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Antibacterial Effect of Chitosan Against *Streptococcus mutans*: An Alternative for Mouthrinse on Dental Caries Control and Prevention?

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Abstract

Objective: This study aimed to review the literature on chitosan antibacterial activity against *Streptococcus mutan*s, assessing this biopolymer's potential for caries control and prevention. **Materials and Methods:** A search for studies in the "Pubmed" database was carried out, using the terms "Chitosan and *Streptococcus mutans*." Eighty-eight articles were found, and no filter was used in the search. **Results:** In general, chitosan inhibited the adhesion and biofilm of Streptococcus mutans, tested in different concentrations, exposure time, molecular weight, and pH. Therefore, chitosan has antibacterial activity against *Streptococcus mutans*. **Conclusion:** Although it can demonstrate the potential for use as a component of an oral product, more studies need to be conducted and analyzed to prove its ability to prevent and control dental caries.

Keywords: Chitosan; Antimicrobial agents; *Streptococcus mutans*.

Introduction

The biofilm formed on the dental structure is associated with developing oral diseases such as caries, gingivitis, and periodontitis.[1,2] *Streptococcus mutans* (*S. mutans*) is the critical microorganism that contributes to caries development, despite not being a primary colonizer, and has a recognized role in the disease initiation.[3,4] Its ability to use diet sucrose to produce exopolysaccharides may favor other bacteria's adhesion (secondary colonizers), making *S. mutans* a significant contributor to the biofilm formation.[3,5,6]

The mechanical control of dental biofilm through frequent and adequate brushing is one of the primary mechanisms for preventing caries disease.[1,7] In cases of difficulty in hygiene and maintenance of motivation for good teeth cleaning, chemical agents in mouthrinse solutions can be an adjunct to the mechanical method to control microorganisms.[1,8] Among the active compounds most used as chemical agents is chlorhexidine; however, its daily use has undesirable side effects: dental and tongue staining, loss of taste, and burning sensation in the oral mucosa.[9,10] Also, the extensive and unrestricted use of chemical agents can lead to side effects and microbial resistance.[11]

Thus, alternative products, natural or biopolymer-containing, have been investigated mainly due to their antimicrobial and biocompatibility properties.[12] The main objective of bioproducts development is to intervene in the caries process, acting in disease prevention, control, and development through antimicrobial activity. The bioproducts can spread within the biofilm and/or effect demineralization and remineralization of tooth enamel.[13] Due to this fact, nanoscale products, such as nanoparticles, can not only be used as biological carriers within the biofilm but allow continuous and controlled release of the therapeutic agent, keeping it at the desired levels at the site.[14] Among the naturally available biopolymers, chitosan is one of the most investigated for nanoparticles' preparation due to its biodegradation properties, absence of toxicity, biocompatibility, and antimicrobial activity.[12,15,16]

Chitosan is obtained through chitin's deacetylation, which is a linear biopolymer and is present in most crustaceans' exoskeletons. It is considered the second largest natural polymer available in nature. With the partial deacetylation of chitin, it becomes soluble in aqueous acidic conditions and is called chitosan[17]. Due to the amine groups (NH_2), chitosan has a cationic character, and at low pH, they are protonated in amino (NH^{3+}). They can interact with negatively charged components, such as proteins, anionic polysaccharides, and phospholipids.[15] Although studies have shown that chitosan has antimicrobial activity against *S. mutans,* [3,12-13] there is variation in this activity according to chitosan chemical properties (degree of deacetylation, pH, molecular weight, among others). They could affect its action as a product for oral use to control and prevent dental caries.

Narrative (Rajoka *et al*. 2020)[18] and systematic (Cicciu *et al*. 2019)[19] reviews on the interaction of chitosan with microorganisms and their use in the field of dentistry were conducted. Rajoka *et al.* (2020)[18] reported the antimicrobial activity of chitosan against infectious disease pathogens in animals and its wide application in the food industry. Cicciu *et al.* (2019)[19] showed chitosan action in the remineralization

of dental tissues, reducing biofilm when incorporated in dental cement, and in the desensitizing effect when present in dentifrices. Thus, there is still little in the literature to compile scientific information on chitosan antibacterial activity against the main microorganism related to dental caries, *Streptococcus mutans*, investigating its potential effect to control and prevent this disease.

