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Abstract

High incidence of bacterial infections and antibiotic resistance as a growing problem has urged the need for novel antibacterial agents. The main purpose of this *in vitro* study was to evaluate the antibacterial activity of the chitosan-based selenium nanoparticles (Cts-Se-NPs) solution against gram-positive and gram-negative bacteria. The Cts-Se-NPs solution was synthesized using a simple chemical reduction method and characterized using ultraviolet-visible spectrophotometry, a particle size analyzer, and atomic force microscopy. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Cts-Se-NPs solution were determined against gram-negative (i.e., Pseudomonas aeruginosa, Salmonella typhimurium, and *Escherichia coli*) and gram-positive (i.e., Streptococcus sanguinis, *Staphylococcus aureus* and Enterococcus faecalis) bacteria using a broth microdilution method. According to the results, S. sanguinis, *S. aureus*, and E. faecalis showed MIC values of 0.068, 0.137, and 0.274 mg ml^{-1}, respectively. Moreover, the results revealed that the concentration of 0.274 mg ml^{-1} had a higher bactericidal effect than the other concentrations. As the concentration of Cts-Se-NPs increased to 0.274 mg ml^{-1}, S. sanguinis, *S. aureus*, and E. faecalis were completely killed after 1, 2, and 6 h, respectively. Further, *S. aureus* and S. sanguinis were totally killed after 24 and 6 h, respectively, at the concentration of 0.137 mg ml^{-1}. The Cts-Se-NPs solution did not elicit any significant antibacterial effect against P. aeruginosa, S. typhimurium, and *E. coli*. The Cts-Se-NPs solution can be further investigated for various antibacterial applications in the fields of medicine and dentistry, such as disinfection and sterilization of medical devices, as well as mouthwash in periodontal diseases and anti-caries agents.

Introduction

Selenium (Se) was discovered in 1817 by a Swedish chemist, Jons Jakob Berzelius. It is an essential trace element, and its low level in humans leads to an increased risk of various diseases, such as cancer and heart disease [1]. This micronutrient has been studied over the last years, and a great deal of evidence has revealed its vital role in the antioxidant defense system, thyroid hormone metabolism, and redox control of cell reactions [2–4].

The nanoform of Se is more interesting for researchers due to its lower toxicity and higher bioavailability compared to other forms [5]. This property results from using Se in zero oxidation state (Se0) [5–7], which is highly unstable and easily transformed into an inactive form [5]. Accordingly, researchers have focused on finding materials and methods to stabilize Se in Se^0.

Chitosan (Cts) is one of the natural materials that can be used for this purpose [8]. This biopolymer was reportedly first discovered by Rouget in 1859. The important properties of Cts, such as antimicrobial activity, biocompatibility, and biodegradability, motivated researchers, and industrialists to discover more effective and

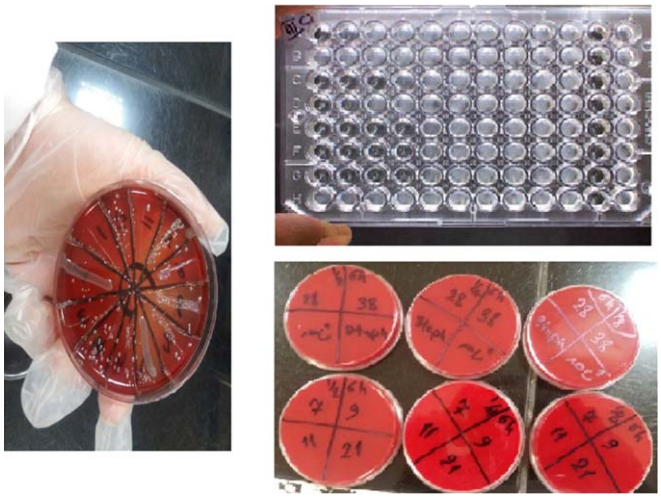

Figure 1. The antibacterial test of Cts-Se-NPs.

novel medical applications for it [9]. The Cts is used in a variety of biomedical fields, including drug delivery systems, wound dressings, as well as food and chemical industries [10].

