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ORIGINAL ARTICLE

Antimicrobial Activity of Colloidal Selenium Nanoparticles in Chitosan Solution against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*

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ABSTRACT

Objective: To investigate the antimicrobial activity of colloidal selenium nanoparticles in chitosan solution (Cts-Se-NPs) against *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Candida albicans*. **Material and Methods:** Cts-Se-NPs solution was prepared using a simple chemical reduction method. The MIC and MBC against *S. mutans*, *L. acidophilus*, and *C. albicans* were determined using the broth dilution assay. **Results:** The Cts-Se-NPs had remarkable antimicrobial activity against *S. mutans*, *L. acidophilus*, and *C. albicans*. The MIC values of the Cts-Se-NPs were lowest for *S. mutans* (0.068 mg/ml) compared to *L. acidophilus* (0.137 mg/ml), and *C. albicans* (0.274 mg/ml). The MBC values of the Cts-Se-NPs against the microorganisms after one, two, six, and 24 hours indicated that the concentration of 0.274 mg/ml of Cts-Se-NPs completely killed *S. mutans*, *L. acidophilus*, and *C. albicans* after one, two, and six hours, respectively. At the concentration of 0.137 mg/ml, *S. mutans* and *L. acidophilus* were killed after six and 24 hours, respectively. **Conclusion:** These findings encourage the potential use of Cts-Se-NPs in dentistry, while further clinical research is required in this area.

Keywords: Selenium; Chitosan; Streptococcus mutans; Lactobacillus acidophilus; Candida albicans.

Introduction

Dental caries is one the most common disease problem in the world. The findings of a recent survey by the Global Oral Health Data Bank indicated the prevalence of dental caries to be 49-83% [1]. Several types of microorganisms are associated with dental caries, but *Streptococcus mutans* and *Lactobacillus acidophilus* are the main bacteria involved in the initiation and progression of caries [2,3]. *S. mutans*, a gram-positive bacterium, is the most significant contributor to dental caries [4]. *Lactobacillus* species are the microbial markers of dental caries risk and a strong correlation has been reported between *Lactobacillus* counts and dental caries [5-7]. *Lactobacillus acidophilus* is the best-known species of gram-positive bacteria in the genus *Lactobacillus* [8].

Candida albicans is a commensal fungus that colonizes the human mucosal surfaces in the oral cavity [9] and is considered the most important *Candida* species, which cause oral infections. *C. albicans* is present in 30-60% of healthy individuals and 60-100% of denture wearers [10]. The coadhesion between *S. mutans* and *C. albicans* could lead to tooth surface colonization and enhance the microbial burden and production of the biofilm matrix, which is followed by severe tooth decay [11].

Numerous attempts have been made to develop optimal anticaries and antifungal agents. Recent findings have indicated that nanomaterials could be used as novel agents to prevent and treat dental caries [12]. In addition, several metal and metal oxide nanoparticles (NPs) have proven effective against cariogenic bacteria, such as silver, zinc oxide, magnesium oxide, titanium dioxides, gold, and copper oxide [13]. On the other hand, non-metallic NPs have been utilized as antibacterial and remineralizing agents; such examples are chitosan [14], hydroxyapatite [15], bioactive glass [16], and casein phosphopeptide-amorphous calcium phosphate [17-20]. Research development regarding nanomaterials' anticaries properties could result in the discovery of novel, effective agents against dental caries. The nano-size of selenium has attracted researchers' attention due to its excellent bioavailability and lower toxicity compared to the other forms of selenium [21]. The zero oxidation state of selenium (Se0) has low toxicity and excellent bioavailability, although it is unstable and should be stabilized by other biocompatible materials, such as chitosan [22]. Chitosan holds a special position among other biomaterials and is considered an ideal option for medical applications considering its biocompatibility, safety, biodegradability, and anti-inflammatory and antimicrobial activities [23].