Thus, the present study aimed to conduct a narrative review of the literature on the antibacterial activity of chitosan against *Streptococcus mutans* evaluating its chemical and biological properties to investigate this biopolymer's potential helping to prevent and control dental caries.

Material and Methods

The study consists of a narrative review carried out by searching the Medline-Pubmed database. The search strategy was to use the keywords *"Chitosan* and *Streptococcus mutans"* in April 2020, published in international journals in English, without a filter for restricting the date. Inclusion criteria were clinical, review, or *in vitro* studies that addressed the mechanism of action and antibacterial activity of chitosan and studies that assessed the antibacterial effect of chitosan in the control of dental caries. The exclusion criteria were lack of relationship with chitosan antimicrobial activity or anti-caries effect, assessing demineralization and remineralization in tooth enamel.

Literature Review

Chitosan

Chitin is a linear biopolymer of N-acetyl glucosamine units linked by ß-(1-4) glycosidic union present in most crustaceans' exoskeleton. It is considered the second largest biopolymer available in nature, with an estimated production of 10^9 - 10^{11} tons per yearo.[17] When the degree of deacetylation of chitin reaches about 50% (depending on the polymer's origin), it becomes soluble in aqueous acidic conditions called chitosan.[15,17,20] Chitosan is considered a copolymer with 2-amino-2-deoxy-D-glucose and 2-acetamide-2-deoxy-D-glucose units joined by β (1-4)-type glycosidic bonds.[21] The process of converting chitin to chitosan must be carried out correctly to ensure chitosan production with high quality and purity, free from contaminants, such as proteins, endotoxins, and toxic metals.[22,23] The chitin deacetylation process takes place in a basic medium with 40% sodium hydroxide solution, at a temperature of 120°C, for 3 hours, resulting in removing the chitin's acetyl groups chain and in free amino groups (NH_2). This chitin deacetylation process occurs partially. A structurally 100% deacetylated homopolymer cannot be obtained, making chitosan soluble only in weak acids, such as acetic acid and formic acid.[5]

While chitin is considered an abundant and undesirable polysaccharide, since it is related to environmental contamination, chitosan has shown excellent biocompatibility, low or no toxicity in humans and animals, bioactivity, biodegradability, the possibility of the reaction of the deacetylated amino group other molecules, selective permeability, antimicrobial activity, ability to form gel and film, and chelating action.[24] These properties make chitosan a highly suitable polymer in several science areas: industrial, textile, agricultural, medical, pharmaceutical and dental.[15] Cicciù *et al*. (2019)[19] carried out a systematic review to verify chitosan use in different areas of dentistry. Twelve studies of clinical trials and randomized clinical trials were evaluated, four studies using chitosan as an analgesic in oral surgery, three studies of use as a mouthrinse, three studies of use in toothpaste, 1 study of use in restorative material, and 1 study of use as a canal irrigation solution. The results showed that chitosan is a safe compound for use, with many favorable properties for oral surgery applications, reducing the clinical signs of inflammation and bone regeneration and bone repair capacity.

The chitosan obtained can be characterized according to the degree of deacetylation and molar mass since these characteristics can influence the degradation and hydrolysis of the polysaccharide.[22,23] According to the mean deacetylation level, chitosan can be obtained with different physical and chemical properties concerning solubility, pKa, and viscosity parameters.[24] Different deacetylation degrees influence the chitosan molecule conformation in an aqueous solution, leading to a variation in the solution viscosity.[25,26] Thus, a high degree of deacetylation leads to an expansion of the polymer chain, as repulsion occurs between the molecules' charges, increasing the viscosity. The opposite happens when the degree of deacetylation is low, as the charge density decreases and the polymer becomes more coiled.[25]

Chitosan is a weak base, and its pKa ranges from 6.2 to 7.0, being insoluble in neutral and alkaline pH. As the chitosan amino groups are protonated, this biopolymer forms salts with organic and inorganic acids resulting in a positively charged soluble polysaccharide. The salts formed are soluble in water depending on the deacetylation degree and solution pH. If the deacetylation degree of the chitosan salts is low (< 40%), they are soluble at pH up to 9, while with a high deacetylation degree (> 85%), they are soluble at pH up to 6.5.[26] Thus, it may be challenging to obtain chitosan with a high deacetylation degree because, as the process increases, the polymer degradation also increases.[22]

