Arch Health Invest (2023)12(7):1482-1487
http://doi.org/10.21270/archi.v12i7.5804

Original Research

Synthesis of an Experimental Gel Containing Biologically Synthesized Silver Nanoparticles used in the Biofilm Disruption

Síntese de um Gel Experimental contendo Nanopartículas de Prata Sintetizadas Biologicamente utilizadas na Ruptura do Biofilme

Síntesis de un Gel Experimental con Nanopartículas de Plata Sintetizadas Biológicamente utilizadas en la Ruptura de Biopelícula

Karina Eleonara Klein Graupen Roldan **ANTUNES**
Department of Orthodontics, Araras Dental School, Herminio Ometto Foundation, FHO, Araras - SP, Brazil
https://orcid.org/0009-0007-2932-5844
Daniel Navarro da **ROCHA**
Department of Mechanical and Materials Engineering, Military Institute of Engineering- IME, Rio de Janeiro - RJ, Brazil
https://orcid.org/0000-0003-2413-345X
Larissa **MARCELINO**
Department of Restorative Dentistry- Dental Materials Area, Piracicaba Dental School. State University of Campinas - UNICAMP, SP, Brazil
https://orcid.org/0000-0002-7199-9013
Roberto do Nascimento **SILVA**
Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto 14049-900 - SP, Brazil
https://orcid.org/0000-0002-8992-7130
Ana Paula **NOGUEIRA**
Federal University of São Paulo, Department of Science and Technology, São José dos Campos - SP, Brazil
https://orcid.org/0000-0002-5322-998X
Analia Gabriella Borges Ferraz **FACURY**
Department of Restorative Dentistry- Dental Materials Area, Piracicaba Dental School. State University of Campinas - UNICAMP, SP, Brazil
https://orcid.org/0000-0001-9255-3937
Ana Rosa **COSTA**
Department of Restorative Dentistry- Dental Materials Area, Piracicaba Dental School. State University of Campinas - UNICAMP, SP, Brazil
Department of Orthodontics, Araras Dental School, Herminio Ometto Foundation, FHO, Araras - SP, Brazil
https://orcid.org/0000-0002-1020-3210
José Guilherme **NEVES**
Department of Restorative Dentistry- Dental Materials Area, Piracicaba Dental School. State University of Campinas - UNICAMP, SP, Brazil
Department of Orthodontics, Araras Dental School, Herminio Ometto Foundation, FHO, Araras - SP, Brazil
https://orcid.org/0000-0003-1613-8496

