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Review Article

# Biochemical Significance of Biomaterials Based on the Chitin-Chitosan Axis

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## Abstract

Chitin is the second most available polysaccharide in nature after cellulose. It is found abundantly in the exoskeleton of arthropods such as insects, crabs, lobsters, and its presence in the fungi cell wall. The deacetylation of Chitin yields the formation of Chitosan, which has multiple exceptional properties. These include biocompatible, biodegradable as well as anti-microbial characteristics. Chitin and its biopolymer Chitosan have been a subject of research for their potential use in biomedical application. Their interesting properties meet the criteria needed to be used as biomaterials, especially in tissue engineering. This review explains the biochemical significance of biomaterials based on the chitin-chitosan axis by showing the biochemical structure and the properties of the Chitin and Chitosan, which include biodegradability, anti-inflammatory, anti-oxidant and some other beneficial properties which make Chitosan a very functional biomaterial that can adapt with living mammalian cells and induce their proliferation. We mention their use in the biomedical field as biomaterial, with a particular focus on Chitin and Chitosan-based scaffolds in tissue engineering (chitosan hydrogels and chitosan sponges), scaffolds in wound dressing (chitosan films, porous chitosan nanofiber membranes, and chitosan-based sutures), and some other chitin/chitosan-based materials. In fact, besides being biocompatible and biodegradable, they also display some other properties that allow them to be formed as scaffolds, using various methods, including lyophilization, freeze gelation, and gas foaming. Their non-toxic, soft and flexible properties will give them advantageous characteristics for their use as hydrogels. Their porous structure, antibacterial and hemostatic characteristics will make them ideal biomaterials for wound dressings and sponge materials in tissue engineering. Chitin/chitosan-based biomaterials have also proven significance in Veterinary medicine with their high nutritional characteristics that should be present in animals' diets. Additionally, it was also recognized for its high biomedical importance that has been used widely in medicine. Chitin and chitosan-based biomaterial are thus a revolutionary discovery in the biomedical field, as they improved the performance of biomaterials by being natural substitutes to the petrochemical products precedents and renewable and environmentally friendly.

**Keywords:** Chitin; Biomaterial; Chitosan; Biodegradability; Biomedical; Biocompatible; Scaffolds; Hydrogels

## Introduction

A biomaterial is a material that interacts with the body to either replace an organ or body part or increase its function. Biomaterials should be biocompatible and biodegradable to be functional and usable in an ideal way [1]. Chitin is a natural polysaccharide that is the most plentiful after cellulose and is a crucial component of the exoskeleton of crustaceans, such as shrimps and crabs and a significant constituent of the fungi cell wall [2]. Chitosan is the deacetylated derivative of Chitin; it has multiple excellent properties, including biocompatibility, biodegradability and non-toxic anti-microbial property, which arise from its primary amines [3]. Chitin has an essential role in the food industry and cosmetic field [4]. In addition, the significance of Chitin and Chitosan-based biomaterial has been a subject of research in the biomedical field for an extended time [5].

This review explains the biochemical significance of biomaterials based on the chitin-chitosan axis by showing the biochemical structure and properties of the Chitin and Chitosan, as well as their use in the biomedical field as biomaterial, with a particular focus on Chitin and chitosan-based scaffolds in tissue engineering (chitosan hydrogels and chitosan sponges) and scaffolds in wound dressing (chitosan films, porous chitosan nanofiber membranes, and chitosan-based sutures), in addition to other chitin/chitosan-based biomaterials [6].