For clinical use with a more significant therapeutic and antimicrobial effect, chitosan can be synthesized in microspheres or nanoparticles. The synthesis of nanoparticles has numerous therapeutic applications, mainly for parenteral or oral administration. The objective is that the nanoparticles increase the polymer bioavailability since they are small in size and have high surface energy.[27] Most advanced drug-

carrying nanoparticle systems are produced using synthetic or natural polymers or a combination of both, with chitosan being one of the most used biopolymers in the synthesis of nanoparticles.[28] Chitosan nanoparticles can be formed by various processes, e.g., electrospray, emulsification, solvent diffusion, microemulsion, ionic gelation.[29] Among these, ionic gelation is one of the most widely used methods. It is fast and straightforward, using crosslinking agents, such as sodium tripolyphosphate. This salt is non-toxic and has a fast gelation capacity, and electrostatically interacts with cationic chitosan.[16,30] Electrostatic interactions occur through inter and intramolecular bonds between TPP phosphates and the amino groups of chitosan. After mixing these two phases, the formation of nanoparticles immediately occurs.[16]

Antimicrobial activity of chitosan against *Streptococcus mutans*

Chitosan exhibits antimicrobial activity against a range of oral pathogens such as the fungus *Candida albicans* (*C. albicans*) and the bacteria *Streptococcus aureus* (*S. aureus*) and *S. mutans,*[15,31,32] being considered a suitable drug delivery system in the oral cavity. The antibacterial activity of chitosan may be due to electrostatic interactions between the amino groups of the molecule and the anionic groups of bacterial cell walls, derived from residues of carboxylic acids and phospholipids.[33] Among the microorganisms that can be inhibited by chitosan, *S. mutans* was highlighted in some studies.[6,12,13,21,34,35,36,37]

It is already known that bacteria adhesion on the tooth surface can lead to biofilm formation. Busscher *et al.* (2008),[34] aiming to determine the treatment effects of chitosan films on adhesion and bacterial growth, observed that the number and growth of *S. mutans* and biofilm viability were significantly reduced in 0.1% chitosan-treated saliva. Therefore, the viability of the biopolymer-treated biofilm was three times lower compared to the control group. The authors concluded that chitosan is a promising antimicrobial for use in oral health. Likewise, Costa *et al.* (2013),[3] aiming to assess the potential use of high and low molecular weight chitosan as potential oral antimicrobials, found that chitosan can also inhibit *S. mutans* adhesion. In this study, high molecular weight chitosan at concentrations of 1 and 2 mg/mL showed no significant differences in *S. mutans* adhesion inhibition at 30s and 90s exposure times, reaching almost 100% of bacterial growth. On the other hand, for low molecular weight chitosan (2 and 4 mg/mL), there was a significant difference between 30s and 90s exposure times. The longer time showed more significant *S. mutans* inhibition between 94 and 99% inhibition. The authors concluded that chitosan could minimize *S. mutans* colonization in the oral cavity, with high molecular weight showing more significant inhibition of the microorganism than low molecular weight.

Hayashi *et al.* (2007),[38] aiming to assess chitosan activity in chewing gum in suppressing oral cariogenic bacteria's growth, revealed that the number of oral bacteria decreased significantly in the chitosan group. Especially for *S. mutans*, the number of bacteria in the chitosan group was the same before and after using the gum, even one hour later. Furthermore, they stated that chitosan action in microorganisms should not be explained by the cell wall's biopolymer performance since the antimicrobial activity can depend on both chitosan (for example, deacetylation degree and molecular weight) and microorganisms properties.