Abstract
The aim of the present study was to synthesize and evaluate the biological and mechanical properties of a polymeric gel associated with silver nanoparticles. The gelswere composed of a Chitosan-Xanthan (CX) polyelectrolytic biopolymer complex associated or not with silver nanoparticles (Ag_{nano}), which were grouped into the following groups: G1) Gel CX; G2) CX Gel + 5% Ag_{nano}; G3) CX Gel + 2.5% Ag_{nano}; 4) CX Gel + 1.25% Ag_{nano}. The characterization of nanoparticles was performed through dynamic light scattering analysis, and gels through rheological analysis. The antimicrobial activity for Streptococcus mutans was performed by the inhibition halo method. The results obtained from DLS showed a Zeta potential of -30 mV and particle size approximately 100 nm. The rheological analysis shows that all groups had viscosity between 5 and 6.5 Pa, and with increasing shear stress the viscosity of all groups wasdecreased. For the analysis of antimicrobial activity, the antimicrobial potential was observed only for the CX gel containing 5% Ag_{nano}. Thus, it is concluded that the gelcontaining 5% Ag_{nano} is promising for use in orthodontic patients with a high rate of bacterial plaque and can be used to prevent white spot lesions during and after treatment.
Descriptors: Nanoparticles; Orthodontics; Silver; Streptococcus mutans; Chitosan.
Resumo
O objetivo do presente estudo foi sintetizar e avaliar as propriedades biológicas e mecânicas de um gel polimérico associado a nanopartículas de prata. Os géis foram compostos por um complexo biopolímero polieletrolítico quitosana-xantana (CX) associado ou não a nanopartículas de prata (Ag_{nano}), que foram agrupados nos seguintes grupos: G1) Gel CX; G2) Gel CX + Ag_{nano} 5%; G3) Gel CX + Ag_{nano} 2,5%; 4) CX Gel + 1,25% Ag_{nano}. A caracterização das nanopartículas foi realizada por meio de análise dinâmica de espalhamento de luz, e dos géis por meio de análise reológica. A atividade antimicrobiana para Streptococcus mutans foi realizada pelo método do halo de inibição. Os resultados obtidos de DLS mostraram um potencial Zeta de -30 mV e tamanho de partícula de aproximadamente 100 nm. A análise reológica mostra que todos os grupos apresentaram viscosidade entre 5 e 6,5 Pa, e com o aumento da tensão de cisalhamento a viscosidade de todos os grupos diminuiu. Para a análise da atividade antimicrobiana, o potencial antimicrobiano foi observado apenas para o gel CX contendo 5% de Ag_{nano}. Assim, conclui-se que o gel contendo Ag_{nano} 5% é promissor para uso em pacientes ortodônticos com alto índice de placa bacteriana e pode ser utilizado para prevenir lesões de mancha branca durante e após o tratamento.
Descritores: Nanopartículas; Ortodontia; Streptococcus mutans; Chitosana.
Resumen
El objetivo del presente estudio fue sintetizar y evaluar las propiedades biológicas y mecánicas de un gel polimérico asociado a nanopartículas de plata. Los geles estaban compuestos por un complejo biopolímero polielectrolítico Chitosan-Xanthan (CX) asociado o no a nanopartículas de plata (Ag_{nano}), los cuales se agruparon en los siguientes grupos: G1) Gel CX; G2) CX Gel + 5% Ag_{nano}; G3) CX Gel + 2,5% Ag_{nano}; 4) CX Gel + 1,25% Ag_{nano}. La caracterización de nanopartículas se realizó mediante análisis dinámico de dispersión de luz, y de geles mediante análisis reológico. La actividad antimicrobiana para Streptococcus mutans se realizó por el método del halo de inhibición. Los resultados obtenidos de DLS mostraron un potencial Zeta de -30 mV y un tamaño de partícula de aproximadamente 100 nm. El análisis reológico muestra que todos los grupos tenían una viscosidad entre 5 y 6,5 Pa, y con el aumento del esfuerzo cortante, la viscosidad de todos los grupos disminuyó. Para el análisis de la actividad antimicrobiana, el potencial antimicrobiano se observó solo para el gel CX que contenía 5% de Ag_{nano}. Por lo tanto, se concluye que el gel que contiene 5% de Ag_{nano} es prometedor para su uso en pacientes de ortodoncia con una alta tasa de placa bacteriana y puede usarse para prevenir lesiones de manchas blancas durante y después del tratamiento.
Descriptores: Nanopartículas; Ortodoncía; Plata; Streptococcus mutans; Chitosana.

INTRODUCTION

A major challenge in orthodontic therapy is the use of fixed appliances, since orthodontic devices such as brackets, tubes, archwires and bands become bacterial retainers, which, associated with other factors, can lead to the occurrence of dental caries and periodontal problems[1]. Dental caries is a multifactorial infectious disease, and biofilm is a necessary etiological agent for the development of this

disease[2]. Streptococcus mutans (S. mutans) are the main microorganisms involved in the initiation and development of caries, thus generating the dissolution of tooth enamel and, with advancement, the formation of cavities, compromising other dental structures[3].

Enamel white spot lesion (WSL) is the clinical sign of caries that is manifested by an intact surface zone and a porous subsurface[4]. WSL formation in the enamel around brackets is a serious and very common complication during orthodontic treatment[5], with its prevalence being 73% to 95%[6]. Through this phenomenon, aesthetics can be compromised after the removal of the fixed orthodontic appliance and be a cause of post-treatment dissatisfaction[7]. Within this context, continuous efforts have been made to control white spot lesions by destructuring the biofilm, preventing enamel demineralization or promoting its remineralization[8].

