan can form intramolecular and intermolecular hydrogen bonds, so that it has the physicochemical properties of swelling, water retention and adsorption, and therefore is hard to be digested and absorbed [20].

The single-chain is linear, every 10.1–10.5 Å along the chain axis undergoes one full twist [21]. Linear aggregates and rigid crystalline domains are formed since abundant hydroxyl groups, highly reactive amino groups and *N*-acetyl counterparts have strong intramolecular and intermolecular hydrogen bonding tendencies. According to the different biological functions and natural sources, and the different structural forms of chitosan are differentiated by the different arrangements of carbohydrate chains [22]. Nuclear magnetic resonance spectroscopy (NMR) and X-ray model are helpful to clarify the three crystal types of chitosan: α, β and γ chitosan, each of which has diverse natural sources and biological functions.

The α conformation is the most common heteromorphic conformation, consisted of two reverse parallel chains, where the units are orthogonal, and has high thermodynamic stability due to the hydrogen interaction between the chains probably, usually separated from the exoskeleton of the crustacean, cell wall of yeast and cuticle of the arthropod. It is generally deposited with minerals to form a hard shell and compact structure. The β-conformation is the second common allomorph, composed of two parallel chains, where the units are monoclinic, and has weaker intermolecular forces perhaps owning to the polymer chains arranged in a parallel fashion, demonstrating a certain degree of hardness, flexibility and fluidity, usually combined with collagen, reside in squid cartilage [23]. The γ-form rarely has been seen and possesses two identical chains in association with one reverse and top-down chain, which seems to be a combination of α and β forms rather than a different variant,

existing in the thick epithelium of the squid stomach [24]. Chitin and chitosan both contain active hydroxyl and amino groups, however, chitin is usually more crystalline than chitosan, so chitosan may be more suitable for the preparation of reagents and biomaterials. The crystallinity of chitosan is affected by the degree of deacetylation. The completely deacetylated chitosan has the characteristics of a uniform molecular chain, good regularity and high crystallinity. As shown in Figure 2, the crystal structure of α-chitosan, β-chitosan and γ-chitosan are respectively matched to anti-parallel, parallel and alternated arrangements of polymer chains.

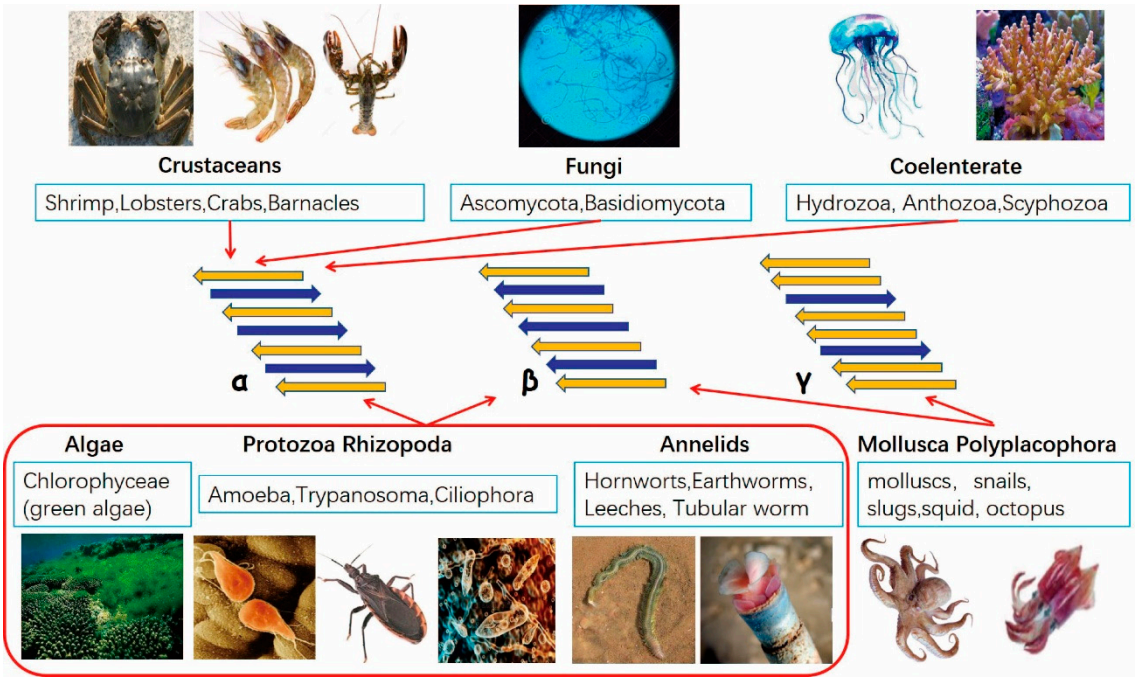

Figure 2. The crystalline structure of chitosan and its main marine organisms sources.

4. Factors Influencing Chitosan Properties

Chitosan has a complex double helix structure, its final structural unit after enzymatic hydrolysis of chitosan is chitobiose, which usually contains two structural units, 2-acetylaminoglucan and 2-glucosamine, and the ratio of two units varies with the degree of deacetylation. Chitosan possesses many active groups such as the amino group and a hydroxyl group, which has high reactivity and can be chemically modified, to obtain unique physical, chemical properties and physiological functions [25]. Chitosan properties are highly influenced by their degree of deacetylation and molecular weight. Different reaction settings like temperature, time, concentration and types of reagents may affect the efficiency of the final product of chitosan. However, both degrees of deacetylation and molecular weight can be lowered by reacetylation and acidic or enzymatic polymerization, respectively [26].

4.1. Molecular Weight (MW)

The physicochemical properties (viscosity, solubility, adsorption on solids, elasticity, tear strength, bio-functional activities, crystal size and morphological character) are affected by the MW of chitosan [27–29]. The crystallinity of the membrane could be decreased by increasing MW. The MW of chitosan plays an important role in its performance as a polymer flocculant. The larger the relative MW is the smaller the solubility and greater the degree of entanglement between molecules.

The relative MW of chitin in marine organisms is 1×10^6–2×10^6, after extraction, about 3×10^5–7×10^5. The relative MW of chitosan is lower, about 2×10^5–5×10^5. Chitin, chitosan and chitosan oligosaccharide are all called chitin substances [30]. Chitin is not soluble in alkali, water, general acid and organic solvent and is only partially soluble in concentrated acid. It can be

partially decomposed by chitinase and lysozyme in the human gastrointestinal tract, so its absorption rate is low, the dosage is large and the taking reaction is over 70%. Chitosan can be dissolved in dilute acid, which is better than chitin. However, chitosan is still a large molecule and insoluble in water, degraded into small molecule chitosan oligosaccharide (Figure 1), which can be directly soluble in water, thus the absorption rate is greatly increased, the dosage and the reaction after taking are greatly reduced. Chitosan with MW 10,000 possesses many excellent functions, such as inhibiting the growth of tumor cells, reducing cholesterol, blood sugar and blood lipids in serum and liver, enhancing body immunity, strengthening liver function, promoting the generation of spleen antibodies, promoting the proliferation of Bifidobacterium, inhibiting the growth of *Escherichia coli* and absorbing and resenting moisture.

