

# Chitosan: An Antimicrobial Polymer

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Antibiotic resistant infections are a rising problem in the United States and globally. These infections are listed as a top concern by the Center for Disease Control and Prevention (CDC) as well as by the World Health Organization. Antibiotic resistance is a phenomenon where microorganisms acquire or innately possess resistance to antimicrobial agents. Antibiotic resistant infections significantly reduce the effectiveness of the treatment causing patients to remain infectious longer and increasing the risk of spreading the resistant microorganisms. Antibiotic resistant infections are incredibly detrimental to society and are threatening many of the medical advances made in the past century.<sup>1</sup>

The World Health Organization's 2014 report states that these antibiotic resistant infections are no longer a problem of the future and are in fact impacting the lives of people worldwide currently. The world is headed towards a "post-antibiotic era" where common infections and minor injuries, which have been treatable for decades, can once again lead to disabilities and death.<sup>1</sup> The CDC estimates the annual death toll in the United States alone to be 23,000 and the British government estimates the current death toll worldwide to be 700,000 per year.<sup>1,2</sup> The projected mortality rate by 2050 is to be 10 million annually, which is more than the projected rate for cancer.<sup>2</sup> In addition to higher mortality rates, health care costs significantly increase as more expensive therapies must be sought to treat these antibiotic resistant infections. Furthermore, antibiotic resistant infections are impairing the success of major medical procedures, such as organ transplantation and cancer chemotherapy. These infections can also severely prolong or completely prevent the wound healing process.<sup>3</sup> Due to the imminent risk of these antibiotic resistant infections, an immense amount of research is being done to find new ways of curbing this chronic wound problem. One area of focus has centered on the exploration of biomaterials with antimicrobial properties.

Antimicrobial polymers have emerged as promising deterrents to the spread of antibiotic resistant infections. One polymer known especially for its antimicrobial properties is chitosan. Chitosan, formed by the deacetylation of chitin, is a natural polymer derived from crustacean shells. It is also biocompatible, biodegradable, nontoxic, anti-inflammatory, and anti-fungal, making it an extremely suitable polymer for biomedical applications.<sup>4</sup> The discovery of chitosan dates back to 1859, when Charles Rouget described in his paper the deacetylation of chitin.<sup>5</sup> Following the discovery, research of chitin and chitosan burgeoned in the 1930s and 1940s. The focus of all the research was on the potential bioactivity of chitosan.<sup>4</sup> A major issue with currently available antimicrobial products is their toxicity to mammalian cells. The natural polymer, chitosan, is a solution as it is non-

toxic towards mammalian cells and has been shown to be extremely effective against bacteria.<sup>4</sup> However, chitosan does have some drawbacks to being utilized in biological applications.

The use of chitosan is heavily limited by its inability to solubilize in water. Due to excessive hydrogen bonding in the structure, chitosan degrades prior to melting, and therefore, it is necessary to dissolve chitosan in a solvent to impart functionality. Improving the solubility of chitosan is crucial for the material to be used to its full potential. Chitosan has the ability to dissolve only in certain acids such as hydrochloric acid, lactic acid, succinic acid, acetic acid, and formic acid etc. Chitosan is insoluble in water, alkali or aqueous solutions above a pH of 7. The solubility of chitosan has become an essential aspect of antimicrobial research.<sup>4</sup>

Chemical modifications have been attempted to improve its solubility in order to widen its applications.<sup>6</sup> Chemical techniques such as PEG-grafting, sulfonation, partial N-acetylation, chitosan branching with oligosaccharides, chitosan-saccharide derivatives, as well as O-succinyl-chitosan have been conducted to make water soluble derivatives of chitosan. More methods such as alkylation, acetylation, carboxymethylation chitosan and quaternization have been attempted as well.<sup>6-8</sup>