A variety of antimicrobial compounds have been introduced into experimental dental restorative materials with the aim of reducing biofilm formation[9], and these biomaterials may have particles such as chlorhexidine, glutaraldehyde, and silver nanoparticles[10]. These biomaterials are used for total or partial replacement, restoration or augmentation of biological tissues, through the use of natural, artificial or synthetic materials and present a satisfactory set of physical, chemical and biological properties[11]. Within the biomaterial synthesis process, the use of nanotechnology offers the possibility of controlling the formation of oral biofilms through the use of nanoparticles with biocidal, anti-adhesive, and delivery capabilities[12].

Silver nanoparticles are best known for their applications in different areas, such as human and veterinary medicine, pharmacology, dentistry, food industry, among others[4,13]. There is great interest in the use of silver nanoparticles (Ag_{nano})[14] due to their remarkable properties, such as high surface area, excellent antimicrobial activity and as alternatives for reducing bacterial adhesion and preventing biofilm formation[15].

The biological synthesis of silver nanoparticles from microorganisms, has become environmentally friendly[16], as well as being a simpler and cost-effective alternative with great bactericidal potential against gram-positive and gram-negative pathogens[17]. In addition, it has high yield and high stability, are non-toxic and have ready solubility of the prepared nanoparticles in water[18].

Within this context, it would be of great importance to synthesize and characterize a polymeric gel associated with silver nanoparticles and that is able to destructure the biofilm, inhibiting the growth of S. mutans and prevent white spot lesions in patients undergoing orthodontic treatment, being this of easy access and manipulation of the clinician. Thus, this study aimed to synthesize and characterize a polymeric gel, associated or not with different concentrations of silver nanoparticles.

MATERIAL AND METHOD

- *Experimental Design*

The gels were composed of a complex of polyelectrolyte Chitosan-Xanthan (CX) biopolymers, associated or not with silver nanoparticles (Ag_{nano}), which were grouped into the following groups: G1) CX Gel; G2) CX Gel + Ag_{nano} 5%; G3) CX Gel + Ag_{nano} 2.5%; 4) CX Gel + Ag_{nano} 1.25%. The characterization of the nanoparticles was performed by dynamic light scattering and the gels were characterized by rheological analysis and antimicrobial activity (zone of inhibition).

- *Synthesis of the Ag Nanoparticles by Biological Means*

The fungus Trichoderma reesei was grown in a Petri dish containing MEX-agar culture medium, being kept in the oven at a temperature of 28ºC, until growth and sporulation, which occurs in approximately 7 days. Then, the spores were collected and inoculated in a volume of 100 ml in potato medium (Potato Dextrose), where it was kept at rest (condition with low oxygen content) in an oven at 30ºC for 7 days. In conditions of low oxygen concentration, Trichoderma reesei starts to produce enzymes capable of producing silver nanoparticles. The biosynthesis of silver occurred by mixing, in amber-colored conical flask, the culture supernatant and an aqueous solution of silver nitrate (AgnanoO3, at a concentration of 5 mM (can vary from 1 to 10mM). After mixing, the pH was adjusted to 8.5. The mixture is kept in the dark at 40°C under 200 rpm rotation for 10 days[19].

Characterization of Ag Nanoparticles

Measurements of size, dispersion index (PDi) and Zeta Potential of the NLS were performed by dynamic light scattering (DLS) using Zetasizer Nano ZS90 (Malvern, UK). The measurements (n=3) were diluted in Potassium Chloride (1:100) and were performed in triplicate[20].