The deacetylation process of chitosan brings about the change of MW. In the manufacturing process, the relative MW of chitosan is generally expressed by the value of viscosity, and the products with different viscosity have different purposes. A high concentration and degree of deacetylation increase the viscosity, whereas high temperature decreases it. It is used as a tackifier because of its high molecular weight and linear and unbranched structure [31].

4.2. Degree of Deacetylation (DDA)

The deacetylation degree is calculated by the amount of deacetylated glucosamine units present in the total glucosamine units. It is one of the most basic structural parameters to investigate chitin/chitosan. The degree of deacetylation (DDA) has a great influence on the solubility, viscosity, crystallinity, ion exchange capacity and flocculation of chitosan. Generally, chitin with more than 55% *N*-acetyl can be dissolved in 1% acetic acid or hydrochloric acid, which is called chitosan, the DDA varies from 60% to 100% due to different preparation conditions and requirements, but only the chitosan with more than 70% DDA can be used as industrial products, the completely deacetylated chitosan is extremely difficult to prepare. The deacetylation degree classification standard of chitosan is shown in Figure 3.

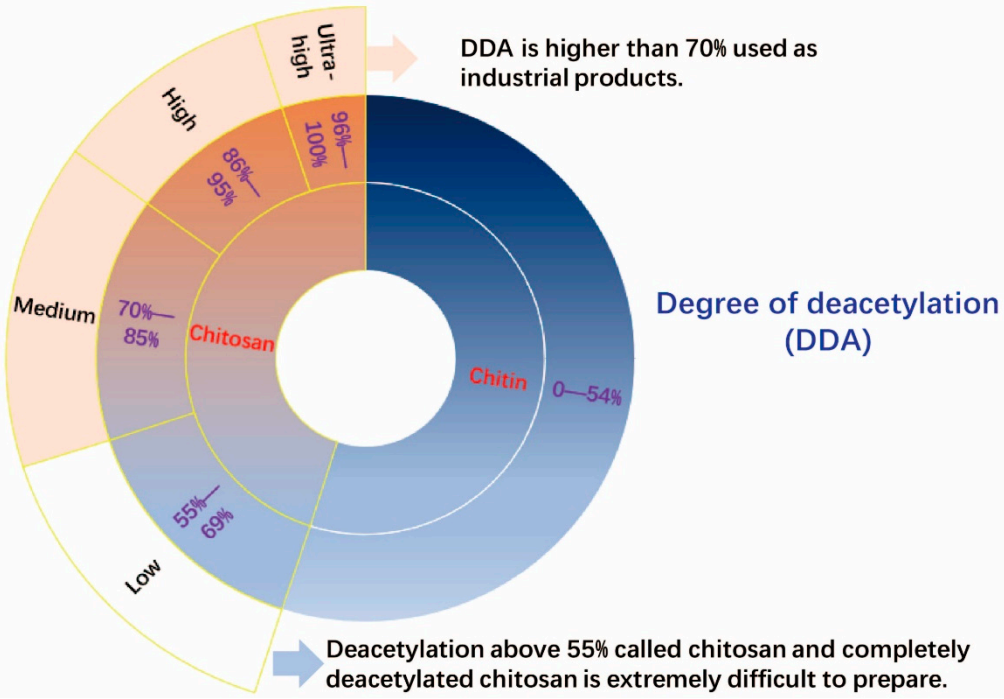

Figure 3. The deacetylation degree classification standard of chitosan.

N-deacetylation degree is the basis for determining the chitosan solubility. The charge density on the carbon chain increases with the increase of DDA, and the -NH2 group in the chitosan molecule

will be protonated to form the NH_3^+ ion in the acid environment, so it will dissolve in the acid condition. The higher the deacetylation degree is, the more free-amino groups are on the molecular chain and the better the solubility is in the acid. The chain elasticity of chitosan can be changed by changing DDA, with the increase of deacetylation, a more flexible chitosan chain is produced, consequently forming a random spiral structure in the chain and more intramolecular hydrogen bonds. The mechanical properties of the microspheres are generally weaker than those of the deacetylated microspheres. The weaker the interaction between molecules, the less tangled the chitosan chain. The preparation of chitosan with high DDA is very important in the process of developing chitosan products, because the DDA can determine the chitosan solubility, and it is also the precondition for its chemical modification and functional modification. Chitosan with high DDA and low molecular weight and low viscosity usually need to be further hydrolyzed and degraded. By the X-ray diffraction test, with the increase of the deacetylation degree, the X-ray diffraction peak becomes sharper and the crystallinity is higher. Furthermore, acetylation regulates cell proliferation and adhesion, however not altering the cytocompatibility of chitosan. It was reported that decreased DDA is beneficial to cell growth and adhesion [32–35].

5. Solubility of Chitosan

Pure chitosan is a white or gray transparent flake or solid powder, tasteless, odorless, non-toxic and stable at room temperature. There is a strong hydrogen bond between chitin molecules, forming a highly crystalline structure, so chitin is highly insoluble in dilute alkali, dilute acid, water and most organic solvents, only soluble in concentrated acid and some solvents. The solubility of chitosan is better than that of chitin because the active group of chitosan molecules is an amino group rather than the acetyl group. Chitosan can be soluble in dilute acid, formic acid and acetic acid, but not in water and most organic solvents. It is easy to react with acid to generate salt due to the existence of an amino group in the structural unit of chitosan, forming a positive cationic group.

Chitosan solubility is related to the degree of deacetylation, relative molecular weight and viscosity. The higher degree of deacetylation leads to reducing relative molecular weight and makes it easier to be soluble in water, whereas the lower degree of deacetylation leads to an increase in relative molecular weight and high viscosity. Chitosan is soluble in dilute acid and presents a viscous shape, with strong adsorption capacity. Chitosan contains hydroxyl, amino and other polar groups, hygroscopicity is very strong and can be used as a humectant. Chitosan, a high-performance metal ion collector, can chelate the heavy metal ions in vivo because the ortho of the free amino group of chitosan is hydroxyl, which can chelate the divalent metal ions.

Chitosan, as a solution, needs to be stored and used in an acidic environment, but because of the presence of acetal structure, the glucose ring is opened and degraded when it is placed in an acidic solution for a long time, the β-1,4-glycoside bond of chitosan will be hydrolyzed slowly to form low molecular weight chitosan. Chitosan can be developed as the immobilized carrier of antigen, antibody, enzyme and other physiologically active substances due to the presence of the free amino group, and has a broad application prospect because of its good physical, chemical and biological properties, excellent stability to organic solvents and convenience for secondary processing [36].

