Review Article

Chitosan Biopolymer and its Anti-biofilm Activity

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Received: 20-06-2020; **Revised:** 24-08-2020; **Accepted:** 30-08-2020. **DOI:** 10.47583/ijpsrr.2020.v64i01.009

ABSTRACT

Biofilm formation leads to significant industrial economic losses and serious health problems. Most of these health problems such as gastrointestinal, eye, ear, dental, etc. are permanent hospital infections associated with medical devices such as catheters, heart valves, and prosthetic joints. It is known today that it is not possible to eliminate biofilm with classical methods. Therefore, in order to protect surfaces against biofilm, there has been an increasing expectation of developing new alternative strategies by limiting the use of toxic antimicrobial agents. Chitosan is a natural biopolymer obtained plenty from resources in nature. It has some properties such as non-toxic to living organisms, biological degradability, biocompatibility and many forms high availability, a renewable resource and environmentally friendly, antibacterial, antifungal and antiviral activities. Chitosan will be presented as an approach, alternative to conventional anti-biofilm strategies, in order to prevent microbial biofilm formation in this review.

Keywords: Anti-biofilm, Biopolymer, Chitosan.

INTRODUCTION

Microorganisms universally attach to surfaces and develop biofilms. Biofilms are a form of life formed by microorganisms living in a gel layer in their polymeric structure by adhering to a surface.[1] The substances in the biofilm structure are attached to each other by extracellular matrix components. Water is present in bacterial cell capsules or is bound to exopolysaccharide (EPS), which is a high molecular weight polymer formed by the monosaccharides of microorganisms.[2] Microbial EPSs provide microorganisms the ability to colonize and bind to a surface by forming biofilm. They can also protect the microorganisms against osmotic stress, phage wastes, toxic compounds, and antibiotics.[3] A variety of environmental factors such as bacterial strain, surface properties, pH, nutrient content, and temperature are effective in the formation and development of biofilms.[4] Biofilms may contain a single or a large number of microorganisms. The most common are the genera *Pseudomonas*, *Enterobacter*, *Flavobacterium*, *Alcaligenes*, *Staphylococcus*, and *Bacillus*.[2]

Modern medicine is increasingly facing biofilms and biofilm-forming bacterial infections every day. Biofilms involve mainly a number of viable and lifeless surfaces such as catheters, contact lenses, prosthetic heart valves and pacemakers, intrauterine devices, and kidney stones in the human body. Increased use of interventional techniques and permanent medical devices in medical applications has also led to an increase in biofilm infections. Particularly in immunocompromised patients and those patients with a permanent medical device or catheter may acquire serious infections.[5] Biofilm cells are more resistant to antimicrobial agents than planktonic cells and have a barrier to prevent or reduce contact with antimicrobial agents[6] and this complicates bacterial control methods. The removal of pathogenic microorganisms by chemicals or physical methods reduces the risk of infection. However, these classical methods are expensive and require a heavy workload. In recent years, problems with the use of antimicrobial agents have led to the need for alternative new agents that do not produce unknown by-products. This approach has limited the use of toxic antimicrobial agents that tended to disrupt the ecological balance, making it possible to investigate the possible source of novel and effective agents. For this purpose, chitosan, which is a biopolymer that can be obtained in large amounts from sources found in nature, has antibacterial, antibiotic, antifungal, and antiviral activity in terms of its non-toxicity against organisms, its biodegradability, its biocompatibility, its chemical and physical properties, and its superior properties compared to other biopolymers. Thus, in recent years, as a new functional material with high utilization potential in various fields, it has found use in many different sectors such as food, cosmetics, agriculture, medicine, pharmacy, paper, and textile.[7] Here, the use of chitosan in the treatment of clinically related microbial biofilm diseases will be examined as an alternative anti-biofilm agent to classical methods.