- *Synthesis of the Experimental Gel*

To obtain the chitosan-xanthan gels, adaptations were made to the protocol described by Bellini et al.[21]. The solutions employed were 3% (w/v) xanthan in deionized water (Milli-Q system, Millipore) and 1% (w/v) chitosan in 2% (v/v) acetic acid solution. In the protocol performed, 200 ml of the chitosan solution was added to 200 ml of the xanthan solution with the aid of a Gilson peristaltic pump, model Minipuls 3, at a flow rate of 10 ml/min. The mixture occurred in a jacketed stainless steel cylindrical reactor with an internal diameter of 10 cm and height of 20 cm, with a flat bottom. During the addition of the chitosan solution to the xanthan solution, the system was kept under stirring at 1000

rpm with the aid of a Quimis Q-251 D mechanical stirrer, with a propeller of the naval type with inclined blades of 3 cm radius, about 4 cm away from the base of the reactor. The temperature of the system was controlled at 25°C using a thermostatic bath (Q-214 M2, Quimis). After the chitosan solution was added to the xanthan solution, the rotation rate was increased to 1200 rpm and remained there for 10 minutes.

- *Rheological Analysis of the experimentals gels*

The viscosity of each sample was measured using a viscoelasticity measuring instrument (HAAKE RheoStress 600; Thermo Fisher Scientific, Yokohama, Japan). Each specimen (0.04 mL) was placed on a measuring plate and a gap of 0.052 mm was maintained between the measuring plate and a cone-plate (1°). The viscosity was then measured at a temperature of 25 °C over 180 seconds with a shear rate of 0-200/s[22].

- *Antimicrobial evaluation- agar diffusion*

For the agar diffusion test, the Streptococcus mutans strain (UA159) was inoculated on the entire surface of the plate using a sterile swab, turning 60° on its edge in order to distribute the inoculum uniformly. Using a sterile 100 µL tip, holes were made on the top of the agar to make the 'wells', where the gel was dispensed to evaluate the antimicrobial activity.

The gels were decontaminated in a laminar flow hood with UV light for 30 minutes (theoretical ref), after which, using a pipette, a sufficient amount of gel was dispensed into each well (according to the study groups) to fill it, and the plate was stored for 24 hours in an incubator in microaerobic conditions with 10% CO2 at 37ºC. After the proposed period, it was possible to verify the diameter of the growth inhibition zones (visible to the naked eye) called the inhibition halo, which was measured using a millimeter scale with the aid of a ruler. Three plates (with the wells containing the study groups) were made in independent assays.

RESULTS

Through DLS analysis, size and Zeta Potential values were obtained. Figure 1 shows the graph of the Zeta potential, which represents the surface charge of the nanoparticles. It was possible to observe an ascending peak in the region of -30 mV, which characterizes a particle in the nanometric scale. In Figure 2 we could observe in the measurements taken, peaks ranging from 60 to 130 nanometers, which also characterizes a nanometer scale particle.

The graphs of the rheological analysis (viscosity) of the experimental gels are shown in Figure 3. It could be observed that all gels submitted to no shear stress presented mean values between 5.5 and 6.5 Pa of viscosity. With the continuous increase in tension, a decrease in the viscosity of gels from all experimental groups was observed.

The antimicrobial activity of the gel using the agar diffusion technique showed well-defined halos of inhibition only in the 5% CX Gel + Ag_{nano} concentration, indicating the absence of Streptococcus mutans growth (Figure 4). The other concentrations of CX Gel + Ag_{nano} addition were not able to inhibit microbial growth. Thus, they did not form the halo of inhibition, making the 5% Ag_{nano} concentration the dose-response.

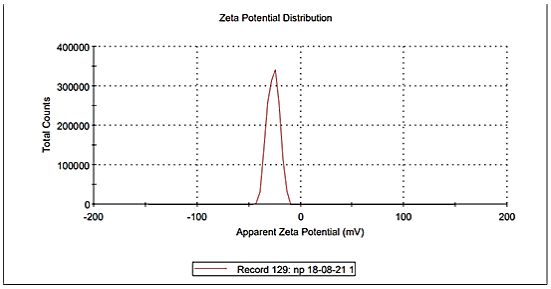

Figure 1: Zeta Potencial Distribution of Ag nanoparticles.