## Chitin and chitosan: Biochemical structures and properties

Chitin is a long-chain polymer of *N*-acetyl-D-glucosamine. It is a natural polysaccharide that resembles cellulose in its structure [7]. However, the hydroxyl groups on the second carbon atom are replaced by an acetamido group in Chitin [3]. Chitosan is the deacetylated derivative of Chitin, and unlike Chitin, it is soluble in acidic solutions [8]. Some enzymes, such as chitin deacetylase, catalyze the hydrolysis of the glycosidic bonds, resulting in the conversion of Chitin to Chitosan [9]. Chitin usually appears as a white inelastic nitrogenous polysaccharide [10]. It is present in nature as ordered crystalline microfibrils present in the exoskeleton of some crustaceans and insects and the fungi cell wall and in small amounts in yeast and the cell wall of green algae [11]. However, it is most abundantly provided by shrimps and crab shells [12]. Despite being similar to cellulose, both in its structure and its low solubility, Chitin is not produced in the same cellulose producing organisms, and its percentage of nitrogen (5.9%) is way higher than cellulose (1.25%) [13]. Another characteristic distinguishing Chitin and Chitosan from other polysaccharides is their high alkali nature instead of most natural polysaccharides' acidic or neutral nature (cellulose, alginic, agar, dextran) [14]. Chitin also exists in three different allomorphs, which differ by the orientation of their microfibrils: α-, β- and γ-chitin, with α- chitin being the most common [15].

As for Chitosan, as mentioned above, it is the deacetylated derivative of Chitin. It is composed of (1→4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) as well as (1→4)-2-acetamido-2-deoxy-β-D-glucan (N-acetyl D-glucosamine) units and is defined as a semi-crystalline, linear polysaccharide [4,16]. To distinguish Chitosan from other chitin derivatives, the Deacetylation Degree (DD), which indicates the number of amino groups across the chain, should be calculated. To be recognized as "chitosan", the DD can vary from 60 to 100% [17].

Chitosan differs from Chitin in many ways. Although they are similar in their low solubility, Chitosan is soluble in acidic mediums, unlike Chitin [3]. Moreover, Chitosan differs from Chitin in the presence of amino groups in its structure, which will be responsible for the unique properties of this polymer [17]. The protonation of the amino groups is the cause of the solubility of Chitosan in acidic solutions. Chitosan with protonated amino groups is called polycation and will allow this polymer to form ionic complexes with many diverse anionic structures (e.g., lipids, DNA, proteins) and take on different shapes and forms [18].

## Chitin and chitosan properties as biomaterials

Biomaterials are natural or man-made equipment that interferes with the biology of living organisms. These biomaterials are used for medical purposes on a wide range; they facilitate the healing of organism tissues [3]. According to Gobbio., *et al*. [19], to be selected as a biomaterial, some requirements should be met, such as biocompatibility, mechanical compatibility, wear resistance, and biodegradability. Chitosan is found to have remarkable biological properties that put it under medical interest. The unique molecular structure of Chitosan is what makes it biomedically worthwhile [15].

### Biodegradability

Chitosan is a polysaccharide, which means it consists of many monosaccharide molecules joined by glycoside bonds. The presence of these bonds gives Chitosan the biodegradability properties, which means that chitosan molecules can be broken down inside the body by specific enzymes [20].

### Anti-microbial property

The molecule of Chitosan has amine groups which make this molecule polycationic [21]. This positivity of the cations attracts the negative charges in carbohydrate and protein residues present on the surface of some bacteria, thus disrupting the homeostasis of the membrane and eventually leading to the death of bacteria. So, the proliferation of bacteria is nearly impossible in the presence of Chitosan [22]. However, this mechanism of Chitosan is applicable in acidic mediums only. A study was held to find out more about the anti-microbial property of Chitosan: CM (chitosan microparticles) were used on cows with uterus infections to estimate the anti-microbial action of Chitosan [23].

### Anti-inflammatory property

Chitosan was found to reduce the irritant effects that accompany an infection. It reduces the number of factors provoking the inflammation by lowering the concentrations of interleukins (such as IL-6, IL-12, IL-10), thus stopping the inflammatory reaction [24].

### Anti-oxidant activity

The body produces free radicals during metabolism; they have harmful effects on animal/human bodies. These radicals can alter lipids, proteins, and DNA if present in excess amounts, causing oxidative stress [25-27]. The anti-oxidant property of Chitosan depends on the $NH_2$ group present in the chitosan molecule [3]. It can scavenge the free radicals and cancel their oxidative activity [28].