Researchers studied if nitric acid could dissolve chitosan only to discover that a white gelatinous precipitate would instead form. They also tried sulphuric acid which did not dissolve the chitosan and instead formed chitosan sulphate, a white crystalline solid. It was determined that the solubility of chitosan very heavily depends on the pKa values of these acids and their concentrations.<sup>4</sup>

In one study, quaternary ammonium chitosan was prepared and tested for its antimicrobial activity. The quaternized chitosan derivatives in fact exhibited stronger antibacterial activity than just chitosan.<sup>4</sup> However, another study reported otherwise, claiming that the quaternized chitosan failed to exemplify antibacterial activity against *S. aureus*, an antibiotic resistant infection that causes over 11,000 deaths annually in the United States alone.<sup>6</sup>

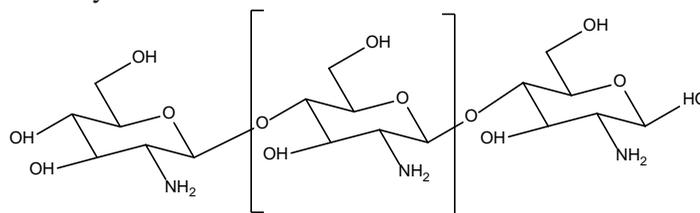


Figure 1: Structure of Chitosan

The partial hydrolyzation of chitosan by enzymatic processes has also been studied in order to enhance the solubility of chitosan. This process decreases the molecular weight of chitosan, shortens the chain lengths, and results in free amine groups. This makes the chitosan readily soluble in water. The greater solubility and low viscosity of the partial hydrolyzed chitosan has drawn many researchers to use chitosan in its lower molecular weight form.<sup>4,7</sup>

The antimicrobial mechanism of chitosan is still not completely understood, and the search continues to find better ways of processing chitosan to acquire the desired properties. The characteristics of chitosan are influenced by molecular weight, as well as the degree of acetylation. These factors impact the solubility of the chitosan, which in turn determines the rheological and physical properties.<sup>9</sup> In several papers, researchers have reported that they improved the solubility of the chitosan by reducing the degree of deacetylation. However, the reacylation of chitosan reverts the polymer back to chitin. The question then arises as to why chitin is not used directly instead since theoretically it should exhibit higher antimicrobial activity.

The  $-NH_2$  groups in the chitosan molecule, as shown in Figure 1, are highly reactive. Therefore processes to increase the free amine groups will directly correlate with a higher antimicrobial effect.<sup>4</sup> The more deacetylated the molecule is, the higher the antimicrobial impact. Since reducing the degree of acetylation increases the antimicrobial activity, using chitin would not result in equivalent antimicrobial results in comparison to using the deacetylated chitosan in the development of these antimicrobial hydrogels.

The distribution of the acetyl groups as well as the free amino groups make chitosan a very unique polymer as it impacts the bonding behavior. It also makes chitosan soluble in solutions below pH of 6.5. The amino groups make chitosan a cationic polyelectrolyte, unlike other polysaccharides.<sup>10</sup> Chitosan is protonated in aqueous acidic solutions and, when dissolved, takes on high positive charges on  $NH_3^+$  groups.<sup>4</sup> The resultant polysaccharide is positively charged making it attracted to negatively charged surfaces. The solubility of chitosan in acidic solutions as well as its aggregation with polyanions contribute to chitosan having excellent properties for gel-forming.<sup>4</sup>

The increasing prevalence of antibiotic resistant infections, especially those stemming from impaired wound healing as well as biomedical implant failure, has led to the development of materials with antimicrobial activity such as hydrogels. Hydrogels are biocompatible and can be incredibly useful in drug delivery, wound healing, and several other biomedical applications. Chitosan-based hydrogels are extremely sought after materials for use as wound dressings since the wound area is aided by the hydrated environment the hydrogel offers while simultaneously imparting antimicrobial activity.<sup>11</sup> Additionally, hydrogels can also incorporate a controlled release system to deliver drugs.