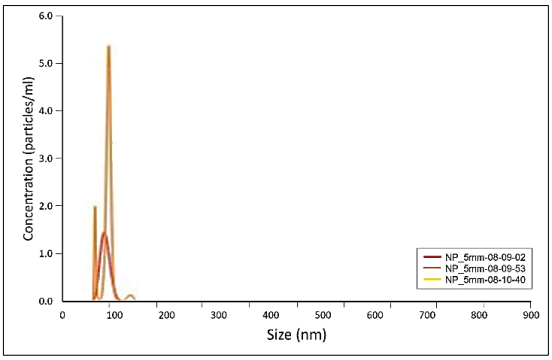

Figure 2: Size Distribution of Ag nanoparticles by intensity.

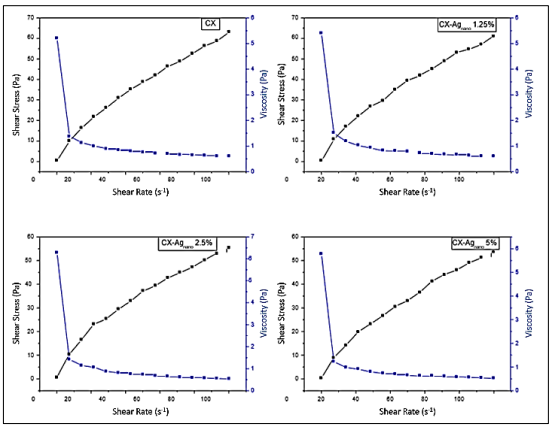

Figure 3: Shear Stress and Viscosity of each experimental gels. [CX] Chitosan-Xanthan Gel; [CX-Ag_{nano} 1.25%] Chitosan-Xanthan Gel and silver nanoparticles 1.25%; [CX-Ag_{nano} 2.5%] Chitosan-Xanthan Gel and silver nanoparticles 2.5%; [CX-Ag_{nano} 5%] Chitosan-Xanthan Gel and silver nanoparticles 5%.

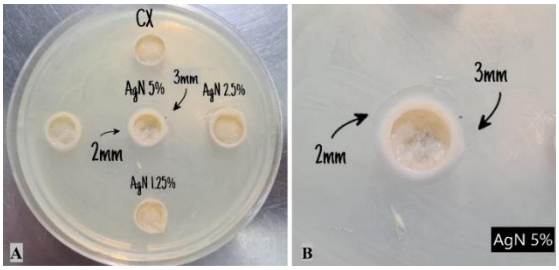

Figure 4: Antimicrobial activity of the experimental gels. (A) Vision of all groups and (B) 5% CX Gel + Ag_{nano} group inhibition halo.

DISCUSSION

WSL formation in the enamel around brackets is still a major challenge during orthodontic treatment[5]. Within this context, continuous efforts have been made to control white spot lesions by destructuring the biofilm, preventing enamel demineralization[8]. In the present study, the synthesis of a polymeric gel containing Ag nanoparticles was proposed to generate a biofilm destructuring process during orthodontic treatment, preventing white spot lesion.

The characterization of Ag_{nano} was performed by dynamic light scattering (DLS), evaluating the physicochemical properties of the particles. The Zeta potential analysis verifies the surface charge of the particles, in which negative charges could be observed, this fact being explained by the anionic characteristic of the Ag particle[23]. Surface charge values equal to or greater than -30 mV are necessary to promote electrostatic stability, due to the fact that it causes the highest repulsion between the particles[24,25]. In the present study, the average particle size around 100 nm also confirms the effectiveness of biological synthesis in making nanoscale particles.

In the present study, rheological analyses indicate favorable viscosity values for surface delivery of particles[26-28]. The high viscosity would not be of great value for the application proposed in the present study, since this can originate from a high crosslinking rate of the polymeric complex, thus generating the entrapment of the particle inside, preventing its action on the surface[27,28]. The polymeric complex of Chitosan Xanthan presents a dense polymer network formed, however, due to the screening of the excluded volume effects, they strongly favor the interpenetration of the polymer, enabling the communication of the particles with the surface[29].