Costa *et al.* (2012),[39] aiming to evaluate the mechanism of action and the antimicrobial capacity of chitosan as an alternative to traditional antimicrobials in the treatment of oral infections, showed that chitosan in concentrations of 3.0 mg/mL and 5.0 mg/mL (high and low molecular weight, respectively) showed antimicrobial activity against anaerobic bacteria: *Prevotella buccae* (*P. buccae*), *Tannarella forsythensis* (*T. forsythensis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *S. mutans, Porphyromonas gingivalis* (*P. gingivalis*) tested in minimal inhibitory concentration (MIC), concluding that chitosan has rapid and effective bactericidal activity against the pathogens tested. In a later study, Costa *et al.* (2014),[12] aiming to investigate the effect of experimental chitosan rinses on biofilm formation, used a high and low molecular weight chitosan in a concentration of 0.4% together with the microorganisms *C. albicans, Enterococcus faecium* (*E. faecium*), *Prevotella intermedia* (*P. intermedia*), *Lactobacillus acidophilus* (*L. acidophilus*), and *S. mutans*. The authors found that chitosan-containing mouthrinse could interfere with microorganism adhesion, biofilm formation, and mature biofilm dissolution. Also, compared to the two commercially tested types of mouthrinse: one essential oils-based and another chlorhexidine-based, the chitosan-based mouthrinse showed significantly superior antimicrobial activity in all the study essays.

Likewise, Chen e Chung (2012)[40] conducted an *in vivo* and *in vitro* study to analyze the antibacterial effect of water-soluble chitosan (500 μg/mL) and as a mouthrinse solution. In the *in vivo* study, the volunteers used the test solutions, and the colonies were counted after 18 h of incubation. In the *in vitro* study, six types of mixed bacteria solution were tested, and after 18h, the colonies were also counted. Both studies showed that the chitosan solution as a mouthrinse, effectively reduced oral bacteria (*S. mutans* and *Lactobacillus brevis*). The antibacterial activity was 95.3% at pH 5.0 and 96.2% at 37°C, against *S. mutans*. The minimum bactericidal concentration found for *S. mutans* was 400 µg/mL. The 5 s solution with this microorganism promoted a 99.8% reduction in bacterial growth, reaching 99.9% in the 20 s-contact, at pH 7.0. n view of the satisfactory results, the authors concluded that water-soluble chitosan could be added later in mouthrinse solutions.

To assess the antibacterial effect of chitosan nanoparticles on the 24-hour biofilm of *S. mutans,* Chávez de Paz *et al*. (2011)[13] used chitosan with different molecular weights and deacetylation degrees to produce the nanoparticles using the ionic gelation method. The results showed that different deacetylation degrees of chitosan did not affect the nanoparticles' antimicrobial activity but that the molecular weight had interference. The low molecular weight chitosan nanoparticles showed high antimicrobial activity at neutral pH, homogeneously interfering with *S. mutans* membrane's integrity at varying depths of the biofilm. Thus, the authors stated that this nanoparticle system could release chitosan more uniformly over the biofilm at neutral pH. In the study by Neilands *et al*. (2011),[28] the acid tolerance response of *S. mutans* to chitosan nanoparticles was evaluated. The cells were initially exposed to pH 5.5 for 2 h and then to pH 3.5 for 30 minutes in the experiment. After adding chitosan nanoparticles in the pH 5.5 exposure, the number of dead cells was noted to increase in the immersion pH of 3.5. According to the authors, the chitosan nanoparticles interfered in the biosynthesis of fatty acids and protein synthesis in response to stress, which are two crucial factors in inducing the response to the acid tolerance of *S. mutans*. Chitosan nanoparticles were shown to have no direct effect on the cell membrane since almost all cells at pH 5.5 were viable with intact membranes (94.5%). However, there was a very low cell viability (1.1%) when exposed to pH 3.5. Thus, they concluded that chitosan nanoparticles inhibited the acid tolerance response of *S. mutans*.

The main property of chitosan nanoparticles against microorganisms, which can make it more interesting clinically than the pure chitosan solution, is the nanometric dimensions that may have greater absorption capacity and adhesion to the dental structure. Also, they penetrate more easily inside the biofilm, allowing a continuous and controlled release.[41,42]

Results

Between the years 1991 to 2020, 88 scientific articles were found, including 16 articles that evaluated the mechanisms of chitosan action and factors that interfered in its antimicrobial activity to prevent and control caries disease. Table 1 presents the included studies and main results obtained, discussed in this review.