Definition of chitosan and its applications

Chitosan is a biopolymer obtained by the deacetylation of chitin, which is found in the skeletal structure of shellfish (Crustacea), in the skeletal structure of insects, and in the cell walls of fungi, and is a natural compound.[8] Now, chitin and chitosan are used in various fields such as medicine, food, agriculture, cosmetics, pharmacy, wastewater treatment, and textile. In the textile sector, it is used for many purposes such as providing antimicrobial properties

to products, giving impermeability to woolen fabrics, reducing the amount of salt in reactive coloring, giving coloring matter with acid dyes, giving antistatic properties, and using it as deodorant material. In addition, there are also various antimicrobial fibers such as Crabyon (chitosan and viscose mixture) and chitosan (chitosan and polynoic fiber blend) produced from the mixture of chitosan and other fibers. The use of chitosan in medical textiles is very common. Chitosan is widely used for wound treatment, especially for tissue integrity. In addition, many purposes such as artificial blood vessels, medical artificial skin, surgical sutures, contact lens production, controlled drug release, dressings, bandages, tumor inhibitors, cholesterol control (fat binding), antibacterial, antifungal, and haemostatic effects.[9] Berger et al.[10] investigated the use of chitosan hydrogels in the biomedical field. While covalently bonded hydrogels can be used in making implants and bandages, ionic bonded hydrogels are used especially in drug release systems. Chitosan has no detrimental effect due to its non-toxic nature, its green and biodegradable properties, its in-vivo testing, and its degradation into non-toxic products (amino sugars). Chitosan has been shown to play a very active role in accelerating wound healing. Because of this feature, it is a promising treatment for patients in terms of rapid recovery of body wounds, especially in diabetic patients. The reason for the active role of chitosan in wound treatments is based on the formation of polyelectrolyte complexes between negatively charged heparin and positively charged chitosan. Heparin is an anticoagulant polysaccharide that promotes tissue development by increasing the complexity of cell growth factors. Some investigators applied water-soluble chitosan to the wounded backs of rabbits and observed that the wound healed rapidly.[11] Muzzarelli et al.[12] obtained 5-methylpyrrolidinone, which is a chitosan derivative, and found that when applied on the wound, it transformed into an oligomer structure under the influence of the lysozyme enzyme.

Controlled drug release is important. The biocompatibility of chitosan has led to the use of many drugs as a matrix and various drugs have been inserted into the chitosan matrix (film, microcapsule, coated tablet, etc.). The free amino group of the chitosan allows the interaction of chitosan with negatively charged drugs, polymers, and bioactive molecules because of its cationic character. The biggest advantage of using it in a drug release system is that it can form gels and can be found in various forms such as copolymer. Antacid and antiulcer activity due to chitosan to prevent irritation of the stomach makes it an ideal material. It is also used in orthopedic and periodontal applications due to its ability to produce composites with calcium-derived materials with functional groups of chitosan.[13]

In addition to all the above-mentioned areas of chitosan use, it can be used in many other areas.[13] This is thanks to its properties such as its biocompatibility, its ability to allow cellular adhesion and proliferation, to form a composite with other materials, and its antimicrobial activity. The antimicrobial activity is mainly due to the polycationic structure of chitosan. Although the antimicrobial activity mechanism of chitosan has not been completely proven, several theories have been proposed. The first is the electrostatic interaction between the positively charged part of chitosan and the negatively charged parts of many fungi and bacteria on the cell surface. This interaction leads to the infiltration of intracellular components such as electrolytes, UV (ultraviolet) adsorbent, proteins, amino acids, glucose, and lactate dehydrogenase by altering the cell surface and cell permeability. It inhibits the normal metabolism of microorganisms and leads to the death of these cells.[14]

Another theory is that some fungi and bacteria inhibit mRNA and protein synthesis due to the interaction between DNA and chitosan. Chitosan must have a molecular weight low enough for the microorganisms to penetrate into the cell.[15] The antimicrobial activity of chitosan is influenced by a variety of factors, such as cations and polyanions, chitosan concentration, molecular weight, temperature and Ph, degree of deacetylation, chain length, type of microorganism tested, the type (basic or derivative) of chitosan, the substrate and / or nutritional composition, and environmental conditions.[16]