The active adsorption center of chitosan is a surface free amino group. Many inorganic acids, organic acids and acid compounds, even amphoteric compounds, can be adsorbed by chitosan. The adsorption rate decreases with the decrease of the dielectric constant of the adsorption medium. The surface energy of the chitosan solution first decreases with the increase of the solubility parameter and then increases rapidly. The dissociation constant pKa of chitosan is not significantly related to the change of deacetylation degree, but the ionic strength and species in the solution. A cationic polyelectrolyte (pKa 6.5) produced by amino groups of chitosan. The dissolution of chitosan in the acidic-aqueous solution produces a proton, which led the soluble polysaccharide to become positively charged, especially a more positive charge on amino groups. Chitosan aggregates and chelates with polyanionic compounds and heavy metal ions, respectively. Both the aggregation with polyanions

and solubility in acidic solution produce chitosan with admirable gelling properties [37]. Under the same hydrolysis time, the reciprocal of the relative molecular weight of the hydrolysate is directly proportional to the temperature. Chitosan emerges as an obvious self-polymerization phenomenon in 0.1 mmol/L acetic acid solution. With the increase of chitosan concentration, the molecular chain of chitosan changes from self-polymerization of the stretch chain structure to the single-chain coil group structure, and the single-chain coil group structure was further transformed into an intertwined coil group structure.

6. Modifications of Chitosan

Although chitosan owns exceptional biological activity, such as biodegradability, hemostasis, antiseptic, antibacterial, biocompatibility and promoting wound treatment and so on, its poor water solubility and mechanical properties greatly limit its application in biomedicine. Improving the solubility of chitosan is the key factor for the rational utilization of its various uses.

To improve the water-insoluble defects of chitosan, by hydrolysis of the main chain, it was degraded into chitosan oligosaccharide, which has good water solubility, is easy to disperse and absorb and has many unique physicochemical properties and biological activities. The most suitable hydrolytic enzyme of chitosan is chitosanase. Chitosanase from different microbial sources hydrolyzes different substrates. According to the action model of chitosanase, it can be divided into two types: endo- and exo-type. The endo chitosanase mainly releases a dimer, trimer or oligosaccharide, while the exo chitosanase produces a monosaccharide residue, namely glucosamine, from the non-reducing end of chitosan or chitooligosaccharide [38]. Both chitosan and cellulose are polysaccharide compounds formed by D2 glucose linked and polymerized by glycosidic bonds. Due to the structural similarity, cellulase should also have a similar degradation effect on chitosan [39]. The acetylated amino group in chitosan can also be modified by chitinase, lysozyme, tyrosinase, laccase and peroxidase, which can effectively improve the biological properties of chitosan, such as antioxidant and antibacterial properties [40–42].

Chitosan can be modified by acylation, carboxylation and etherification to produce a series of chitosan derivatives with different properties, which can improve its water solubility, biological activity and mechanical properties, and expand the application of chitosan in various fields because the basic unit of chitosan is glucosamine, which contains a lot of active amino and hydroxyl groups. Modified chitosan derivatives have attracted more and more attention because they are superior to unmodified chitosan in chemical, biological and functional aspects such as solubility and gelation. As shown in Figure 4, previous studies also extensively reported the chemical modification of chitosan, such as acylation, carboxylation, etherification, etc. [43,44]. The present review only focuses on the study of graft copolymerization and the blending modification of chitosan in recent years.

(1) Carboxylation: chitosan molecules contain more free -NH_2 and -OH, introducing carboxyl functional groups, among which carboxymethyl is the most common, that replace the side chain ammonium salt, can obtain water-soluble, alcohol soluble, organic solvent-soluble, surface-active and fibrous polymer derivatives. Dumont et al. [45] suspended the chitosan powder in isopropanol, added isopropanol chloroacetate solution to the solution, reacted at room temperature and continuously stirred with magnetic force to obtain carboxymethyl chitosan with a high degree of protonation. Feng et al. [46] successfully prepared *N*,*O*-carboxymethyl chitosan from chitosan and chloroacetic acid under alkaline condition. The obtained carboxymethyl chitosan not only retains the original superior properties, but also improves the solubility more effectively, and has the function of moisturizing.

(2) Etherification: the hydroxyl of chitosan can react with methyl ether, ether, benzyl ether and other alkylating agents to form an ether. By the way of cellulose modification, hydroxyalkyl chitin and carboxyalkyl chitin can be obtained by the reaction of basic chitin and etherification reagent.

(3) Crosslinking: chitosan can be crosslinked in or between molecules through -OH and -NH_2 with aldehydes, anhydrides or epoxides with two functional groups, and grafted to form network

polymers to obtain crosslinked products with improved mechanical properties, providing conditions for further grafting modification.

(4) Chelation: -OH and -NH_2 have coordination and chelation. They can form complexes with transition metal ions first, and then cross-linked with the cross-linking agent. Chitosan with template memory and selective adsorption can be prepared.

(5) Acylation: the hydroxyl group and amino group on the sugar residue of the chitosan molecular chain can react with some derivatives of organic acids, such as anhydrides and acyl hydrides, including *O*-acylation to form esters and *N*-acylation to form amides. *N*-acylation products are obtained usually after the introduction of aliphatic or aromatic acyls with different molecular weights, whose solubility in organic solvents is greatly improved.

(6) Oxidation: the -OH of chitosan can be oxidized, among which H_2O_2 is the most widely used method to degrade chitosan. The C6 hydroxyl group can be oxidized to the aldehyde or carboxyl group, and the C3 hydroxyl group can be oxidized to a carbonyl group. If CrO_3 is used as an oxidant in the perchlorate suspension of chitosan, C6 hydroxy can be oxidized to carboxyl.

(7) Alkylation: the hydroxyl and amino groups on chitosan form respective water-soluble derivatives through alkylation. Hydroxypropyl chitosan can be obtained by the reaction of chitosan and propylene oxide on the hydroxyl group in the basic condition, and *N*-alkylated chitosan can be obtained in an acid condition.