Extremely few studies synthesized and investigated various aspects of chitosan-selenium nanoparticles (Cts-Se-NPs). In their recent study, Zhai *et al* [8] evaluated the antioxidant capacities of Se-NPs stabilized by Cts. To our knowledge, there have been no previous studies investigating the antibacterial properties of colloidal Se-NPs in Cts solution. Therefore, the main purpose of this *in vitro* study was to evaluate the antibacterial activity of the Cts-Se-NPs solution against gram-positive and gram-negative bacteria.

Materials and methods

Preparation and characterization

In total, 0.15 g Cts (low molecular weight, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 50 ml double distilled water by adding some drops of acetic acid (1.0%) solution at room temperature. Afterward, 25 ml of ascorbic acid (0.01 g ml^{-1}, Sigma-Aldrich, St. Louis, MO, USA) solution was added to the Cts solution and stirred. Following that, 25 ml of sodium selenite solution (0.005 g ml^{-1}, Sigma-Aldrich, St. Louis, MO, USA) was added to the Cts-ascorbic acid solution. Finally, the solution was stirred to obtain a reddish-orange homogeneous colloid. The ultraviolet-visible (UV–vis) analysis of the solution was characterized using UV–vis spectrophotometry (Cecil, UK). The UV–vis wavelength covered a range from 200 to 700 nm.

The average particle size distribution was determined using a particle size analyzer (PSA) (Horiba Model SZ-100). Moreover, the surface charge of Cts-Se was determined using zeta potential measurements with the same equipment. In addition, the atomic force microscopy (AFM) images were taken using an Autoprobe CP Research AFM system (JPK NanoWizard II Instrument, Berlin, Germany).

Antibacterial activity

The minimum inhibitory concentration (MIC) of the Cts-Se-NPs solution was determined against gram-negative (i.e., Pseudomonas aeruginosa, Salmonella typhimurium, and Eschericha coli) and gram-positive (i.e., Streptococcus sanguinis, *Staphylococcus aureus*, and Enterococcus faecalis) bacteria using a broth microdilution method in sterile 96-well microplates (figure 1). The serial dilution with sterile Mueller Hinton broth (MHB) was transferred into wells of a microplate and inoculated with 100 μl of bacterial suspension (1–2 $\times$ 10^8 CFU/ml). Afterward, the microplate was incubated at 37 °C for 24 h. Finally, the lowest concentration of the Cts-Se-NPs solution, which prevented the growth of bacteria, was defined as MIC. The positive control contained bacteria inoculated into MHB without Cts-Se, while the negative control included culture media without bacteria and Cts-Se-NPs. To determine the minimum bactericidal concentration (MBC) in various contact times (1, 2, 6, and 24 h), 10 μl of suspensions from wells without turbidity were inoculated into blood agar medium and incubated at 37 °C until sufficient growth was obtained. The lowest concentration that killed 99.9% (>3 log10) of the initial inoculum after 1, 2, 6, and 24 h was regarded as MBC in each contact time. Figure 1 demonstrates the MIC and MBC tests of Cts-Se-NPs.

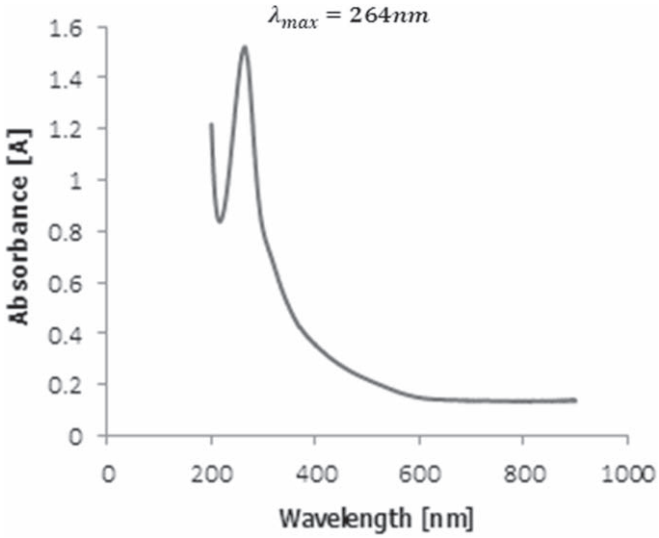

Figure 2. UV–vis spectrum of Cts-Se-NPs.