Antimicrobial activity for S. mutans was observed only for the gel containing 5% Ag_{nano}, and a possible dose-response can be observed. The first mechanism of action of these nanoparticles is due to their surface-to-volume ratios, i.e., a large number of silver ions are released and achieve high penetration potential, occupying more easily the surfaces[30,31].

It is known that the cell membrane of S. mutans has an anionic characteristic, similar to that found on the surfaces of nanoparticles in the present study. Based on this, the inhibition mechanism can be justified by the potential of Ag_{nano} to reduce the production of extracellular polysaccharide matrix of the biofilm, and thus, not letting it form/organize[30-32]. In addition, Ag nanoparticles have an effect on lactic acid produced by bacteria, preventing the dissolution of the mineral content of tooth enamel[30].

In addition to the aforementioned benefits on the use of Ag nanoparticle-containing compounds in preventing dental caries, the study by Scarpelli et al.[33] addresses the ability of Ag to increase the microhardness of enamel caries. The mechanism is through the penetration of the Ag nanoparticle and its adhesion on hydroxyapatite crystals. In addition, silver ions released from silver nanoparticles can generate insoluble silver chloride, which increases the mineral density of hard dental tissue[30]. Thus, future studies are suggested, evaluating the tooth enamel strengthening effect of the gels developed in the present study, as well as their application in restructuring collagen exposed to the bacteria involved in caries disease. It is concluded that the gel containing 5% Ag_{nano} is promising and can be used for the inhibition of S. mutans, consequently preventing white spot lesions during and after orthodontic treatment.

REFERENCES

1. Oz AZ, Oz AA, Yazıcıoglu S. In vivo effect of antibacterial and fluoride-releasing adhesives on enamel demineralization around brackets: A micro-CT study. Angle Orthod. 2017;87(6):841-46.
2. Krasniqi S, Sejdini M, Stubljar D, Jukic T, Ihan A, Aliu K et al. Antimicrobial Effect of Orthodontic Materials on Cariogenic Bacteria Streptococcus mutans and Lactobacillus acidophilus. Med Sci Monit Basic Res. 2020;26:e920510.
3. Jing D, Hao J, Shen Y, Tang G, Lei L, Zhao Z. Effect of fixed orthodontic treatment on oral microbiota and salivary proteins. Exp Ther Med. 2019;17(5):4237-243.
4. Zhang J, Lynch RJM, Watson TF, Banerjee A. Remineralisation of enamel white spot lesions pre-treated with chitosan in the presence of salivary pellicle. J Dent. 2018;72:21-8.
5. Fernández-Ferrer L, Vicente-Ruíz M, García-Sanz V, Montiel-Company JM, Paredes-Gallardo V, Almerich-Silla JM et al. Enamel remineralization therapies for treating postorthodontic white-spot lesions: A systematic review. J Am Dent Assoc. 2018;149(9):778-86.e2.
6. Liu Y, Zhang L, Niu LN, Yu T, Xu HHK, Weir MD et al. Antibacterial and remineralizing orthodontic adhesive containing quaternary ammonium resin monomer and amorphous calcium phosphate nanoparticles. J Dent. 2018;72:53-63.
7. Qin D, Wang Y, Levey C, Ngan P, He H, Hua F; Core Outcome Set for trials on the prevention and treatment of enamel White Spot Lesions (COS-WSL) group. Protocol for the development of a Core Outcome Set for trials on the prevention and treatment of Orthodontically induced enamel White Spot Lesions (COS-OWSL). Trials. 2021; 22(1):507.
8. Nam HJ, Kim YM, Kwon YH, Kim IR, Park BS, Son WS et al. Enamel Surface Remineralization Effect by Fluorinated Graphite and Bioactive Glass-