### Biocompatibility

Biocompatibility is defined by the ability of living cells of animals to bind, stick and proliferate in a particular medium. Chitosan can be used as a biomaterial, and it must be biocompatible as it will be interacting and/or replacing living tissues [29]. Again, thanks to the positive charges present in Chitosan, animal living cells with negative charges on their surface bind to chitosan scaffolds, divide and increase in number in a chitosan medium. Some studies were made to determine which mammalian cells fit mostly on a chitosan matrix [30,31]. Hsu., *et al*. [30] and Chatelet., *et al*. [31] showed that chondrocytes were the most to proliferate on a chitosan matrix, than fibroblasts and keratinocytes. It was deduced that the cells with higher negative charges on their surface were the best to adapt to the chitosan scaffold. Because of the significant properties of Chitosan, scientists innovated some forms to use Chitosan as a natural biomedical substance. Chitosan was then processed into nanoparticles, hydrogels, bandages, films, capsules and beads. These forms have been used in many fields, including the food industry, pharmacy, and biomedicine [23].

### Chitin and chitosan scaffolds in tissue engineering

Tissue engineering uses bioactive factors, cells, and supporting structures to regenerate or substitute damaged tissue structures and functions [32]. Scaffolds are materials used in tissue engineering; they enhance cellular interactions, forming a new functional tissue [18]. Chitin and chitosan-based scaffolds can be engineered because of their high porosity, biodegradable property, non-toxicity, biocompatibility, and ability to be fabricated into different types of scaffolds, such as hydrogels, sponges, and other three-dimensional porous structures [18,32].

### Chitosan hydrogels

Chitosan hydrogels are an essential class of scaffolds. They are composed of a solid (10% of the total gel volume) and a liquid phase. The solid phase is responsible for absorbing a large quantity of water without mixing with the liquid phase [18]. It is characterized by high water content, which makes it compatible with most living tissues, and it is bendable and soft, which protects and lowers the percentage of damage of the surrounding tissue during or after the implantation, as well as shows very high biocompatibility and antibacterial ability [33]. Besides, hydrogels mimic body tissues' mechanical and functional properties and they are used in other biomedical applications such as drug delivery and growth factor delivery [34]. According to Croisier and Jérôme [18], three main types of chitosan hydrogels exist: Non-covalent cross-linked physical chitosan hydrogels, Chitosan hydrogel by coordination cross-linking, and Covalent (chemically) cross-linked chitosan hydrogels.

### Non-covalent cross-linked physical chitosan hydrogels

This type of chitosan hydrogel is based on non-covalent reversible interactions. Various parameters, such as the temperature, pH, and concentration, can alter these interactions making this hydrogel unstable, hence the reversible nature of the gelation, depending on the number of interactions, the swelling and consistency of the hydrogel changes [32]. The decrease of interaction will make the gel softer, and the increase will make it stiffer [18]. The production of chitosan hydrogels can be formed without any additives, which is possible due to the neutralization of the amino groups, leading to the repulsion between the chitosan chains [35].

### Chitosan hydrogel by coordination cross-linking

The formation of this type of hydrogels occurs via the bonding of Chitosan with specific metal ions, such as Mo (IV), Pt (II), Pd (II). However, this type of chitosan hydrogel is not very suited for biomedical use [36].

### Covalent (chemically) cross-linked chitosan hydrogels

This type of hydrogel is based on covalent cross-linking between polymer chains. As opposed to the physically cross-linked hydrogel, this one is much more stable and irreversible. However, some alteration should be made to the structure of Chitosan, which may alter its properties and put it at risk of contamination by catalyst traced or the residual reactant that may be toxic [18,37]. The most straightforward way to form stable, irreversible chitosan hydrogel is by using dialdehyde cross-linkers, such as glutaraldehyde and glyoxal, which will bond with the amino chitosan backbone, or its hydroxyl groups [36]. Other cross-linkers can be used, such as genipin, a natural derived cross-linker, which degrades slower than the other [33].

