ISSN 2563-9218

REVIEW ARTICLE

Chitosan: A Review of its' Antimicrobial and Biological Properties and Use in Wound Care

V Edwards-Jones[1,2*] and Mohsen Miratab[3,4]

Edwards-Jones V, Miratab M. Chitosan: A Review of its' Antimicrobial and Biological Properties and Use in Wound Care. Int J Biomed Clin Anal. 2022;2(2):48-57.

Abstract

Chitin is the second most abundant natural polymer on the planet and chitosan is a deacetylated derivative of chitin. Chitosan has a number of natural biological properties including broad spectrum antimicrobial activity mediated by the cationic /anionic interaction between the chitosan and bacterial cell wall respectively. Chitosan is used extensively in the food industry but is used only minimally in the wound care industry. This makes chitosan an alternative option to antibiotics for the treatment of wound infection, especially those organisms that are antibiotic resistant. Many common wound pathogens have developed resistance to first line antibiotics and those wound infections can be difficult to resolve. Resistance to chitosan has been observed by the production of chitosanase by some bacterial strains and modification of the cell envelope in a yeast strain but reports are limited. This review will discuss the potential of chitosan in wound care.

Key Words: *Antimicrobial; Chitosan; Medical device; Wound dressings*

Introduction

Chitosan was discovered by Rouget in 1859 [1], and is a high molecular weight, polycationic linear polymer, consisting of two monosaccharides, N-acetyl-D-glucosamine and D-glucosamine linked together by β-1-4 glycosidic bonds usually containing more than 5000 glucosamine units [2]. It is made by deacetylation of chitin, a natural structural component of the exoskeleton of crustaceans (e.g., prawn, crab, shrimp) and fungal cell walls and is very abundant in nature, second only to cellulose [3]. The relative amounts of the two monosaccharides vary in the deacetylated chitosan giving rise to varying properties. The characteristics of chitosan can therefore be varied as required for a particular application and is dependent upon the degree of deacetylation (compared to chitin) and its resultant molecular weight (MW) [4].

[1]*Emeritus Professor of Medical Microbiology, School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom*

[2]*Visiting Professor of Microbiology, Institute of Skin Integrity and Infection Prevention, University of Huddersfield, Huddersfield, United Kingdom*

[3]*Honorary Professor of Materials, University of Manchester, M13 9PL, United Kingdom*

[4]*CEO and Director of Medical Device Consultancies, Manchester Sk9 4JJ, United Kingdom*

[*]*Corresponding author: V Edwards-Jones, Emeritus Professor of Medical Microbiology, School of Healthcare Science, Manchester Metropolitan University, Manchester, United Kingdom, Tel: +44 7734062958; E-mail: v.edwards-jones@sky.com*

Received: October 13, 2022, Accepted: November 10, 2022, Published: November 28, 2022

Production of Chitosan from Chitin (Understanding the Chemistry)

There are four major steps in the processing of chitosan from chitin. 1) deproteinization, 2) demineralisation, 3) decolouration and 4) deacetylation [5]. The resultant chemical properties of chitosan following processing are a linear polyamine, reactive amino groups, reactive hydroxyl groups and a chelator of metal ions [5].

Chitosan is insoluble in most solvents (including water) but is soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid when the pH is <6. Solubilization occurs by protonation of the –NH2 group of the D-glucosamine repeating unit and can be examined by dissolving it in 1% or 0.1M acetic acid [6]. As the degree of protonation is progressively increased, so is the solubilisation of chitosan [7]. Solubility differences between chitosan with different degree of deacetylation may influence accessibility of chitosan to enzymes and the resultant biological effects [8]. Chitosan normally has high viscosity, and this can be altered by temperature and pH, and tends to coagulate with proteins at a high pH [2]. It is a polycationic polymer (has positive charges at several sites) and this property enables binding to negatively charged cells such as bacteria and red cells [2].