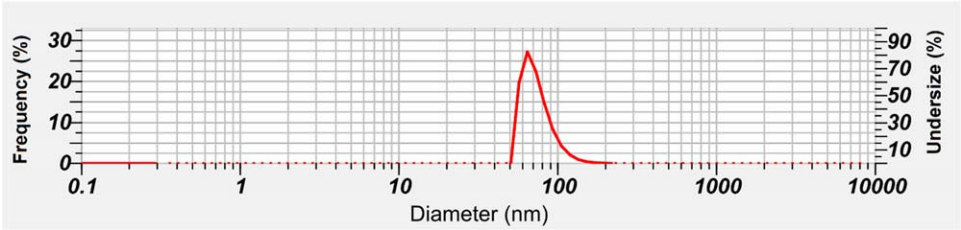

Figure 3. The particle size distribution of the Cts-Se-NPs.

Results

Figure 2 illustrates the UV–vis spectrum of the Cts-Se-NPs solution. It can be observed that the absorbance peak of the Se-NPs was located at 264 nm. The obtained result is in line with the findings of a study conducted by Chen *et al* who reported the UV–vis spectrum of Cts-Se-NPs [11]. Their results showed that the absorption peak of Se-NPs was located at 267 nm.

The PSA technique was utilized to determine the average size of small particles in suspension. As shown in figure 3, the size of Cts-Se-NPs varied in the range of 50–105 nm, and the mean diameter of the nanoparticles was approximately 69.6 nm. The zeta potential value for the obtained Cts-Se-NPs was 45.4 mV, indicating the suitable stability of the nanoparticles.

Figure 4 depicts a well-dispersed heterogeneous shape of the Cts-Se-NPs using AFM. The size distribution of the nanoparticles was less than 100 nm.

The Cts-Se-NPs revealed good antimicrobial activity against the gram-positive bacteria. Table 1 summarizes the MIC values of the Cts-Se-NPs solution against the gram-positive bacteria within the range of 0.068 to 0.274 mg ml^{-1}. As indicated in the table, S. sanguinis, *S. aureus*, and E. faecalis had the MIC values of 0.068, 0.137, and 0.274 mg ml^{-1}, respectively. The Cts-Se-NPs did not elicit any significant antibacterial effect against the gram-negative bacteria of P. aeruginosa, S. typhimurium, and *E. coli*.

Table 2 shows the MBC values of the Cts-Se-NPs against the tested bacteria after 1, 2, 6, and 24 h. The results revealed that the concentration of 0.274 mg ml^{-1} had a higher bactericidal effect. As the concentration of the Cts-Se-NPs increased to 0.274 mg ml^{-1}, S. sanguinis, *S. aureus*, and E. faecalis were completely killed after 1, 2, and 6 h, respectively. Moreover, S. sanguinis and *S. aureus* were respectively killed after 6 and 24 h at the concentration of 0.137 mg ml^{-1}. However, the concentration of 0.068 mg ml^{-1} did not show any significant bactericidal effect.

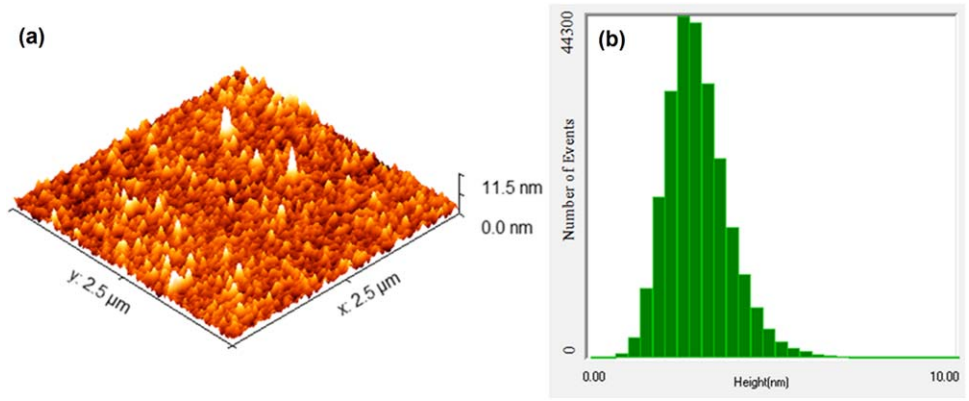

Figure 4. AFM image of the Cts-Se-NPs (a) and its size histogram (b).