Recently, we have investigated the antibacterial activity of colloidal selenium nanoparticles in chitosan solution (Cts-Se-NPs) as a new antibacterial agent [24], and the obtained results indicated that the Cts-Se-NP solution had excellent antibacterial activity against gram-positive bacteria (*Streptococcus sanguinis*, *Staphylococcus aureus*, and *Enterococcus faecalis*). In a continuation of our previous research, the present study aimed to investigate the antimicrobial activity of Cts-Se-NPs against *S. mutans*, *L. acidophilus*, and *C. albicans*. The novelty of the study lies on the antimicrobial and antifungal properties of Cts-Se-NPs against three cariogenic planktonic microorganisms.

Material and Methods

In this study, Cts-Se-NPs was synthesized according to our previous research [24]. Cts (0.15 g) was dissolved in a solution of 1.0% acetic acid solution at room temperature, 25 milliliters of ascorbic acid (0.01 g/ml-1) was added to the Cts solution, and the solution was stirred. Afterwards, 25 milliliters of sodium selenite solution (0.005 g/ml-1) was added drop-wise to the solution and stirred to obtain a reddish-orange homogeneous colloid. Particle size measurements were performed using a particle size analyzer (SZ100, Horiba Ltd., Kyoto, Japan).

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were used as the most important predictive tools for the evaluation of antimicrobial activity [25]. MIC is defined as the lowest concentration of an antimicrobial agent that could inhibit the activity and growth of a microorganism after incubation. The broth microdilution method [26] in 96-well microplates was used to measure the MIC of the Cts-Se-NP solution against *S. mutans*, *L. acidophilus*, and *C. albicans*. Three oral microorganisms, *S. mutans* (PTCC No: 1683), *L. acidophilus* (PTCC No: 1643), and *C. albicans* (PTCC No: 5027), were obtained from Persian Type Culture Collection.

The twofold serial dilution was prepared using the Mueller Hinton broth (MHB). The twofold serial dilutions of the Cts-Se-NP solution in the MHB were transferred into the wells of a microplate and inoculated with 100 microliters of the bacterial suspension ($1\text{-}2\times10^8$ CFU/ml). McFarland Standard No. 0.5, used in this study, contains approximately cell density ($1\text{-}2\times10^8$ CFU/ml) and was determined by measuring the optical absorbance at a wavelength of 600 nm. The absorbance was kept in the same range as equivalent to that of the McFarland standard 0.5 (Optical Density - O.D.) at 600 nm between 0.08–0.1 corresponds to $1\text{-}2 \times 10^8$ CFU/ml) [27]. The microplate was incubated at 37°C with 5% CO_2 in aerobic conditions for 24 hours, and the lowest concentration of the agent that prevented bacterial growth was recorded as the MIC. The bacteria inoculated into the MHB without Cts-Se NPs and culture media without the bacteria were considered as the positive and negative controls, respectively.

MBC is defined as the lowest concentration of an antibacterial agent killing most bacterial inoculums (99.99%). To measure the MBC, 10 microliters of the bacterial suspensions in the wells were inoculated into the blood agar medium without turbidity and incubated at the temperature of 37°C until sufficient growth. The lowest concentration that killed 99.9% (>3 log 10) of the initial inoculum after one, two, six, and 24 hours was considered as the MBC at each contact time. Figure 1 depicts the MIC and MBC tests of Cts-Se-NPs.