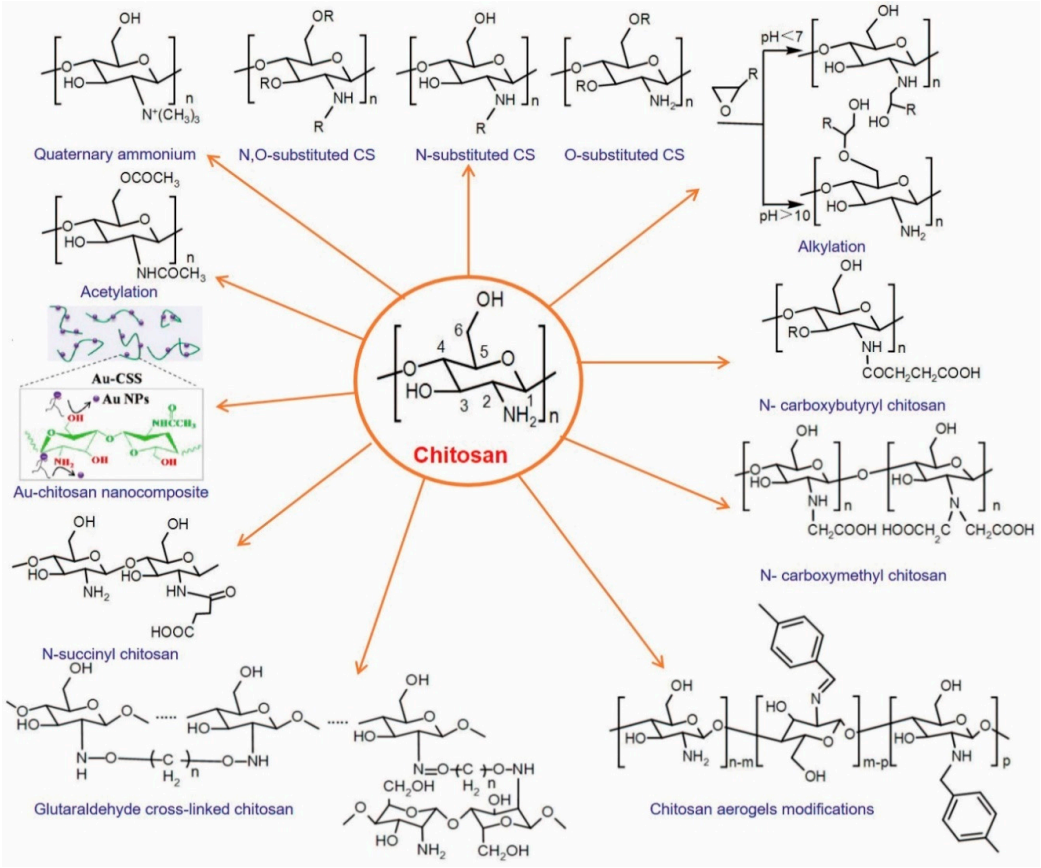

Figure 4. Chemical structure of different types of chemical modification of chitosan. The information obtained from the literature such as quaternary ammonium, acetylation, N-succinyl chitosan [40], N-carboxymethyl and N-carboxybutyryl chitosan [47], glutaraldehyde cross-linked chitosan [48], alkylation [49], Au-chitosan nanocomposite [50], chitosan aerogels modifications [51], and N,O-/N-/O-substituted chitosan [52].

6.1. Graft Copolymerization of Chitosan

Grafting copolymerization, which can change the physicochemical properties of chitosan, is an effective method to broaden the practical use of chitosan. The molecular structure, number and length mainly affect the properties of the final graft copolymerization products. These methods include free radical graft copolymerization, condensation copolymerization, oxidative coupling copolymerization, ring-opening copolymerization of ring mounted monomers, polymer grafting, etc. Chitosan C6 primary hydroxyl, C3 secondary hydroxyl and C2 amino groups can all be grafted points. The side chain of chitosan C6 can be grafted selectively to obtain the branched polysaccharide with unique immuno promoting activity, that is, the polysaccharide with the side chain of α-(1→6) on the main chain of α-(1→4). Glycosyl, polypeptide, polyester and alkyl chain can be introduced into chitosan by the grafting reaction, which can improve the affinity of chitosan to the solvent, as well as the antibacterial and immune activity. They are mainly used as drug membranes, gelatin, microspheres (microcapsules), nanoparticles, slow-release materials, gene delivery vectors, polymer drugs, drug-loaded micelles, etc.

Kweon et al. [53] reported the synthesis of chitosan grafted polyvinyl alcohol (PVA) and its release of prednisolone. As a drug release coating, chitosan has some disadvantages, such as poor solubility, too strong dependence on pH and poor mechanical properties, but grafting PVA with good water solubility and biocompatibility can greatly improve the drug release behavior.

Panda et al. [54] prepared chitosan with three different molecular weights through the p-coumaric acid method to improve their antioxidant property and water solubility. Further, they reported that the water solubility and antioxidant property of modified products decrease with an increased molecular weight of corresponding native chitosan. Liu et al. [55] used chitosan alkalization and carboxymethylation reactions to prepare carboxymethyl chitosan followed by polyethyleneimine (PEI) grafting through an amidation reaction. Figure 5 shows the steps involved in carboxymethylation–PEI copolymer synthesis. The carboxymethylation–PEI copolymer formed nanoparticles through high complexation capability with DNA and attained high transfection efficiency and minimum cytotoxicity than 25 kDa PEI against 3T3 and 293T cells. Furthermore, the carboxymethylation–PEI copolymer with <0.05 mg/mL concentration showed a minimum effect on the morphology, lysis of human red blood cells or aggregation or on blood coagulation, demonstrating excellent blood compatibility. Therefore, the copolymer serves as an alternative, safe and effective non-viral vector for practical application.

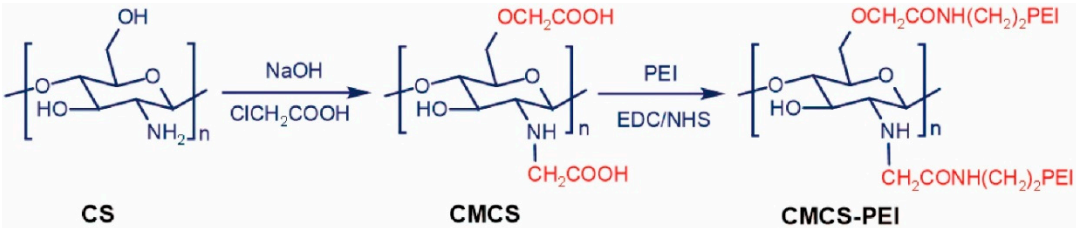

Figure 5. Scheme of the preparation of the CMCS–PEI copolymer [55].

Two varying forms of nanostructured carbon were grafted by functionalized single-walled carbon nanohorns to the amine group of chitosan and covalently linking the carboxylic group existing on graphene oxide, respectively (Figure 6). Compared to unmodified chitosan, the nanostructured carbon significantly accelerated the biological functions using a comparative analysis of substrate-osteoblast cell line (MC3T3-E1) communication. Moreover, protein adsorption and nanostructured carbon have a synergistic effect to create favorable modulating biological functions such as cell proliferation, viability and adhesion, with a pronounced effect on nanostructured carbon-modified scaffolds. A study also highlighted that nanostructured carbon-modified scaffolds favored protein adsorption, bioactivity and other biological tasks [56].