Over the years, researchers have been attempting to perfect the process of creating hydrogels using chitosan. Scientists at the University of Otago attempted to make antimicrobial chitosan dextran-based hydrogels. However, they determined that the antimicrobial activity was to be credited to the dextran aldehyde instead of the chitosan because their processing of the hydrogel led to the chitosan losing its polycationic nature.<sup>8</sup> Others have immobilized the chitosan, encapsulating it into a gel to prepare a wound dressing using glutaraldehyde as the cross-linker. The authors in that study showed that the chitosan hydrogel layer did lower the microbial activity.<sup>8</sup>

Antimicrobial hydrogels have also been developed as coatings for medical implants. Scientists developed an antimicrobial hydrogel based quaternized chitosan-graft-PEG methacrylate. Several samples were created with different variability in each from degree of acetylation to degree of quaternization.<sup>6</sup> Of the hydrogels that were tested, the dimethyldecylammonium chitosan (highly quaternized)-graft-PEG methacrylate gel showed the most antimicrobial activity. The researchers proposed that the polycationic hydrogel is contact active, working as a sponge and attracting the anionic microbial membranes into the hydrogel nanopores. The cationic amino groups of the chitosan bind with the anionic groups of the microorganisms and fungi, resulting in the inhibition of the bacterial growth.<sup>7</sup>

Over the last couple decades, chitosan has grabbed researchers' attentions due to its potential as an antimicrobial agent. While a significant amount of progress has been done in the characterizing of chitosan, there are many areas that require further research. Various results reported by researchers under similar or even identical conditions tell different and even completely opposing stories.<sup>7</sup> Testing methods have not been standardized, thereby making it difficult to compare different studies. Furthermore, the development of a process for increasing chitosan's solubility that does not diminish its antimicrobial properties is a priority. This discovery alone can open a whole world of applications for chitosan in, the biomedical field and beyond. Future work in this field can provide clarity to the underlying mechanisms furthering our understanding of chitosan and its behavior and characteristics. The development of materials that are active against these infectious microorganisms is proving essential, particularly in biomedical implants as well as wound healing. Chitosan is a game changing polymer with its anti-inflammatory, antifungal, and antimicrobial properties as well as its biocompatibility, biodegradability, and ability to bypass mammalian cells and specifically attack foreign microbes.<sup>7</sup> Additionally, chitosan-based antimicrobial hydrogels are ideal for injectable, topical, and coating applications among others.<sup>9</sup> Needless to say, the focus on chitosan will continue to proliferate, especially as antibiotic resistant infections are on the rise.

## References

1. Antimicrobial Resistance: Global Report on Surveillance; World Health Organization: Geneva, 2014.
2. O'Neill, J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations; Government of the U.K. and Wellcome Trust, 2014.
3. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States. U.S. Department of Health and Human Services, 2013.
4. Goy, R. C.; de Britto, D.; Assis, O.B.G. Polimeros: Ciencia e Tecnologia **2009**, 19, 241-247.
5. Rouget, C. Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences **1859** 48, 729-795.
6. Tan, H.; Ma, R.; Lin, C.; Liu, Z.; Tang, T. International Journal of Molecular Sciences **2013**, 14, 1854-1859.
7. Kong, M.; Guang Chen, X.; Xing, K.; Jin Park, H. International Journal of Food Microbiology **2010**, 144, 51-63.
8. Aziz, M.A.; Cabral, J.D; Brooks, H.J.L; Moratti, S.C; Hanton, L.R. American Society of Microbiology **2012**, 280-287.
9. Salome Veiga, A.; Schneider, J.P. Biopolymers **2013**, 637-644.
10. Chen, Y.L. Preparation and Characterization of Water-Soluble Chitosan Gel for Skin Hydration. Universiti Sains Malaysia, 2008.
11. Li, Y.; Fukushima, K.; Coady, D.; Englier, A. Angewandte Chemie International Edition in English **2013**, 52, 647-648.



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