Several approaches have been proposed to prevent biomaterial-related infections, and infection-resistant biomaterials have been produced according to these approaches. Nanoparticles are generally preferred for surface coating to prevent adhesion or to produce antimicrobial effects. Biofilm formation with nanoparticles is prevented by designing the surfaces to prevent limited bacterial colonization.[17]

Bacterial attachment and biofilm formation is inhibited by coating, impregnation, or embossing nanomaterial surfaces by nano-functionalization.[17] Thus, nanoparticles have emerged as a new field of struggle against biofilms.[18] There are several studies on the antimicrobial and antibiotic activities of silver nanoparticles, and it has been reported that such nanomaterials can be used in the production of medical biomaterials. Gubta et al.[19] reported that silver nanoparticles prepared with *Psidium guajava* leaves (AgNPs) have broad-spectrum antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas diminuta*, *Mycobacterium smegmatis*, *Fusarium oxysporum* and *Candida albicans* and they could have potential applications in the biomedical field. The use of chitosan nanoparticles is important because of their benefits in controlled drug release systems. Controlled release can be carried out with chitosan gels, tablets, microspheres, and microcapsules.[20] Natural and synthetic polymers can be used in the preparation of nanoparticles. The main natural polymers used in the preparation of nanoparticles are lipids, proteins (such as albumin, gelatin, collagen), and polysaccharides (such as alginate, chitosan, and dextran). In recent years, the use of polymeric carriers

in the formulations of therapeutic carrier systems is preferred due to their superiority. Nanoparticles are widely used in particulate carrier systems prepared with these biodegradable polymers. Chitosan, one of the natural polymers used in the preparation of nanoparticles, has been extensively researched on its use potential due being biocompatible, biodegradable in biological systems, having low toxicity, absorption-enhancing property on epithelial surfaces, and its potential as a mucosal immunity and gene delivery system.[21] Chitosan, a natural biopolymer, has also been currently used as a stabilizing agent for the preparation of nanoparticles in recent years. Chitosan-stabilized nanoparticles can be easily integrated into relevant systems for drug, biomedical, and biosensor applications. Maki and Mermel[22] used chitosan as a polymer-based preservative agent to stabilize metal nanoparticles.

Anti-biofilm effect of chitosan

The virulence and pathogenicity of microorganisms is usually enhanced when a biofilm is formed. Therefore, new strategies are needed to control biofilm formation and development. Many pathogenic microorganisms produce biofilm and these biofilms cause additional problems when designing new anti-microbial agents.[23]

The elimination of biofilm often causes serious environmental damage as well as the emergence of resistant microorganisms and high concentrations of disinfectant or antibiotic use. For this reason, public health concerns, as well as the economic loss associated with biofilm formation, require the development of new biofilm-resistant systems. Therefore, alternative therapeutic and prophylactic approaches are needed to compete against biofilm-forming microorganisms.[24] The most important of these approaches are anti-biofilm agents. These anti-biofilm agents are classified as quorum sensing inhibitors, bacteriophages, enzymes, and surfactants.[25]

In a study on surface modification, the surface of the modified polyhydroxialkaonate was loaded with disperse in B, which is an antibiotic agent, and it was determined that it inhibited the attachment and aggregation of *S. epidermidis*.[26] In another study, the surface of polylactide medical material was modified by binding it to quaternary poly (2- (dimethylamino) ethyl methacrylate) with covalent modification, which provided biomaterial retention and antibiotic properties.[27] However, Domingues et al.[28] emphasized that macrophage phagocytosis should be taken into account when developing an infection-resistant biomaterial and suggested that further studies on biomaterial surface modification should be directed towards increasing phagocytosis.