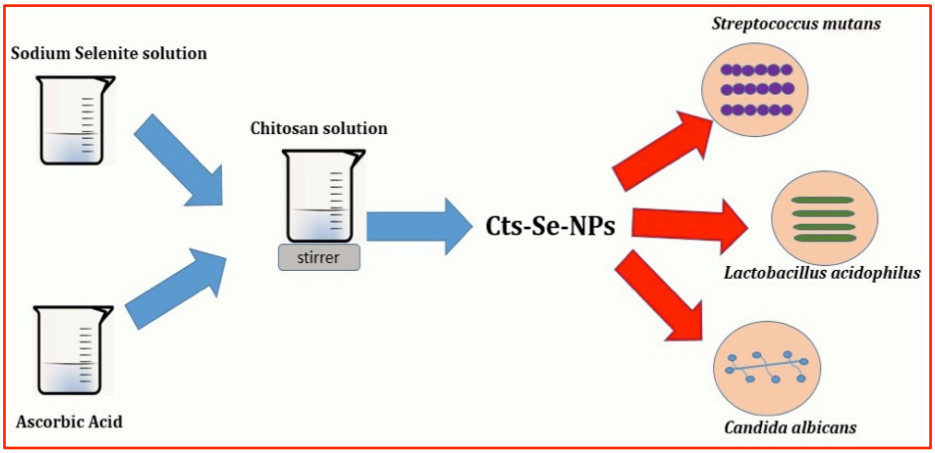

Figure 1. Scheme of synthesis of Cts-Se-NPs.

Results

As is observed in Figure 2, the mean particle size distribution of the Cts-Se NP solution was approximately 81.4 nanometers.

According to the obtained results, Cts-Se-NPs had significant antimicrobial activity against *S. mutans*, *L. acidophilus*, and *C. albicans*. The MIC values of Cts-Se-NPs were lowest for *S. mutans* (68 µg/ml) compared to *L. acidophilus* (137µg/ml) and *C. albicans* (274 µg/ml) (Table 1).

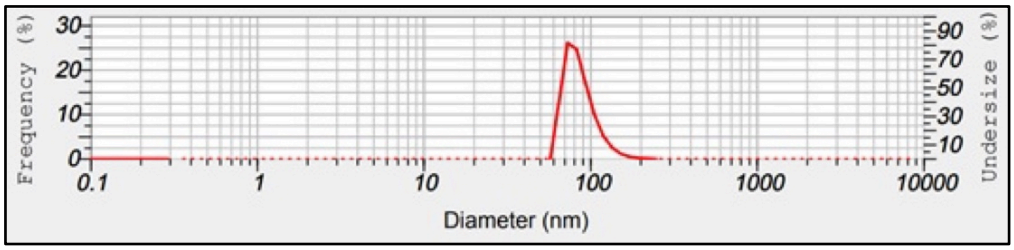

Figure 2. Particle Size Distribution of Cts-Se NPs.

Table 1. MIC values of Cts-Se-NPs.

Microorganisms	MIC
Streptococcus mutans	68 µg/ml
Lactobacillus acidophilus	137 µg/ml
Candida albicans	274 µg/ml

Table 2 shows the MBC values of Cts-Se-NPs against the microorganisms after one, two, six, and 24 hours. As can be seen, Cts-Se-NPs displayed more rapid bactericidal activity against *S. mutans*. Concentration of 274 µg/ml completely killed *S. mutans*, *L. acidophilus*, and *C. albicans* after one, two, and six hours, respectively. At the concentration of 137 µg/ml, *S. mutans* and *L. acidophilus* were killed after six and 24 hours, respectively.

Table 2. MBC values of Cts-Se-NPs after one, two, six, and 24 hours.

Microorganisms	MBC Values			
	After 1 Hour	After 2 Hours	After 6 Hours	After 24 Hours
Streptococcus mutans	274 µg/ml	274 µg/ml	137 µg/ml	137 µg/ml
Lactobacillus acidophilus	*	274 µg/ml	274 µg/ml	137 µg/ml
Candida albicans	*	*	274 µg/ml	274 µg/ml

*Not killed.

Discussion

The present study aimed to investigate the antimicrobial activity of Cts-Se-NPs against three major microorganisms involved in dental caries, including *S. mutans*, *L. acidophilus*, and *C. albicans*.

The current research aimed to assess the antimicrobial activity of Cts-Se-NPs against *S. mutans*, *L. acidophilus*, and *C. albicans*. Previous research [24] confirmed the significant antibacterial effects of a Cts-Se-NP solution against three gram-positive bacteria (*Streptococcus sanguinis*, *Staphylococcus aureus*, and *Enterococcus faecalis*), while no such effects were observed on gram-negative bacteria.