Table 1. MIC values of the Cts-Se-NPs against different bacteria.

Bacteria name	MIC (mg ml^{-1})
Pseudomonas aeruginosa	NA□
Salmonella typhimurium	NA□
Escherichia coli O157	NA□
Enterococcus faecalis	0.274
Staphylococcus aureus	0.137
Streptococcus sanguinis	0.068

NA*: Not killed at any concentration in the range of examination.

Table 2. MBC values of the Cts-Se-NPs against bacteria after 1, 2, 6, and 24 h.

Bacteria name	MBC after 1h (mg ml^{-1})	MBC after 2h (mg ml^{-1})	MBC after 6h (mg ml^{-1})	MBC after 24h (mg ml^{-1})
Pseudomonas aeruginosa	NA□	NA□	NA□	NA□
Salmonella typhimurium	NA□	NA□	NA□	NA□
Escherichia coli O157	NA□	NA□	NA□	NA□
Enterococcus faecalis	NA□	NA□	0.274	0.274
Staphylococcus aureus	NA□	0.274	0.274	0.137
Streptococcus sanguinis	0.274	0.274	0.137	0.137

NA*: Not killed at any concentration in the range of examination.

Discussion

In this study, we utilized a simple synthesis method using ascorbic acid as a green reducing agent. The advantage of Se-NPs is the possibility of using Se in Se0, which presents low toxicity and excellent bioavailability compared to other oxidation states such as Se (IV) and Se (VI) [6, 7]; however, it is highly unstable and easily transformed into an inactive form. Although, as can be observed in several studies, Se-NPs can be stabilized by Cts [5, 12–14]. Therefore, we used Cts as a stabilizer for Se0 and evaluated the antibacterial activity of Se-NPs stabilized by Cts.

The results showed the antibacterial effects of the Cts-Se-NPs solution against the various gram-positive bacteria, but not the gram-negative bacteria. Several studies found that Cts generally had stronger effects on gram-positive bacteria than on their gram-negative bacteria [15]. Tran *et al* [2] reported that Se-NPs did not show any significant effect against *E. coli*.

Gram-negative bacteria are surrounded by lipopolysaccharide molecules, which carry a negative charge [16]. Therefore, there is a strong electrostatic repulsion between Se-NPs and the outer bacterial membrane [2]. The results of the current study revealed that the Cts-Se-NPs solution had a significant effect on the gram-positive bacteria (i.e., S. sanguinis, *S. aureus*, and E. faecalis).

S. aureus is a major human pathogen that causes a wide range of clinical infections [17]. It is a leading cause of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, septic arthritis, device-related infections, pulmonary infections, gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections [18].

The results of the present study also showed that the Cts-Se-NPs solution had a remarkable effect on *S. aureus*. Tran *et al* [2] found that Se-NPs showed strong growth inhibition toward *S. aureus*. The cell wall in gram-positive bacteria contains a thick layer of peptidoglycan without any outer lipopolysaccharide membrane, and their net surface charge is much less negative compared to that of gram-negative bacteria [19]. Therefore, Se-NPs could penetrate much more easily through the peptidoglycan layer of *S. aureus*, reducing bacterial cell division. This consequently inhibits the growth of *S. aureus* [2, 20].

Guisbiers *et al* [20] observed that Se-NPs had a significant inhibitory effect on *S. aureus*. Moreover, Goy *et al* [21] found that Cts was more active against *S. aureus* compared to the gram-negative *E. coli*. In the same line, Tao *et al*[22] studied the effect of Cts on the membrane permeability and cell morphology of *S. aureus*. Their results showed that Cts had a significant effect against *S. aureus* and performed its antibacterial activity via increasing the permeability of cell membranes. Therefore, in the present study, Cts and Se might have a synergic antibacterial effect on the gram-positive bacteria, such as *S. aureus*.