The prevention of microorganism-microorganism interactions, which is the basis for biofilm formation, has emerged as a new field that allows the identification of natural anti-biofilm compounds (eg, polysaccharides released by natural bioprotective agents and mature biofilms, etc.). For example, *P. aeruginosa* secretes cis-2-decanoic acid, which breaks down the biofilm matrix molecules of biofilms formed by a wide variety of bacteria and yeasts.[29] Some studies have shown that polysaccharides extracted from mature biofilms result in the prevention of biofilms generated by commensal and antagonist bacterial species. Similarly, Rendueles et al.[30] have shown in vitro that fluids extracted from *E. coli*, which form biofilm, inhibit the adhesion of *S. aureus* and thus reduce biofilm formation.

This activity has been reported to be caused by a new, high molecular weight polysaccharide, Ec300p, which is not found in planktonic *E. coli* cultures. Agents that exhibit detergent-like activity against matrix components, which can degrade or chelate them, have been proposed as a promising treatment in biofilm infections.[31] Petrova and Sauer[32] reported on more than one enzyme capable of reducing biofilm components. For example, microyaline glycosyl hydrolases capable of disrupting matrix components are presented as a potential alternative treatment for biofilm removal or prevention.

Natural compounds derived from plant extracts, mammals, insects, and marine organisms have potential advantages due to their low price, abundance, and safety. These advantages enable them to be applied as natural biofilm inhibitors in biofilm infections due to other potent anti-infective properties such as anti-inflammatory and wound healing activity.[33] To date, many other natural anti-biofilm agents have been identified, including garlic, ginseng, chitosan, berberine, curcumin, flavonoids[34] and *Vibirnum opulus*. [35] In addition to alternative topical therapies, organic acids such as acetic, citric, oxalic, and trichloroacetic acids have been proposed to prevent biofilm formation in wound infections. Organic acids can effectively kill *P. aeruginosa* strain, and acetic acid has been shown to destroy *P. aeruginosa* and *S. aureus* biofilms respectively at a rate of 0.5% and 1%, in vitro.[36] Chitosan show the antimicrobial activity on the biofilm-forming microorganisms such as *C. albicans*, *S. aureus*, *S. epidermidis*, *Acinetobacter baumannii*, *Streptococcus mutans*, *P. aeruginosa*.[37] Costa et al.[38] investigated the effect of chitosan on *S. mutans* biofilm formation and reported that it inhibited biofilm formation and had a significant reduction (94%) on mature biofilm structures.

Pu et al.[39] used Minimal Inhibitor Concentration (MIC), (XTT) reduction test [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5 carboxanilide], and Scanning Electron Microscope (SEM) to investigate the effect of chitosan on *C. albicans* biofilms. In their analysis, they reported that chitosan had a significant inhibitory effect on both planktonic cells and *C. albicans* biofilms depending on the dose. In addition, a morphological defect on the biofilm formation of the chitosan treated group seemed by SEM analysis. Carlson et al.[40] studied biofilm formation on chitosan coated surfaces of biofilm-forming bacteria such as *S. epidermidis*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*, and showed a reduction in

the biofilm formation of these bacteria on chitosan coated surfaces. Martinez et al.[41] investigated the effect of chitosan on biofilms produced by the pathogenic fungus *Cryptococcus neoformans* and showed that chitosan significantly reduced both the metabolic activity and cell viability of *C. neoformans* biofilms. In addition, using confocal and SEM, they also demonstrated the damage of chitosan to biofilms and fungal cells and suggested chitosan as an alternative option to prevent or treat fungal biofilms in cryptococcal biofilms in established medical devices.

CONCLUSION

Consequently, effective anti-biofilm agents are an urgent need in the medical industry. Therefore, chitosan may be used as alternative an antibiofilm agent against the treatment of clinic-associated biofilm diseases.

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Source of Support: None declared.

Conflict of Interest: None declared.