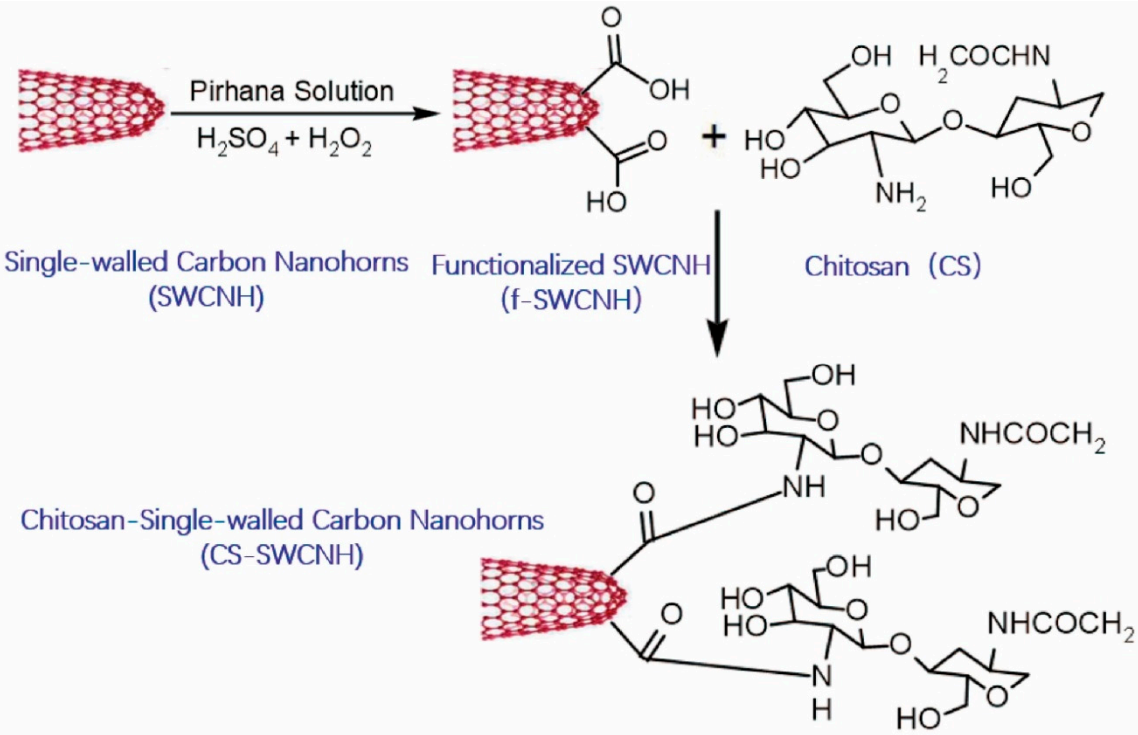

Figure 6. Schematic representation of subsequent covalent reaction between single-walled carbon nanohorns (SWCNH) and the amine group of chitosan (CS) during CS-SWCNH hybrid scaffolds fabrication.

In a word, various side chains can be grafted on the primary, secondary hydroxyl and amino groups of chitosan and new properties endowed. However, because the reactivity of the three functional groups is a little different, it is not easy to introduce the side chain in the fixed point and quantity, and it is difficult to separate and purify the intermediate or end product of the reaction, which limits the study of the chitosan grafting reaction to a certain extent. The research focuses on finding the appropriate reaction reagent, mild experimental conditions and efficient separation methods.

6.2. Blending Modification of Chitosan

The blending modification is a common method of polymer modification. Through some physical and chemical methods, chitosan is blended with other natural polymer materials or synthetic polymer materials, including starch, glucomannan capsule, gelatin, glycerin, cellulose, polyvinyl alcohol, polyacrylonitrile, polyacrylamide, etc., which can synthesize the excellent characteristics of each component to prepare for the needs of various fields, expanding the application range of polymer materials effectively. The blending modification of chitosan can not only give new excellent properties to new materials but also greatly reduces the cost of polymer materials. It is extensively used in adsorption of heavy metal ions, biomedical membrane materials, biological tissue functional materials, environmental protection fresh-keeping materials and other fields.

Du et al. [57] fabricated chitosan biomaterials with collagen using a cross-linker alginate dialdehyde (ADA) that has been presented in Figure 7. Intact retaining of the classical triple-helical structure after crosslinking was confirmed by in vitro fiber formation and FTIR analysis. More compact microfibril structural interactions of collagen side-chains established by SEM analysis. Crosslinking of chitosan and collagen could improve the thermostability of final products. There was no significant effect of ADA in chitosan/collagen scaffolds on antibacterial activity. Accordingly, the fabrication of chitosan/collagen composites crosslinked with ADA improves stabilization, conserves the standard triple-helical structure, sustains good biocompatibility and discloses the new medical uses.

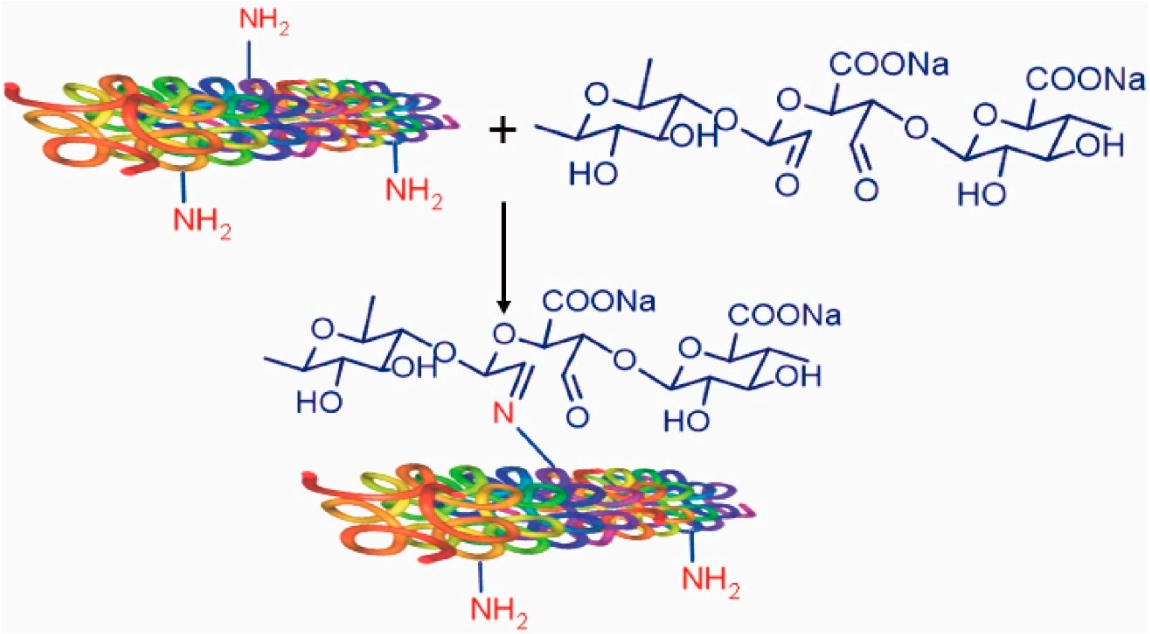

Figure 7. The diagram of the reaction for possible crosslinking between collagen and alginate dialdehyde (ADA).

Chitosan and carboxymethyl glucomannan are two kinds of polyelectrolytes with an opposite charge, which can form the polyelectrolyte complex and cross-linked membrane with the network structure in the process of film formation. Sun et al. [58] prepared the chitosan carboxymethyl glucomannan blend film by solution blending (Figure 8). The results showed that there was durable communication and better compatibility between chitosan and carboxymethyl glucomannan in the blend membrane and the mechanical properties of the blend membrane increased with the increase of the carboxymethyl glucomannan content. Liu et al. [59] reported that collagen, glucomannan and chitosan have good compatibility, and there are strong interactions among the three polymers, such as an electrostatic attraction and hydrogen bond. It is precise because of this interaction that the membrane has a uniform and smooth cross-section, high transmittance and better mechanical properties than a single polymer and binary blend membrane, the adsorption and permeability were also significantly lower than that of the glucomannan collagen binary membrane. The blend membrane containing glucomannan has good compatibility with endothelial cells and can be used as the carrier of endothelial cells. These characteristics indicated that the blend membrane of glucomannan collagen and chitosan has broad prospects as a potential scaffold material for tissue engineering, membrane carrier for cell transplantation, or biomaterial for organ damage repair.