E. faecalis is a gram-positive bacterium that colonizes human gastrointestinal tracts. It is one of the main causing agents of hospital-acquired infections, which is regarded as a major concern in public health [23]. In our study, the Cts-Se-NPs solution showed noticeable antibacterial activity against E. faecalis. Khiralla *et al* [24] found that Se-NPs demonstrated an antimicrobial effect against E. faecalis. Furthermore, Perelshtein *et al* [25] observed that Cts-NPs had more effects on E. faecalis than on *E. coli*. This is probably attributed to this property that E. faecalis only has a single membrane and is more susceptible to the effects of surface disruption than *E. coli*, which has a double outer membrane [26].

S. sanguinis is a member of the viridans streptococcal group and is a leading cause of infective endocarditis, which is a life-threatening infection of the cardiovascular system [26, 27]. It is also one of the pioneering tooth colonizers and is one of the most abundant species in oral biofilm, dental plaque, dental caries, and periodontal diseases [28].

Further, the results of the present study revealed that the Cts-Se-NPs solution had a significant effect on S. sanguinis. In a similar vein, Aliasghari *et al* [29] showed that Cts and nano Cts had anti-growth and anti-adherence effects against cariogenic bacteria, such as S. sanguinis.

In addition, Archana *et al* [30] found that chlorhexidine along with Cts combination mouthrinse was superior in antimicrobial activity than chlorhexidine alone. The effect of Se against S. sanguinis has not been so far studied. In a study performed by Tran *et al* [2], it was demonstrated that Se-NPs had an extremely low hemolysis rate with only 18% of maximal hemolysis. Other studies determined the hemolytic tendency of other nanoparticles, such as silver and gold, at 100% and 60%, respectively [31, 32]. Therefore, Se-NPs have low hemolytic activity and can be potentially used for medical devices having direct contact with blood [2].

MIC and MBC of an antibacterial substance are the basic and one of the most important criteria for antibacterial activity. Although, the MIC and MBC levels are not a confirmation of the antibacterial activity on the bacterial cell wall. NPs need to be in contact with bacterial cells to accomplish their antibacterial role [33]. With respect to existing studies, the main processes underlying the antibacterial activity of NPs are as follows: (1) contact with bacterial cell walls (various forms of contact include electrostatic attraction [34], Vander Waals forces [35], receptor–ligand [36], and hydrophobic interactions [37]), (2) disruption and penetration of the bacterial cell membrane, (3) generation of reactive oxygen species (ROS) and interact with basic component of microbial cell (i.e., DNA and mitochondria), protein deactivation, electrolyte balance disorder, enzyme inhibition, and modify gene expression levels [38–40].

Conclusion

In summary, in this study, the Cts-Se-NPs solution exhibited excellent antibacterial activity against the gram-positive bacteria. This product can be investigated further for various antibacterial applications in the fields of medicine and dentistry, such as disinfection and sterilization of medical devices, as well as a mouthwash in periodontal diseases and anti-caries agents.

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Conflicts of interest

The authors report no conflicts of interest in the present work.