### Chitosan sponges

Berretta., *et al*. [38] stated that chitosan sponges are porous chitosan structures that possess sponge-like properties. They are usually formed by lyophilization, which consists of freezing a chitosan solution, then exposing it to reduce pressure, resulting in its sublimation [39]. These materials are usually suitable biomaterials because they are porous, flexible and soft while presenting an excellent cell interaction surface [40]. Chitosan sponges are mainly utilized as biomaterial 3D scaffolds for wound healing because of their ability to enhance collagen formation and absorb the wound exudates while assisting tissue regeneration and filling materials and bone tissue engineering [41]. Their porous nature also makes them suitable drug delivery devices [42]. According to Matica., *et al*. [43], the chitosan sponge showed antibacterial and hemostatic activity, showing its efficacity in wound dressing application.

### Chitin and chitosan 2D scaffolds in wound healing and wound dressings

Chitin and chitosan-based biomaterials have been revolutionary discovery for wound healing. Chitin is composed of poly-*N*-acetylglucosamine, which includes favourable tissue regenerations, thus, wound repair [44]. Wound dressings are important for wound healing. Their purpose is to soak up any wound leakage, protect the wounded area and provide an ideal environment for tissue healing. Three primary materials are used in this procedure: chitosan films, porous nanofibers membranes, and chitosan sutures [18,45].

### Chitosan films

As Kumar., *et al*. [46] stated, due to their biodegradable, non-toxic and anti-microbial properties, chitosan films have gained much attention in the past decade; they are prepared either by wet casting method, followed by drying, or by neutralization with NaOH solution. These films have proven to be ideal wound dressing biomaterials as they are biodegradable, flexible, protect the wounded area and prevent its swelling, as well as environmentally friendly [8]. Two methods can modify the film thickness, roughness, and other parameters, to make it more advantageous: the Layer-by-layer deposition technique and the Langmuir Blodgett technique [18].

### Porous nanofiber membranes

There are multiple ways to fabricate chitosan fibres; one of them is electrospinning (ESP) which, the best way is to create chitosan nanofibers. It was proved that the mats of fibres obtained by this spinning method possess numerous ideal properties for their use as biomaterials for wound dressing, such as high specific surface area, ability to mimic the structure and properties of the extracellular matrix, high porosity, biocompatibility, and antibacterial properties [47,48].

### Chitosan sutures

Chitin has been recently used as a suture material due to its numerous biochemical properties. Besides being compatible and absorbable, a study in rats, *in vivo*, highlighted the other advantageous characteristics of Chitin and Chitosan for their use as sutures [44,49,50]. In the experiment, chitosan-based sutures were compared to nylon-based ones. The results showed that chitosan sutures produced a lower inflammatory response, a pain-free removal as it can be dissolved in a couple of hours if dissolved with a low pH solution, or absorbed within four months naturally, and a long term, high bacteriostatic effect due to its positively charged chain [51]. Although Chitin and chitosan-based biomaterials presented many advantageous properties for their use in the biomedical field, they also can show some disadvantages that should be considered.

Table 1 displays the main advantages and disadvantages of the chitin and chitosan biomaterials explained in the previous parts [18].

| Biomaterial type | Main advantages | Main disadvantages | Main biomedical applications |
|---|---|---|---|
| Hydrogels:<br>• Physically cross-linked (reversible).<br>• Chemically cross-linked (irreversible). | Non-toxic, soft and flexible.<br>Stable, soft and flexible, controlled pore size. | Not stable.<br>Might be toxic | Tissue engineering/substitution<br>Also, in drug and growth factor delivery |
| Sponges | High porosity + soft and flexible. | Might wrinkle and shrivel | Tissue engineering (filling material)<br>Wound dressings as skin substitutes |
| films | Material coating + multilayer construction | Many steps | Coatings in variety of scaffolds wound dressings and skin substitutes |
| Porous nanofibers membranes | High porosity and mimic extracellular matrix | ESP of pure Chitosan is very difficult | Coatings in variety of scaffolds wound dressings and skin substitutes |