Although chitosan has outstanding advantages such as non-toxicity, degradability, biocompatibility, does little damage to the drug and is suitable for drug release, it also has disadvantages such as poor solubility, poor mechanical properties and too much dependence on pH. Blending with PVA can improve these disadvantages of chitosan. Chitosan was easy to accumulate on the surface of the airside when it was blended into the film, which indicated that chitosan was more hydrophobic than PVA. The results showed that this blend film is more beneficial to the adhesion and growth of the slender runner compared with PVA due to the surface of the blend membrane being porous and the inner part being dendritic, while the surface of the PVA membrane is almost porous and the inner part is a sponge. More detailed studies on the surface state of the membrane showed that a hydrogen bond interaction between the -NH_2 or -OH of chitosan and -OH of PVA when chitosan and PVA are blended. Compared with PVA, fewer hydrogen bonds formed when blended with chitosan because PEO has less hydroxyl, resulting in poor compatibility. When the PEO content was lower than 20%, it was amorphous, and when PEO content was higher than 20%, it was easy to form crystal [60].

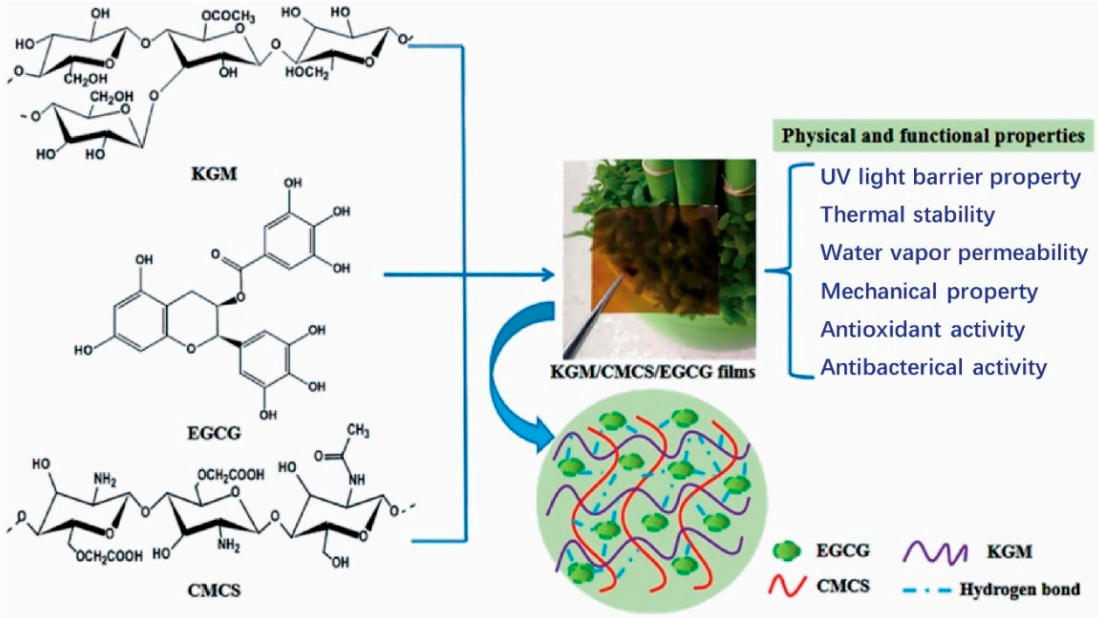

Figure 8. Schematic illustration of epigallocatechin gallate (EGCG) incorporated carboxymethyl chitosan (CMCS)/konjac glucomannan (KGM) biocomposite films [58].

7. Biological Properties

Chitosan, because of the strong hydrogen bond between molecules, possesses a regular molecular chain, good crystallinity and other properties such as adsorption, moisture retention, a film-forming ability, etc. It is a naturally available alkaline polysaccharide, while others like pectin, dextran, cellulose, starch, agar–agar, carrageenan, alginic acid, etc., are either acidic or neutral. It is a biocompatible substance that gradually breaks down to safe products like amino sugars, which absorbed completely in the body. The enzyme lysozyme hydrolyzed chitosan in vivo into oligomers that trigger macrophages to release *N*-acetyl-D-glucosamidase for catalyzation of oligomers to produce substituted glucosamines and D-glucosamine [61]. Several studies reported that MW, DDA, active groups and the bonding mode of chitosan and its derivatives are vital factors for their marvelous biomedical properties. The biological properties and corresponding biomedical applications of chitosan are shown in Figure 9.

(1) Antibacterial. Chitosan is an only natural weak alkaline polysaccharide that easily dissolves in a dilute acid solvent. The dissolved chitosan contains an amino group (NH_3^+), which can inhibit bacteria by binding negative electrons and its antibacterial activity may enhance with concentration. Chitosan with different molecular weight can stop several bacteria growths, and has a strong inhibitory effect on *Escherichia coli* and *Staphylococcus aureus*, showing similar characteristics with antibiotics [62]. The antibacterial mechanism of chitosan can be divided into two ways: One is that chitosan forms a layer of the polymer by adsorbing on the cell surface. The membrane prevents the transport of nutrients to the cells and plays the role of bacteriostasis and decontamination; the second is that chitosan penetrates the cells through osmosis, adsorbs the anionic cytoplasm in the cells and causes flocculation, which disrupts the normal physiological activities of cells and kills bacteria [63–65]. When the molecular weight of chitosan is different and the bacteria that act on it are different, the antibacterial mechanism of chitosan is different, but in essence, its antibacterial property comes from the antibacterial factor NH_3^+ [66,67]. Chitosan has amino and acetyl groups on its molecular chain, so it is amphoteric. Its isoelectric point is pH 6.2. When the pH of the solution is higher than this value, chitosan will not have a positive charge and no bacteriostatic effect. If it is lower than this value, it will have a positive charge. The degree of deacetylation of chitosan may also affect its antibacterial effect. The content of the free amino group and the bacteriostatic rate increased with the increase of the deacetylation degree [68]. The bacterial strains, pH, temperature, salinity, molecular weight, concentration

and degree of deacetylation of chitosan may be closely related to the antibacterial effect of chitosan. However, considering the high number of bacteria strains and the complexity of the bio environment generated, it is not clear the specific interaction between these factors [69,70]. The latest studies have enforced an investigation of the inhibitory activity of biofilm production of chitosan and its derivatives as prominent agents and diminish virulence properties by several pathogenic bacteria. Conjugation of chitosan with other bioactive materials can further promote its antimicrobial activity [71]. Ionic gelation was used to produce chitosan nanoparticles (CNP) and a different concentration of CNP (0–20% *w/w*) was used to prepare starch-based nanocomposite films. An in vitro and in vivo study proved the antimicrobial characteristics of CNP-starch films. For instance, the growth of tested pathogens such as *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* were inhibited by CNP-starch films about 15–20%.