The scientific literature addressed in this review revealed the mechanism of action proposed for chitosan,[43,44] demonstrating its potential for oral products in preventing and controlling dental caries. Of the 16 articles addressed in the present study, eleven focused on the antimicrobial effect of chitosan on dental biofilm,[3,12,13,19,34,36,38,39,41,42,45] and three studies revealed its anti-caries effect through remineralization of dental enamel.[46,47,48] Therefore, all studies showed that it could be used in the oral cavity to prevent dental caries.

Table 1. Studies included in chitosan's antimicrobial activity study.

Authors	Year	Country	Methodology	Main results found
Zheng e Zhu	2003	China	Evaluation of MIC and antimicrobial mechanism.	High molecular weight chitosan showed a better antibacterial effect on gram-positive bacteria (*S. aureus*), explained by the formation of a membrane that would prevent the adsorption of nutrients by the bacteria, causing cell death more easily.
Hayashi *et al*.	2007	Japan	Evaluation of the effect of chitosan-containing chewing gum on inhibiting oral bacteria growth through sample culture analysis.	The number of oral bacteria decreased significantly in the chitosan group. In particular, *S. mutans* was maintained at a level of about 20% compared to before chewing gum, even an hour later.
Busscher *et al*.	2008	Netherlands	Initial adhesion test	The number, growth, and viability of *S. mutans* biofilm were significantly reduced by 0.1% chitosan-treated saliva.
Takahashi *et al*.	2008	Japan	Evaluation of antimicrobial activity through conductivity testing and evaluation of the number of microorganisms (CFU) and minimum inhibitory concentration (MIC).	A more significant chitosan bactericidal effect was observed in gram-positive bacteria (such as S. mutans) than in gram-negative bacteria. Increasing the degree of deacetylation of chitosan was also noted to increase the antibacterial activity (S. aureus).
Arnaud, Neto & Diniz	2010	Brazil	Evaluation of chitosan application interference in enamel demineralization through microhardness, chemical analysis of phosphorus loss, and optical coherence tomography image.	The chitosan solution's best performance was found at concentrations of 2.5 mg/mL and 5.0 mg/mL, with exposure times of 60 s and 90 s to the enamel.
Chávez de Paz *et al*.	2011	Sweden	Evaluation of the antimicrobial effect of chitosan nanoparticles with different molecular weights and deacetylation degrees by laser scanning confocal microscopy.	Different deacetylation degrees of chitosan did not affect the antibacterial activity of chitosan nanoparticles against S. mutans.