**Table 1:** The main advantages and disadvantages of the chitin and chitosan biomaterials.

## Other uses of chitin and chitosan-based biomaterials

### Gastrointestinal diseases

One of the most important characteristics of Chitosan is its anti-inflammatory property. This property has been shown when Chitosan was used on open wounds, where it showed excellent efficiency. Therefore, Chitosan was tested in multiple fields, including the gastrointestinal system, where it is thought to impact the intestinal mucosa during infection significantly. A study was made to determine the effect of chitosan ingestion on piglets with enterotoxigenic *Escherichia coli* intestinal infections [52]. It was later discovered that chitosan-oligosaccharide (COS) could decrease the diarrhoea index in these piglets. This study also came down to the conclusion that Chitosan can reduce the intestinal inflammation severity, in addition to amplifying the cell-mediated immune response. Chitosan was able to achieve these by regulating the inflammatory cytokine release [53]. Other than its anti-inflammatory property, it was shown that a chitin-glucan complex can be used as a prebiotic to enhance the proliferation of beneficial microorganisms in the gut [54].

### Drug delivery system

According to Khor and Lim [55], Chitin and Chitosan's peculiar properties allows them to act as carriers for diverse, active agents, such as drugs. Chitosan hydrogels can be used as drug delivery devices and inserted in the wanted location as implants. For example, CMC (carboxymethyl chitin) is a drug delivery device prepared through cross-linking using $CaCl_2$ and $FeCl_3$. Some studies showed that its drug-loaded nanoparticles were easily carried and released and showed no toxic properties [50]. Chitosan synthesized with nanoparticles and alginate can also be used effectively in targeted drug delivery, showing anti-microbial and immunological properties [56].

### Ophthalmology

Ophthalmology is the study of disorders or diseases related to the eyes. Contact lenses are a perfect example of biomaterials that enhance the function of the eye. Besides the chitin and chitosan properties previously mentioned, Chitosan shows some other specific characteristics that allow it to fabricate contact lenses, such as optical clarity, gas permeability, mechanical stability, immunological compatibility, and wettability [15]. These are formed by partial depolymerization of purified, isolated Chitosan. In addition, its anti-microbial, wound healing, and film-forming properties allow it to act as ocular bandages [57].

### Significance of chitosan in veterinary medicine

As Chitosan has been very helpful as a biomaterial for medical purposes, it has also shown great importance in veterinary medicine. Like in human medicine, chitosan aids with wound healing, tissue regeneration, and bone restoration in animals [58]. However, lately, scientists are looking forward to more benefits of Chitosan in Veterinary Medicine. A possible application of Chitosan is that it would be used in drug and vaccine delivery in animals; it could also be a nutritional ingredient in Veterinary Medicine [59]. It has been suggested that Chitosan will be responsible for delivering the chemotherapeutics to the mucosal tissue of the local mucosal tissues [60].

Moreover, the Chitosan could target the lymphoid tissues in the body to regulate the inflammatory reactions [61]. The delivered substances could be antibiotics, pain killers or immune modulators [60]. The goal to be achieved is that chemotherapeutics should reach the local mucosal tissues rather than just systematic routes to induce more robust and more efficient reactions. However, these suggestions have found some trouble as in the difference of digestive systems in animals, ruminants, for example, have a different and more complex digestive system than other animals, which means they might not ingest the medication properly.

## Conclusion

Chitosan is a very unique biopolymer, with primary amine groups in its structure which gives this polysaccharide interesting physical and chemical properties. In addition, the protonation of this polysaccharide in acidic mediums allows it to become soluble in aqueous solutions and take on different shapes such as sponges, gels, nanofibers mats, and thin films. The biochemical structure of Chitin and Chitosan, which gives them these special properties makes them ideal candidates to be used as biomaterials. This, in addition to its availability, makes it an unlimited source of benefit to the medical field of both animals and humans. Chitosan will be used in drug delivery systems as it is highly efficient and highly nutritional for animals and humans. It will also facilitate the vaccination field as it could be a part of oral/non-oral vaccines. When Chitosan reaches the duodenum, it dilutes the HCl-layer and accumulates fatty acids and cholesterol, thus, reducing their absorption. In the veterinary medicine field, we believe that Chitosan could also be applied as an external factor. As horse hooves are generally sensitive and need much care, especially that they are subjected to many bacteria, Chitosan could prevent the unwanted inflammation of horse hooves. This field of study is important and no doubt new developments will arise with future studies.

## Conflict of Interest

All authors have declared no conflict of interest.

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