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References

[1] Tinggi U 2008 Selenium: its role as antioxidant in human health *Environmental Health and Preventive Medicine* **13** 102
[2] Tran P A, O'Brien-Simpson N, Reynolds E C, Pantarat N, Biswas D P and O'Connor A J 2015 Low cytotoxic trace element selenium nanoparticles and their differential antimicrobial properties against *S. aureus* and *E. coli Nanotechnology* **27** 045101
[3] Navarro R, Vicente A, Ortiz A J and Bravo L A 2010 The effects of two soft drinks on bond strength, bracket microleakage, and adhesive remnant on intact and sealed enamel *The European Journal of Orthodontics* **33** 60–5
[4] Brown K M and Arthur J 2001 Selenium, selenoproteins and human health: a review *Public Health Nutrition* **4** 593–9
[5] Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay-Nedecky B, Peng Q, Baron M, Melcova M, Opatrilova R and Zidkova J 2018 Nano-selenium and its nanomedicine applications: a critical review *Int. J. Nanomed.* **13** 2107
[6] Torres S, Campos V, León C, Rodríguez-Llamazares S, Rojas S, Gonzalez M, Smith C and Mondaca M 2012 Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity *J. Nanopart. Res.* **14** 1236
[7] Wang H, Zhang J and Yu H 2007 Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice *Free Radical Biol. Med.* **42** 1524–33
[8] Zhai X, Zhang C, Zhao G, Stoll S, Ren F and Leng X 2017 Antioxidant capacities of the selenium nanoparticles stabilized by chitosan *Journal of Nanobiotechnology* **15** 4
[9] Ahmed T, Ab Rahman N and Alam M K 2018 Assessment of *in vivo* bond strength studies of the orthodontic bracket-adhesive system: a systematic review *European Journal of Dentistry* **12** 602
[10] Park B K and Kim M-M 2010 Applications of chitin and its derivatives in biological medicine *Int. J. Mol. Sci.* **11** 5152–64
[11] Chen W, Li Y, Yang S, Yue L, Jiang Q and Xia W 2015 Synthesis and antioxidant properties of chitosan and carboxymethyl chitosan-stabilized selenium nanoparticles *Carbohydr Polym* **132** 574–81
[12] Lara H H, Guisbiers G, Mendoza J, Mimun L C, Vincent B A, Lopez-Ribot J L and Nash K L 2018 Synergistic antifungal effect of chitosan-stabilized selenium nanoparticles synthesized by pulsed laser ablation in liquids against Candida albicans biofilms *Int. J. Nanomed.* **13** 2697
[13] Estevez H, Garcia-Lidon J C, Luque-Garcia J L and Camara C 2014 Effects of chitosan-stabilized selenium nanoparticles on cell proliferation, apoptosis and cell cycle pattern in HepG2 cells: comparison with other selenospecies *Colloids Surf., B* **122** 184–93
[14] Zeng S, Ke Y, Liu Y, Shen Y, Zhang L, Li C, Liu A, Shen L, Hu X and Wu H 2018 Synthesis and antidiabetic properties of chitosan-stabilized selenium nanoparticles *Colloids Surf., B* **170** 115–21
[15] Goy R C, Britto D d and Assis O B 2009 A review of the antimicrobial activity of chitosan *Polímeros* **19** 241–7
[16] Slavin Y N, Asnis J, Häfeli U O and Bach H 2017 Metal nanoparticles: understanding the mechanisms behind antibacterial activity *Journal of Nanobiotechnology* **15** 65
[17] Tong S Y, Davis J S, Eichenberger E, Holland T L and Fowler V G 2015 *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management *Clinical Microbiology Reviews* **28** 603–61
[18] Taylor T A and Unakal C G 2017 Staphylococcus aureus *StatPearls [Internet]* (Treasure Island (FL),: StatPearls Publishing) https://scholar.google.com/scholar_lookup?title=Staphylococcus%20aureus%2C%20in%20StatPearls&publication_year=2019&author=TA%20Taylor&author=CG%20Unakal
[19] Chung Y-C, Su Y-P, Chen C-C, Jia G, Wang H.-l., Wu J G and Lin J-G 2004 Relationship between antibacterial activity of chitosan and surface characteristics of cell wall *Acta Pharmacol. Sin.* **25** 932–6 https://scholar.google.com/scholar_lookup?journal=Acta+Pharmacol.+Sin.&title=Relationship+between+antibacterial+activity+of+chitosan+and+surface+characteristics+of+cell+wall&author=Y.C.+Chung&author=Y.P.+Su&author=C.C.+Chen&author=G.+Jia&author=H.L.+Wang&volume=25&publication_year=2004&pages=932-936&pmid=15210068&
[20] Guisbiers G, Wang Q, Khachatryan E, Mimun L, Mendoza-Cruz R, Larese-Casanova P, Webster T and Nash K 2016 Inhibition of *E. coli* and *S. aureus* with selenium nanoparticles synthesized by pulsed laser ablation in deionized water *Int. J. Nanomed.* **11** 3731
[21] Goy R C, Morais S T and Assis O B 2016 Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth *Revista Brasileira de Farmacognosia* **26** 122–7
[22] Tao Y, Qian L-H and Xie J 2011 Effect of chitosan on membrane permeability and cell morphology of Pseudomonas aeruginosa and Staphyloccocus aureus *Carbohydrate Polym.* **86** 969–74
[23] Banla I L, Kommineni S, Hayward M, Rodrigues M, Palmer K L, Salzman N H and Kristich C J 2018 Modulators of Enterococcus faecalis cell envelope integrity and antimicrobial resistance influence stable colonization of the mammalian gastrointestinal tract *Infection and Immunity* **86** e00381
[24] Khiralla G M and El-Deeb B A 2015 Antimicrobial and antibiofilm effects of selenium nanoparticles on some foodborne pathogens *LWT-Food Science and Technology* **63** 1001–7
[25] Perelshtein I, Ruderman E, Perkas N, Tzanov T, Beddow J, Joyce E, Mason T J, Blanes M, Mollá K and Patlolla A 2013 Chitosan and chitosan–ZnO-based complex nanoparticles: formation, characterization, and antibacterial activity *Journal of Materials Chemistry B* **1** 1968–76
[26] Bensing B A, Loukachevitch L V, McCulloch K M, Yu H, Vann K R, Wawrzak Z, Anderson S, Chen X, Sullam P M and Iverson T 2016 Structural basis for sialoglycan binding by the Streptococcus sanguinis SrpA adhesin *J. Biol. Chem.* **291** 7230–40
[27] Turner L S, Kanamoto T, Unoki T, Munro C L, Wu H and Kitten T 2009 Comprehensive evaluation of Streptococcus sanguinis cell wall-anchored proteins in early infective endocarditis *Infection and Immunity* **77** 4966–75