Through its abundant amino groups, chitosan enables chelation of metal ions [9] and has been used as a flocculating agent in the water industry [5,10]. By utilising the primary amino groups and the primary and secondary hydroxyl groups of chitosan in chemical modification reactions, variable products can then be formed allowing a wide range of applications in the cosmetic industry (skin care, hair care and oral care), food industry and now the biomedical industries [5,11]. Chitosan (Figure 1) has been shown to be relatively non toxic to keratinocytes and has improved cell proliferation compared to other biocides [12]. In addition, chitosan is naturally biodegradable *in vivo* via enzymatic breakdown (mainly lysozyme) and transforms to basic, non-toxic components [13]. These attributes of good antimicrobial activity, biodegradability and limited effect on cell proliferation makes chitosan an exciting addition for wound care [14]. Additives can be bound to chitosan to improve or alter the structure and functional activities of the polymer [8] and in the wound care industry, there has been an expansion of new products. Fortunately, as technology has improved and there is a greater understanding of the chemistry there have been some marked successes in the wound care industry and more products including chitosan are becoming available for use. The various physical forms of chitosan can include anything from; chitosan powder [15] films [16,17], hydrogels [18], coatings, fibrous mass, to chitosan in solutions or in combinations with other ingredients [19].

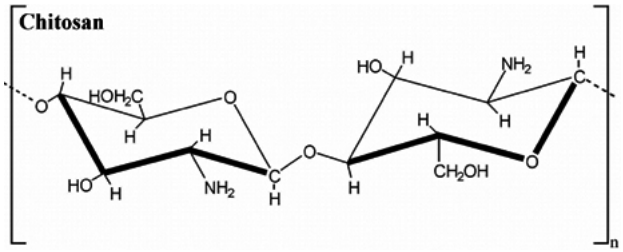

Figure 1) *Chemical structure of chitosan.*

Antimicrobial Activity

Chitosan has broad spectrum of antimicrobial activity against a range of Gram positive and negative bacteria and fungi. This antimicrobial activity is variable and is dependent a number of factors including:

1. Variability between microbes (species, cell age and growth phase) [20-24].

2. Chemical properties of the chitosan, degree of positive charge (acetylation), molecular weight, hydrophilic/ hydrophobic characteristic and chelating capacity [25-27], physical state of chitosan (solid versus water soluble state) [26].

3. Environmental factors when used, e.g., ionic strength in medium, pH, temperature and reactive time [22,24,28].

Gram-positive and Gram-negative bacteria interact differently to chitosan due to their different cell surface characteristics. Teichoic acids, which are found on the surface of Gram positive bacteria, interact with chitosan molecules through non-covalent binding [29] and as they play an important role in pathogenesis and modulating susceptibility to cationic molecules, their binding can alter regulation of the negative charge of the bacterial cell, which also prevents binding of extracellular molecules [24]. Chitosan has a greater antimicrobial effect against Gram negative bacteria [25,30], and it was demonstrated by Chung *et al.* [25] and Davidova *et al.* [31] that chitosan binds to lipopolysaccharide present on the cell envelope. The amounts of absorbed chitosan were related to environmental pH values (pH 4 absorbed more than pH 5) and degree of deacetylation of chitosan (greater positive charge) [32,33].

Concern over antimicrobial resistance is a global concern and in 2020 it was declared an emergency by the World Health Organisation and has become one of its major priorities [34]. Microorganisms have developed novel resistance mechanisms to both antibiotics and antiseptics through acquired resistance or through mutation [35]. Chitosan has good activity against multi-drug resistant strains [36,37].

In yeast cells, only high concentrations of chitosan can have an antifungal effect and inhibit their growth [38] through suppression of sporulation and spore germination [39]. It has a greater antifungal activity at lower pH values [40-42].