(2) Adsorption. Chitosan has forceful adsorption function and can selectively adsorb heavy metal ions, cholic acid, cholesterol, triglyceride and grease [72–75]. It cannot be digested and absorbed in the gastrointestinal tract in a short time, on the contrary, it can absorb the fat that can significantly prevent the digestive system from absorbing cholesterol and triglycerides, avoiding the excessive accumulation of cholesterol and fatty acids. Eating 2 g of chitosan every day can effectively absorb the fat in the food, which is beneficial to losing weight [76]. Researchers try to find out the relationship between the structure of chitosan and the lowering of blood lipids and blood glucose, which is mainly due to the amino group of chitosan itself, which makes chitosan a poly ionomer. One possible way is that the chitosan catabolic compound can be adsorbed with negatively charged fatty acids and cholesterol. The simple chitosan can adsorb many times of its weight of oil, which can effectively prevent the digestive system from absorbing cholesterol and triglycerides, prevent the accumulation of cholesterol and fatty acids in the body and promote its excretion from the body. By reducing intestinal lipid absorption, the levels of cholesterol and triglycerides in plasma were reduced. Another possibility is that chitosan combines with negative bile acids to reduce the amount of bile in the liver and empty the gallbladder. There must be a certain amount of bile acid reserve in the gallbladder, so that the cholesterol in the plasma or liver can be converted into bile acid to maintain the bile acid reserve, thus reducing the cholesterol concentration in the plasma or liver [77].

(3) Moisture retention. The molecular chains of chitosan and its derivatives contain numerous active hydrophilic polar groups, such as -OH, -NH2, -COOH, etc. [78]. The content of the carboxyl group in carboxylated chitosan is far more than other derivatives, and the repulsion of the negative charge on the carboxyl group makes the polymer chain space to be extended especially large, even at a lower concentration, there is a strong interaction between molecules. The force on the water molecule is strengthened due to the hydrophilicity of the carboxyl group and the large extension of the molecular chain so that it has better moisture absorption and retention performance.

(4) Film forming. Chitosan has stable physicochemical properties and outstanding film-forming performance, its film-forming ability is closely related to the internal structure. The higher the deacetylation degree of chitosan, the lower the swelling and tensile strength of the membrane and the more difficult the degradation of the corresponding chitosan membrane in vitro and in vivo [79]. Since there are more crystal structures in chitosan with a high degree of deacetylation, the molecular rigidity is stronger and the water absorption is lower. The film formation and its characteristics are greatly regulated by the relative molecular weight of chitosan. The lower the molecular weight is the lower the tensile strength and the stronger the permeability of the membrane. The larger the molecular weight is, the more the crystal structure is, and the higher the molecular entanglement is. Therefore, the flexibility of the molecule is poor, the tensile strength is high and the permeability of the membrane is poor [80]. Since chitosan is degradable, the changes in the molecular weight might affect the properties of the membrane; the chitosan crosslinking degree also affects the properties of the membrane. With the increase of the crosslinking degree, the spatial network structure formed between the molecules increases, the tensile strength of

the membrane increases and the water permeability decrease. Chitosan membrane biomedical materials are widely used, such as preventing postoperative abdominal adhesions, purifying drugs and serum antibodies, manufacturing artificial renal membrane, artificial skin, contact lens membrane, drug sustained-release, dental surgery and nerve repair materials, etc. [81].

(5) Regulatory. Chitosan can activate lymphocytes with the immune function, distinguish normal cells from cancer cells and kill cancer cells [82]. Besides, it also has the function of regulating the endocrine system, regulating the pH value of the body to weak alkaline, improving the utilization rate of insulin, making the insulin secretion normal, inhibiting the rise of blood sugar, reducing blood lipid and helping to prevent and treat diabetes [83]. Many researchers have found that chitin, chitosan and chitosan oligosaccharide have immunomodulatory effects. The molecular weight of chitin and chitosan is more than 1 million, so its immunogenicity is very weak or almost negligible [84]. The immune response mediated by chitosan and its derivatives is closely related to its chemical structure. After deacetylation, chitin has -NH2 on the chitosan molecule, which can combine with H^+, enhance affinity, chemotactic leukocyte and induce a local macrophage. Macrophages play an important role in the immune response and regulation [85]. There are receptors of bacterial polysaccharides on the surface of macrophages. Chitosan, as a bacterial polysaccharide like substance, can stimulate the activation of macrophages, thus promoting the phagocytic ability and enhancing the activity of hydrolase secreted by macrophages [86]. As a natural high molecular material, chitosan with unique structure presents natural physiological activity in vivo, which can stimulate local tissues, promote cell proliferation and then evolve into macrophages, produce inflammatory mediators and improve the body's resistance to inflammation.

(6) Biodegradability. Chitosan is a kind of natural medical polymer material with excellent biodegradability, which is determined by its chemical structure. Chitosan has obvious degradation under the action of a lysozyme in vitro or in body fluid [87]. Degradation products are methyl sugar and oligosaccharide, which are safe for the body and can be decomposed, absorbed and metabolized. *N*-acetylglucosamine, one of the degradation products, is very important for scar repair of tissues and is toxic to some malignant tumors in vivo, so it can be utilized as cancer chemotherapy drugs [88]. Its degradation products are generally non-toxic to the human body, no accumulation in the body, no immunogenicity, so it can be used to manufacture artificial skin, surgical suture, bone repair materials, contact glasses, artificial dialysis membrane, anticoagulant materials, etc., carried a very broad application prospect in the medical field.

Figure 9. The biological properties of chitosan and its corresponding biomedical applications.

8. Application of Chitosan Biomaterials

According to the Pharmacopoeia of the people's Republic of China (Volume 4), chitosan is used for pharmaceutical excipients, disintegrants, thickeners, etc. [89]. Chitosan has a variety of biomedical properties and is widely used in wound dressings, orthopedics, dentistry, antitumor therapy, vascular repair and other fields [90,91]. Aments of studies have confirmed that the unique biomedical properties of chitosan and its derivatives are closely related to its structure.

Chitosan and its derivatives act as wound healing materials with a broad application prospect, which is attributed to their advantages of promoting wound healing, unique bactericidal and biodegradable properties and providing certain nutrients for cell growth. In the process of wound healing, chitosan can regulate the function of macrophages and the secretion of cytokines such as interleukin and tumor necrosis factor. Aamna et al. [92] synthesized silver nanoparticles in-situ in chitosan sericin composite to prepare chitosan sericin silver nanocomposite film by solvent casting technology and studied its antibacterial activity. The composite films were tested in Sprague Dawley male rats burn wound model for 7 days and confirmed a remarkable wound-healing ability with complete neovascularization, fibrosis, epidermal regeneration and collagen reorganization. Ouyang [93] constructed a new composite sponge by using chitosan/tilapia peptides microspheres as fillers and chitosan as a matrix and proposed for hemorrhage regulation. The findings indicated that by absorbing a high amount of water, the composite scaffolds accelerate platelet adhesion, speed up blood clotting, and stimulate the fibrin formation from fibrinogen. The bleeding volume was significantly reduced by decreasing bleeding time. Chitosan can accelerate the regeneration of epithelial cells, promote wound healing through cell proliferation and attract a large number of polymorphonuclear cells and macrophages through mild acute inflammatory response to remove tissue debris and blood clots. Chitosan can promote the chemotaxis, migration and activation of stromal fibroblasts and accelerate cell proliferation and tissue remodeling. It can also promote the formation of granulation and epithelial tissue, reduce the contraction of the wound surface, thus reducing scar formation.