[28] Zhu B, Macleod L C, Kitten T and Xu P 2018 Streptococcus sanguinis biofilm formation & interaction with oral pathogens *Future Microbiology* **13** 915–32
[29] Aliasghari A, Khorasgani M R, Vaezifar S, Rahimi F, Younesi H and Khoroushi M 2016 Evaluation of antibacterial efficiency of chitosan and chitosan nanoparticles on cariogenic streptococci: an *in vitro* study *Iranian Journal of Microbiology* **8** 93
[30] Archana V, Prabhuji M L, Karthikeyan B V and Selvan A 2013 Control of Streptococcus sanguinis oral biofilm by novel chlorhexidine-chitosan mouthwash: an *in vitro* study *Journal of Experimental & Integrative Medicine* **3** 165–69
[31] Lin J-J, Lin W-C, Dong R-X and Hsu S-h 2012 The cellular responses and antibacterial activities of silver nanoparticles stabilized by different polymers *Nanotechnology* **23** 065102
[32] Khullar P, Singh V, Mahal A, Dave P N, Thakur S, Kaur G, Singh J, Singh Kamboj S and Singh Bakshi M 2012 Bovine serum albumin bioconjugated gold nanoparticles: synthesis, hemolysis, and cytotoxicity toward cancer cell lines *The Journal of Physical Chemistry C* **116** 8834–43
[33] Wang L, Hu C and Shao L 2017 The antimicrobial activity of nanoparticles: present situation and prospects for the future *Int. Journal of Nanomedicine* **12** 1227
[34] Li H, Chen Q, Zhao J and Urmila K 2015 Enhancing the antimicrobial activity of natural extraction using the synthetic ultrasmall metal nanoparticles *Sci. Rep.* **5** 11033
[35] Armentano I, Arciola C R, Fortunati E, Ferrari D, Mattioli S, Amoroso C F, Rizzo J, Kenny J M, Imbriani M and Visai L 2014 The interaction of bacteria with engineered nanostructured polymeric materials: a review *The Scientific World Journal* **2014** 1–18
[36] Gao W, Thamphiwatana S, Angsantikul P and Zhang L 2014 Nanoparticle approaches against bacterial infections *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **6** 532–47
[37] Luan B, Huynh T and Zhou R 2016 Complete wetting of graphene by biological lipids *Nanoscale* **8** 5750–4
[38] Bankier C, Matharu R, Cheong Y, Ren G, Cloutman-Green E and Ciric L 2019 Synergistic antibacterial effects of metallic nanoparticle combinations *Sci. Rep.* **9** 1–8
[39] Shaikh S, Nazam N, Rizvi S M D, Ahmad K, Baig M H, Lee E J and Choi I 2019 Mechanistic insights into the antimicrobial actions of metallic nanoparticles and their implications for multidrug resistance *Int. J. Mol. Sci.* **20** 2468
[40] Panpatte D G *Nanotechnology for Agriculture: Crop Production & Protection* (Berlin: Springer)