Mode of antimicrobial action

The exact mechanism of the antimicrobial action of chitosan is still to be fully elucidated but different mechanisms have been proposed [24,43]. The most likely is the disruption of the cell membrane through interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leading to the leakage of proteinaceous and other intracellular constituents [2] and rupture of the cell. Work undertaken by Lui *et al.* [44], using a variety of analytical techniques showed that chitosan increased the permeability of the outer and inner bacterial cell membranes resulting in the release of cellular contents and disruption of the cell through lysis. It is proposed that cellular damage was likely caused by the electrostatic interaction between NH_3^+ groups of chitosan and phosphoryl groups of phospholipid components of cell membranes [44-46]. It is mediated by a two-step process which involves an initial separation of the cell membrane from the cell wall and then destruction of the cell membrane based on similarities of the antibacterial pattern to polymyxin and Ethylenediaminetetraacetic acid (EDTA) [25]. Transmission electron micrographs showed an altered outer cell membrane with cytosolic components coagulated after treatment for 24 chitosan [47].

A second proposed mechanism is with interaction of DNA and disruption of protein synthesis by inhibition of mRNA [48,49]. Xing *et al.* [49] investigated the binding of oleoyl-chitosan nanoparticles (OCNPs) to DNA/RNA by examining the effect of OCNPs on the electrophoretic mobility of nucleic acids. Migration of DNA and RNA from *E.coli* was inhibited at a concentration of 1000 mg/L. The negatively charged phosphate groups in the nucleic acid interacted with the positively charged amino groups in OCNPs [50].

A third mechanism of action is through metal chelation. Bacterial cell wall components, lipopolysaccharide and teichoic acids, attract divalent cations that are beneficial to stability of the cell envelope (Mg^{2+} and Ca^{2+}) [49]. However, at low pH (below 6,0), the protonated

amino groups in chitosan chelate the metal ions in competition (compete for the divalent cations through chelation for the phosphate groups found in chitosan can chelate metal ions and essential nutrients) [51].

A fourth mechanism is the formation of a dense polymer film on the bacterial cell surface [43], preventing nutrient and oxygen uptake, which then inhibited growth [52], and chitosan aggregates have been observed with electron and fluorescence microscopy [53].

Resistance to chitosan in an occasional bacterial strain has been observed due to the production of enzymes (chitosanases) which break down the polymer [54] and modification of the cell envelope in a yeast strain [55] but reports of resistance are limited.

Biological Activities of Chitosan

Chitosan has been reported to exhibit various biological activities, including anti-tumour activity, haemostatic activity [56], and acceleration of wound healing [57]. As it has haemostatic properties it adheres to red blood cells and encourages adherence, activation and aggregation of platelets at the site of vascular injury [58-60]. This results in reduction of bleeding in the wound bed and is used during excision of necrotic material extensively in the military. It has been used successfully in excisional wounds to control bleeding [61]. Medical grade sterilized chitosan powder after direct application to incisions in the skin of rats showed improved healing capabilities. Early granulation tissue formation with reduced exudation and peripheral swelling was observed in the treatment group compared to the control group (standard dressing material-gauze) with healing completed on day 20-22, compared to day 27-28 in the control group [62]. This data was further consolidated in a study using a chitosan-based film containing tyrothricin (TYR) in rats that were given different wounds including burns, abrasions, incisions and excision models. The results were compared with no-treatment groups and those with sodium fusidate ointments [63]. Improved healing, improved granulation tissue formation and epithelization was observed (clinically and histologically) within the first 6 days in the rats with the chitosan-based films compared to compared with the no treatment group (negative control). The chitosan film-forming gel also enabled significantly better healing compared with the sodium fusidate ointment (positive control) treatment and it was concluded that wound occlusion had resulted due to the chitosan film, enabling improved healing [63].

When chitosan is made into wound dressings it displays several attributes other than its natural haemostatic and antimicrobial abilities [19]. It can also reduce pain by blocking nerve endings where blood clotting occurs and provides a suitable matrix for tissue growth and subsequent encouragement of macrophages for tumoricidal activities [43]. It further, promotes cell proliferation and tissue organisation to restore original architectural features of the skin and its substructure [43]. It degrades gradually or depolymerises to release N-acetyl-b-glucosamine which initiates fibroblast proliferation and deposition of ordered collagen whilst assisting increased level of hyaluronic acid synthesis at the wound bed [11].