Chitosan and its derivatives are also widely used in tissue engineering, especially as biological scaffolds of skin and bone, due to its ability of no expansion in water, high porosity and water absorption, interconnecting pores and uniform pore size, which are suitable for material exchange and growth metabolism of cells [94–98]. Chitosan scaffolds degrade without toxicity or inflammatory reactions eventually for the formation of the new tissue [99]. So, many porous chitosan scaffolds have been used for skin fibroblasts, keratinocytes and bone osteoblasts [100]. Yeh, et al. [101] synthesized chitosan cellulose scaffolds, grafted with sodium tripolyphosphate and polymethyl methacrylate, and finally were coated with gelatin, Schwann cells and fibroblasts. Chitosan provides a growth scaffold for cells, adheres to cells on the surface and makes cells grow rapidly. Shaltookia et al. [102] prepared porous nanocomposite scaffolds containing polycaprolactone and 45s bioactive glass nanoparticles with different nanoparticles (about 40 nm in diameter) by solvent casting technology. This material can play a good role in bone tissue engineering. Many kinds of chitosan composites such as thin film, gel, sponge and granule have been produced [103,104]. Chitosan-based systems for soft tissues like skin, adipose tissue, cornea, liver, nerve, CNS and blood vessel reengineering have been reviewed [105,106]. In skin tissue engineering, the rigid structure of chitosan fibers can enhance the mechanical resistance of the dermal matrix and prolong the degradation of the dermal matrix by wound cell collagenase [107]. In cartilage tissue engineering, chitosan sustained-release microspheres have good drug loading and drug-releasing properties. Microsphere scaffolds can well maintain the phenotype of chondrocytes, promote their adhesion and proliferation, and have a good application prospect in the construction of cartilage and repair of cartilage damage as a carrier of chondrocytes. Chitosan and its derivatives have been extensively applied in the study of artificial nerves because of their excellent biodegradability and biocompatibility. During the construction of artificial nerves, the function of normal peripheral nerves was not affected, which could promote nerve regeneration and provide conditions for the attachment, migration and proliferation of Schwann cells to play their normal functions.

Gels, nanoparticles, films, compressed tablets, beads and microspheres are currently used as potential drug delivery systems [108–110]. Chitosan has excellent biological activities as mentioned before and has been widely used in the study of the drug carrier systems as drug conjugates, hydrogel systems and biodegradable release systems [111–113]. It is mainly used in gene therapy, biological imaging, delivery of proteins/peptides, anti-inflammatory drugs, growth factors, antibiotics and vaccines. Drug delivery routes include oral administration, nose, eye and percutaneous administration [114,115]. The ionic interaction between the negatively charged sialic acid substructure in the mucus and the positively charged primary amino group of chitosan polymer could offer adhesion and permeability properties of chitosan. Self-assembled nanospheres were prepared by chemically-linked active amino groups on the chitosan backbone, which can circulate in the blood for a long time without being engulfed and can be transplanted to the target ligand, which is easy to deposit in the designated lesion site for treatment. Kim [116] used carbodiimide to connect the bile acid to the glycolytic chitosan skeleton so that the chitosan had strong hydrophilicity, and the nano-microspheres circulated in the blood for a long time and could be loaded with doxorubicin, paclitaxel, doxycycline and other anticancer drugs to effectively treat tumors.

Chitosan can attach nucleic acids via electrostatic bonding and also could be used to create non-viral gene delivery vectors, which enter into the cells without alienation of the DNA-chitosan complex [117–120]. It shows the main part in both lysosomal escape and membrane adhesion of the encapsulated DNA for effective cell transfection. Garcia et al. [121] prepared siRNA/folate poly-chitosan lactate nanoparticles by ionic gelation, showing the potential of effective gene therapy for ovarian cancer.

Song [122] investigated the antitumor activities of chitosan with a molecular weight of 3 K, 65 K and 600 kda and the zero-valent selenium (Se^0) nanoparticles stabilized by oligosaccharides. High molecular weight chitosan stabilized nanoparticles are easier to release selenium than low molecular weight chitosan, and to be absorbed by HepG2 cells through electrostatic action. Additionally, they are more effective in inhibiting the activity of HepG2 cells. These nanoparticles could produce highly

toxic Se^{4+} from the less toxic Se^{0} and release selenium upon high ROS production by cancer cells. This high toxic Se^{4+} causes apoptosis and mitochondrial dysfunction via consuming antioxidant enzymes. Chitosan and its derivatives can regulate the immune system through molecular mediation, enhance the body's resistance to various pathogenic microorganisms, and show antitumor activity. The antitumor activity of chitosan varies with the molecular weight and the substituted functional groups. Additionally, there are more negative charges on the surface of tumor cells, and chitosan and its derivatives are polycationic electrolytes, which are easy to adsorb to the surface of cancer cells and neutralize the charges, which can inhibit the growth and metastasis of tumor cells and even kill cancer cells.

In a word, the research and application of chitosan and its derivatives are the important direction of biomedical materials research in recent years, which deliver new materials for the development of biomedicine. At present, the research on chitosan is far more than the above-mentioned applications. With the continuous update of science and technology, chitosan and its derivatives in biological medicine are reported quite more every year, including anticancer, antiviral drugs, wound healing promoting materials, implants or blood components, substitutes of tissue components and applications in biotechnology as carriers of biological separators, fermentation industry, biomacromolecules and biosensors. This explains its importance in various fields, especially as biomedical materials. Although chitosan and its derivatives have a significant effect in biomedicine, due to the shortcomings of poor solubility and mechanical properties of chitosan, which limits the development of pure chitosan in the medical field. Additionally, there are still some key scientific problems to be solved, such as the uneven particle size, the deactivation of entrapped drugs, the inability to entrap hydrophobic drugs, and the difficult regulation of release. Therefore, the modification of chitosan, grafting with other materials and strengthening the development of drug loading system, design and construction of safe and efficient granules for protein-peptide sustained release, antitumor drug targeting, intraocular drug delivery and therapeutic vaccine adjuvant are the research hotspot of chitosan as biomedical materials.

9. Conclusions and Perspectives

Chitosan has good histocompatibility, biodegradability and excellent biomedical properties such as improving immune activity, antitumor, antibacterial, hemostasis and promoting wound healing. These properties are influenced by the degree of deacetylation, molecular weight and groups, especially amino groups of chitosan. To find out the relationship between these properties and its molecular structure has become the focus of many researchers, which provides a theoretical basis for the better development of new materials of chitosan and its derivatives, and makes it have a better application prospect in the field of biomedicine.

The research on the characteristics and application of chitosan in biomedicine has developed rapidly and become one of the hot research fields. Compared with α-chitosan, β-chitosan has weaker binding force, better solubility and biological activity. However, there are a few kinds of research on it at present. Therefore, exploring the economic and environmental protection of β-chitosan production process and modification research may become one of the research hotspots of potential biomedical materials in the future.

With the rapid development of biomaterials, higher requirements and challenges have been put forward for scaffold materials and drug carries. However, chitosan and its derivatives limit their application to some extent due to their defects, which need further study. At the same time, it is also necessary to strengthen the research of composite with other biomaterials to form new functional materials of marine organisms with multiple advantages, which should be one of the research hotspots of biomaterials in tissue engineering.

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