According to the results of the present study, Cts-Se-NPs had remarkable antimicrobial effects against *S. mutans*, *L. acidophilus*, and *C. albicans*. In gram-positive bacteria, the cell wall is thick and consists of several layers of peptidoglycan without an outer lipopolysaccharide membrane. The lipopolysaccharides carry a net with a negative charge, causing the strong negative surface charge of these bacteria. The electrostatic interaction could easily deposit selenium NPs in the peptidoglycan layer of gram-positive bacteria, thereby disrupting the bacterial cell division [28]. The antibacterial activity of chitosan could be attributed to its cationic nature. Cationic materials are able to degrade the cell wall structure and cell membrane of bacteria, which in turn leads to the exposure of the cell membrane to osmotic shock and exudation of the cytoplasmic content, eventually followed by cell death [29]. In a study in this regard, Costa et al. [30] observed that

chitosan was largely effective against the adherence and biofilm formation of *S. mutans*, and its action was evident in the inhibition of the initial adherence and biofilm formation and disruption of the mature biofilms. Similarly, Saita et al. [31] reported that submicron chitosan particles had significant antibacterial activity and anti-adhesive action against *S. mutans*.

In another study, Ikono et al. [32] investigated the effects of nanochitosan against *S. mutans* and *C. albicans* dual-species biofilms, reporting that 15% (v/v) nanochitosan had prominent antimicrobial activity against the dual-species of *S. mutans* and *C. albicans* biofilms through decreasing the survival rate of the microbial cells. In addition, Costa et al. [30] compared the effects of a chitosan-based mouthwash with two commercial mouthwashes on the biofilm formation of *S. mutans*, *L. acidophilus*, *E. faecium*, *C. albicans*, and *P. intermedia*, reporting that the chitosan-based mouthwash exerted significant antibacterial effects against the microorganisms.

Several studies have also evaluated chitosan's antibacterial activity, although its mechanisms against C. *albicans* remain unclear [33]. For instance, Shih et al. [33] reported the mechanism of chitosan action against *C. albicans* through the inhibition of SAGA complex gene expression, which decreased the protection of the cell surface against chitosan. On the other hand, Krasniqi et al. [34] reported that selenium-based orthodontic bonding agent had significant effects against *S. mutans*, while Tran et al. [35] observed that selenium could inhibit bacterial plaque formation on human teeth with durable antibacterial properties.

In a study in this regard, Guisbiers et al. [36] stated that selenium NPs had remarkable antifungal activity against *C. albicans* and could easily adhere onto the biofilm, penetrate *C. albicans*, and disrupt the cell structure through substitution with sulfur. The effective adherence of selenium NPs onto *C. albicans* results from the higher adherence of *C. albicans* to materials with similar surface energy [36]. Due to the similar chemical properties of selenium and sulfur, they compete in biological processes, thereby leading to the substitution of sulfur by selenium in amino acids and the subsequent morphological changes in *Candida* cells; such examples are the increased cell size, shrinkage of yeasts, cytoplasm thickening, and changes in the vacuole structure [37].

Limitations are inevitable with in-vitro studies, such as the results may not correspond to the actual behaviors of Cts-Se-NPs in vivo because they are not exposed to the same conditions found in the oral cavity. In this study, only three cariogenic bacteria were investigated in laboratory, which might not show completely the antiplaque effect and substantivity property of Cts-Se-NPs. In vivo studies are required to support the efficacy of Cts-Se-NPs.

Conclusion

The use of Cts-Se-NPs is an effective approach to the inhibition of the three of the most common bacteria involved in dental caries. However, it is important to note that further studies are required to evaluate its effects on other aspects related to tooth caries.

Authors' Contributions

MD	https://orcid.org/0000-0002-2624-7242	Conceptualization, Methodology, Formal Analysis, Data Curation and Writing - Review and Editing.
AR	https://orcid.org/0000-0002-6323-6112	Methodology, Formal Analysis, Investigation, Writing - Original Draft and Supervision.
KG	https://orcid.org/0000-0002-8538-1425	Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft and Visualization.
HB	https://orcid.org/0000-0003-1269-7434	Methodology, Data Curation and Writing - Original Draft.
AB	https://orcid.org/0000-0001-7943-6306	Conceptualization, Investigation and Writing - Original Draft.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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