Costa *et al*. Costa *et al*. Costa *et al*.	2012 2013 2014	Portugal Portugal Portugal	Evaluation of the antimicrobial activity of chitosan by MIC, inhibition curve, cell membrane integrity, and flocculation assay. Evaluation of the antimicrobial potential of high and low molecular weight chitosan in adhesion and biofilm assays. Evaluation of the effect of incorporating chitosan in a mouthrinse on the formation of oral microorganisms biofilm on microbial adherence, the formation of the initial and mature biofilm.	Chitosan at concentrations of 3.0 mg/mL and 5,0 mg/mL (high and low molecular weight, respectively) showed antimicrobial activity against anaerobic bacteria: *Prevotella buccae, Tannarella forsythensis, Aggregatibacter actinomycetemcomitans, S. mutans, and Porphyromonas gingivalis*. Chitosan was able to interfere in *S. mutans* adhesion and formation of primary biofilm. It was also able to inhibit biofilm formed by two microorganisms and showed the capacity to inactivate mature biofilm, leading to reduced microorganisms in the biofilm (94%). The chitosan-containing mouthrinse was able to interfere in all microorganisms' adherence, the formation of initial and mature biofilm, and demonstrated superior activity to the commercial mouthrinse.
Chang *et al*.	2015	Taiwan	Evaluation of the influence of pH, molecular weight, and temperature on the antimicrobial chitosan activity and the number of microorganisms (CFU).	Chitosan antimicrobial activity increased as the pH decreased so that at neutral pH (pH 7.0), the high molecular weight chitosan solution decreased the antimicrobial activity.
Aliasghari *et al*.	2016	Iran	Evaluation of chitosan antimicrobial effect by inhibition zone, determination of MIC and MBC, and anti-adhesion effect	More significant inhibition zones were observed for chitosan and chitosan nanoparticles with a higher concentration (5 mg/mL). The most significant effect in reducing adherence was found for *S. mutans* with a reduction of 92.5% for chitosan and 93.4% for chitosan nanoparticles, at a chitosan concentration of 5 mg/mL. Thus, the decreased concentration was associated with increased bacterial growth.
Zhang *et al*.	2018	England	Evaluation of chitosan application in the remineralization of white spot lesions using microhardness and scanning electron microscopy.	Chitosan allowed remineralizing the demineralized surface through the interaction between positively charged nitrogenous species and demineralized enamel prisms.
Abedian *et al*.	2019	Iran	Analysis of the antibacterial and anti-biofilm properties of low and high molecular weight chitosan through the assessment of MIC and MBC	High molecular weight chitosan (0.019 mg/mL) was responsible for a 50% decrease in S. mutans. The low molecular weight chitosan solution required a higher concentration (0.039 mg/mL to 0.078 mg/mL) to inhibit the same percentage of S. mutans. The authors conclude that high molecular weight chitosan promoted a more significant antibacterial effect than low molecular weight.
Cicciù, Fiorillo & Cervino	2019	Italia	Evaluation of the use of chitosan in a literature review study	Chitosan has the potential to be used as a component of dental caries prevention products.
Ikono *et al*.	2019	Indonesia	Evaluation of inhibition ability of chitosan nanoparticles against biofilm by cell viability analysis	Cell viability of the analyzed species (*S. mutans* and *C. albicans*) decreased with the incubation times of 3 h and 18 h, following the increase in chitosan nanoparticles' concentration. The species' biofilm showed a reduction trend only after 18 h, also associated with the increase in the nanoparticle concentration.
Zhang *et al*.	2019	England	Efficacy test of *in vitro* remineralization of the chitosan-bioglass complex in artificial lesions of white spots by Knoop microhardness and scanning electron microscopy	Chitosan stabilized amorphous calcium phosphate and induced the complex's transformation into hydroxyapatite, enhancing its ability to benefit remineralization in deeper regions of enamel caries lesion.

Discussion

Some studies[3,12,28,34,37] has reported the antibacterial activity of chitosan solutions against *S. mutans*. It is relevant to analyze how this biopolymer exerts its antibacterial effect against the most common caries disease-related pathogen to evaluate the possibility of inhibition of this microorganism. It is also relevant to evaluate the effect on the demineralization and remineralization of dental structures, aiming to incorporation in products for oral use. Thus, it can be another antimicrobial alternative to aid in caries control and prevention.

Since the biofilm structures represent an anchorage and protection to the composing microorganisms, its reduction or elimination is difficult and challenging for all antimicrobials.[3] *S. mutans* is a gram-positive bacterium with peptidoglycans and teichoic acid in its cell wall, a polyanionic polymer responsible for the bacterium's structural stability wall.[15] The mechanism of chitosan antibacterial action against *S. mutans,* mentioned by some authors, would be by chitosan interaction (polycationic) with the polyanions of teichoic acid on the wall of gram-positive bacteria via electrostatic interactions.[13,15,18,49] Due to competition for the amine group's positive charge, chitosan is known to be responsible for reducing biofilm. It occurs as it interrupts the electrostatic bonds between the biofilm bacteria and the dental surface, as found in the study by Chávez de Paz *et al.* (2011),[13] in which there was homogeneous interference in the integrity of *S. mutans* at varying depths of the biofilm formed by this microorganism.