This depolymerisation allows release of N-acetyl-b-D-glucosamine, which initiates fibroblast proliferation and helps in ordered collagen deposition and stimulates increased level of natural hyaluronic acid synthesis at the wound site. This will help with faster wound healing and may help reduce scarring [64].

Application of Chitosan in Wound Care

The inherent biological properties of chitosan, such as haemostatis, antimicrobial activity, biocompatibility, low toxicity and biodegration are all very positive and should make a useful addition as a dressing in the wound care industry. Chitosan has been combined with alginate [65-68], sulphonamide drugs [69] essential oils [70-

73] and honey [74]. Nano-spun chitosan has also been combined with nanoparticles of silver with enhanced antimicrobial properties [75,76].

The degree of acetylation and its immediate environment can affect the efficiency and potency of the resultant chitosan [77]. The ability to make a variety of wound dressings with different physical and biological attributes, along with having antimicrobial properties enables a whole new portfolio of non-biocidal alternative natural antimicrobial dressings [19].

Hydrogels are popular wound dressings because of their absorbent properties in exudating wounds and ease of removal [78,79]. Chitosan can be made into a hydrogel form by cross linking the polymer by several methods including chemical modification [80] UV light [81] or graft polymerisation [82]. This technique uses reactive groups within the polymer molecule i.e., free amine groups on deacetylated units and the hydroxyl groups to react with polymerisable monomers that form the grafted chains [83]. Genipin has been used as an alternative natural cross-linker with low toxicity. Genipin can also act as an anti-inflammatory agent [84]. A major disadvantage with physical cross-linking can be instability and low mechanical properties, however, to overcome this, freeze-thaw processing has been adopted. In this process, natural polymers such as chitosan are combined with a synthetic biocompatible polymer like polyvinyl alcohol (PVA). This combination improves chitosan hydrogel strength and its biodegradability as well as being readily available [85,8].

Chitosan can be readily made into a soluble solution and hence fibres via Wet Spinning. This is a relatively simple process and if need be, the chitosan fibres can be subsequently converted back to chitin fibres by the acetylation process [86]. Chitosan as a wet extruded fibre could be brittle due to its medium to low absorption properties thus causing processing difficulties. Miraftab *et al.* [87], have successfully combined hydrolysed chitosan with alginate to form a hybrid fibre that is far more process friendly as well as possessing positive attributes of both ingredients i.e., antimicrobial, haemostatic, high absorption etc. To enhance wound dressing interaction with the damaged skin and its substructure, wound dressings made from nanofibers have come to the forefront of research and development. These submicron fibres maybe produced by variety of techniques, but electrospinning is the most efficient and cost-effective method of production. An added advantage is skin regeneration because of the structural similarity to extracellular matrix of the skin and their ability to promote cell growth and proliferation whilst maintaining antimicrobial features as well as being able to deliver bioactive molecules [88].

Conclusion

In conclusion, chitosan, the deacetylated form of chitin is inherently antimicrobial, haemostatic, and biocompatible, and is widely believed to have good healing properties. Chitosan, unlike many other materials, is highly reactive due to its amino and hydroxyl groups carrying a positive charge at a pH below 6.5. Most natural materials including fibres, human skin, bone, hair, and microbes bear negative charges and are therefore potentially capable of interacting with chitosan. Currently, resistance to this substance has been observed through the production of chitosanases and modification of the cell envelope but reports are limited. This compound is unlike most antimicrobials in that it has other functional activities such as a natural haemostat, and it can be made into different structures with varying activities. In wound care, the compound can be made into fibres, powders and ointments and be combined with other substances to enhance activities. Going forward, this natural inherent antimicrobial substance may become a suitable alternative to be used to prevent or treat acute and chronic wounds.

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