Also, another mechanism of chitosan antibacterial action would be the action on the bacterial plasma membrane.[5,15,43] As it has a cationic character due to the amino groups (NH^{3+}), chitosan can adhere to negatively charged surfaces, such as phospholipids in the bacterial membrane, reducing the negative charge on cell surfaces. This action alters the balance between the plasma membrane's internal/external concentration, increasing the plasma membrane permeability, causing loss of cell content and, consequently, cell death.[31] Additionally, chitosan can penetrate the bacterial cell wall and bind to DNA, inhibiting mRNA synthesis, and affecting the production of essential proteins and enzymes.[13,15] A chitosan chelating effect has also been reported, interfering in the bacteria synthesis of fatty acids and protein, which shows another form of antimicrobial action of this compound.[28]

Chitosan antibacterial effect is not only related to its mechanism of action against bacteria. In addition to the microbial factor, other factors such as the physical state and intrinsic properties of chitosan can interfere with its antimicrobial activity.[15] In this context, the scientific literature reports that chitosan nanoparticles, due to their small size, have better action mechanisms against bacteria than chitosan solution. It is due to their greater absorption capacity and adhesion to the tooth structure, penetrating inside the biofilm due to the nanometric dimensions, allowing its continuous and controlled release.[13,31]

The effect of molecular weight on chitosan antimicrobial activity has been reported in some studies.[3,13,36,38] However, there is still controversy regarding chitosan molecular weight and the more significant inhibition of *S. mutans*. Hayashi *et al*. (2007)[38] and Chávez de Paz *et al*. (2011)[13] found that low molecular weight chitosan showed more significant activity against *S. mutans* than high molecular weight. Abedian *et al*. (2019)[36] observed that high molecular weight chitosan (with a concentration of 0.019 mg/mL) was responsible for a 50% decrease in *S. mutans*. Low molecular weight chitosan solution required a higher concentration (0.039 mg/mL to 0.078 mg/mL) to inhibit the same percentage of *S. mutans*. Thus, the authors concluded that high molecular weight chitosan promoted better antibacterial effects than low molecular weight. Likewise, Zheng and Zu (2003)[43] report that high molecular weight chitosan showed a better antibacterial effect on gram-positive bacteria (*S. aureus*) in their study. This fact is explained by forming a membrane that would prevent the adsorption of nutrients by bacteria, causing cell death more easily.

The degree of polymer deacetylation can also influence antimicrobial activity, closely associated with the density of the positive charge of chitosan.[5] Chitosan solutions with a higher degree of deacetylation have higher positive charges. Therefore, they are expected to have a more significant inhibitory effect against gram-positive and gram-negative bacteria.[5] Thus, Takahashi *et al*. (2008)[45] observed a greater chitosan bactericidal effect in gram-positive bacteria (such as *S. mutans*) than in gram-negative bacteria. These authors also observed that by increasing chitosan deacetylation degree, the antibacterial activity was increased, but it is worth mentioning that the microorganism investigated was *S. aureus*. Chávez de Paz *et al.* (2011)[13] found that different deacetylation degrees of chitosan did not affect chitosan nanoparticles' antibacterial activity against *S. mutans*. Therefore, based on the articles included in this review, there is still a variation on chitosan deacetylation degree and its antibacterial effect against *S. mutans*.

The chitosan solution's concentration and the time of exposure to bacteria were also related to antimicrobial activity.[18,39,46,49,50] Various chitosan concentrations have been tested in the literature (ranging from 0.1% to 2% and 0.1 mg/mL and 7 mg/mL),[3,12,28,34,36,37,39,41,42] so that there is no defined concentration of which would be the most suitable to act against *S. mutans*. In general, the higher the concentration and exposure time of chitosan, the greater the antibacterial activity against *S. mutans*.[13,39,51] Busscher *et al*. (2008)[34] showed 20 to 30% inhibition in the initial adherence of *S.*

mutans when in contact for 2 min with the 0.1% chitosan solution. Costa *et al.* (2012)[39] used higher concentrations of the biopolymer, ranging from 0.1 to 7 mg/mL, which showed 99.9% inhibition of the initial adherence of *S. mutans*. In the study by Ikono *et al.* (2019),[42] chitosan nanoparticles at concentrations of 15%, 30%, and 45% were tested for the biofilm inhibition capacity of *S. mutans* and *C. albicans* after 3- and 18-hour incubations. The viability of both species' cells decreased with both incubation times, following the increase in chitosan nanoparticles' concentration. In contrast, the biofilm showed a reduction trend only after 18h, associated with the increase in concentration.

In their study, Aliasghari *et al.* (2016)[41] evaluated the antimicrobial effects of chitosan and chitosan nanoparticles on the biofilm of *S. mutans, Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus sanguis* (*S. sanguis*), *Streptococcus salivarius* (*S. salivarius*) through measures of the diameter of the zones of inhibition, determination of the minimum inhibitory concentration and the effect on the reduction of the adhesion after incubation of 24 h. Among the concentrations of 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL, the largest zones of inhibition by chitosan and nanoparticles were observed with 5 mg/mL concentration. The greatest effect in reducing adherence was found for *S. mutans* with a reduction of 92.5% for chitosan and 93.4% for chitosan nanoparticles and a 5 mg/mL concentration. The authors concluded that the decrease in concentration is associated with increased bacterial growth. Although *S. mutans* was not tested, the study by Arnaud *et al.* (2010)[48] assessing the effect of chitosan on dental enamel after a cariogenic challenge with pH cycling showed that the release of minerals from the enamel was dependent on the biopolymer concentration and the exposure time used. The chitosan solution's best performance was found in the concentrations of 2.5 mg/mL and 5.0 mg/mL, with exposure times of 60 s and 90 s to the enamel. Finally, the chitosan solution's pH must also be considered when analyzing this compound's antimicrobial activity.

Chang *et al.* (2015)[44] analyzed chitosan antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* from solutions with different molecular weights and pH. The antimicrobial activity of chitosan was noted to increase as the pH decreased so that at neutral pH (pH 7.0), the chitosan solution with high molecular weight decreased the antimicrobial activity. At neutral pH, the low molecular weight solution increased the antimicrobial activity. The literature indicates it is more common to find chitosan presenting a more significant inhibitory effect against bacteria at a lower pH. Thus, the inhibitory activity may weaken with increasing pH.[52] A possible explanation for the lower chitosan bactericidal activity at a higher pH would be that there is a more significant amount of non-positively charged amine groups and that chitosan is less soluble under these conditions, thus reducing its effects.[53]

Interestingly, in addition to antibacterial activity, especially against *S. mutans*, chitosan can interfere in the enamel demineralization process. It interacts with the enamel surface by forming an acid penetration-resistant physical barrier, reducing demineralization of the tooth enamel.[47] Also, chitosan was found to have the potential to carry mineral ions in the deepest regions of enamel caries lesions, which explains its role in controlling and progressing caries.[47] Likewise, Zang *et al.* (2018)[52] also showed that chitosan improved the demineralized surface's remineralization effect through the interaction between positively charged nitrogenous species and demineralized enamel prisms. Furthermore, Zang *et al.* (2019)[53] found that chitosan can stabilize amorphous calcium phosphate and induce the transformation into hydroxyapatite, enhancing its ability to benefit remineralization in deeper regions of enamel caries lesions.

In the systematic review study by Cicciù *et al.* (2019),[24] the results showed that chitosan could be used as a component of products to prevent dental caries. In this context, despite the investigated studies being laboratory tests, this literature review showed that chitosan could inhibit and have an antibacterial effect against *S. mutans*, having the potential to help prevent and control caries disease.

Clinical studies still need to be conducted to evaluate chitosan effects in oral products in the oral environment. However, the studies reviewed in this work show the potential of incorporating chitosan into oral products for caries prevention and control. In the oral cavity, other factors can influence the performance of an antimicrobial compound, from the variety and the interaction of the countless microorganisms that colonize it, such as factors linked to the host (such as saliva, surfaces to be colonized, hygiene habits, and diet, among others). The effect of chitosan in multispecies biofilm and biocompatibility tests and the polymer's cytotoxic potential among the variation of the physical-chemical properties shown in the review still require further investigation conducting clinical trials aiming at formulating an oral use chitosan-based product.

Conclusions

Based on the studies analyzed in this narrative review, chitosan had an antibacterial effect against *S. mutans* in the inhibition of adherence and biofilm, demonstrating the potential to be incorporated into a product for oral use to prevent and control caries disease. However, the review's methodological limits, requires to conducting *in vitro, ex vivo* and *in vivo* studies to prove the chitosan-exerted anti-caries effect, also enabling a systematic review of the literature that rigorously highlights this effect.

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