Containing Orthodontic Bonding Resin. Materials (Basel). 2019;12(8):1308.
9. Lale S, Solak H, Hınçal E, Vahdettin L. In Vitro Comparison of Fluoride, Magnesium, and Calcium Phosphate Materials on Prevention of White Spot Lesions around Orthodontic Brackets. Biomed Res Int. 2020;2020:1989817.
10. Ramburrun P, Pringle NA, Dube A, Adam RZ, D'Souza S, Aucamp M. Recent Advances in the Development of Antimicrobial and Antifouling Biocompatible Materials for Dental Applications. Materials (Basel). 2021;14(12):3167.
11. Allaker RP, Yuan Z. Nanoparticles and the control of oral biofilms. Nanobiomaterials Clin Dent. 2019:243–75.
12. Zakrzewski W, Dobrzynski M, Dobrzynski W, Zawadzka-Knefel A, Janecki M, Kurek K et al. Nanomaterials Application in Orthodontics. Nanomaterials (Basel). 2021;11(2):337.
13. Bapat RA, Chaubal TV, Joshi CP, Bapat PR, Choudhury H, Pandey M et al. An overview of application of silver nanoparticles for biomaterials in dentistry. Mater Sci Eng C Mater Biol Appl. 2018;91:881-98.
14. Jasso-Ruiz I, Velazquez-Enriquez U, Scougall-Vilchis RJ, Morales-Luckie RA, Sawada T, Yamaguchi R. Silver nanoparticles in orthodontics, a new alternative in bacterial inhibition: in vitro study. Prog Orthod. 2020;21(1):24.
15. Fernandez CC, Sokolonski AR, Fonseca MS, Stanisic D, Araújo DB, Azevedo V et al. Applications of Silver Nanoparticles in Dentistry: Advances and Technological Innovation. Int J Mol Sci. 2021;22(5):2485.
16. Xu L, Wang YY, Huang J, Chen CY, Wang ZX, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics. 2020;10(20):8996-9031.
17. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. J Nanobiotechnology. 2018; 16(1):14.
18. Kapoor RT, Salvadori MR, Rafatullah M, Siddiqui MR, Khan MA, Alshareef SA. Exploration of Microbial Factories for Synthesis of Nanoparticles - A Sustainable Approach for Bioremediation of Environmental Contaminants. Front Microbiol. 2021;12:658294.
19. Vahabi K, mansoori GA, Karimi S. Biosynthesis of AgNPs by fungus Trichoderma Reesei (A Route for Large-Scale Production of AgNPs). Insciences J. 2011;1(1):65-79.
20. Neves JG, Marcato PD, de Paula E Silva FWG, Mantovani CPT, Prado HS, Aires CP et al. Synthesis and characterization of an experimental primer containing chitosan nanoparticles - Effect on the inactivation of metalloproteinases, antimicrobial activity and adhesive strength. Arch Oral Biol. 2021;127:105148.
21. Bellini MZ, Caliari-Oliveira C, Mizukami A, Swiech K, Covas DT, Donadi EA et al. Combining xanthan and chitosan membranes to multipotent mesenchymal stromal cells as bioactive dressings for dermo-epidermal wounds. J Biomater Appl. 2015;29(8):1155-66.
22. Murakami M, Nishi Y, Fujishima K, Nishio M, Minemoto Y, Kanie T et al. Impact of Types of Moisturizer and Humidity on the Residual Weight and Viscosity of Liquid and Gel Oral Moisturizers. J Prosthodont. 2016;25(7):570-75.
23. Algebaly AS, Mohammed AE, Abutaha N, Elobeid MM. Biogenic synthesis of silver nanoparticles: Antibacterial and cytotoxic potential. Saudi J Biol Sci. 2020;27(5):1340-51.
24. Farhadi S, Ajerloo B, Mohammadi A. Green Biosynthesis of Spherical Silver Nanoparticles by Using Date Palm (Phoenix Dactylifera) Fruit Extract and Study of Their Antibacterial and Catalytic Activities. Acta Chim Slov. 2017;64(1):129-43.
25. Mohammed AE, Al-Qahtani A, Al-Mutairi A, Al-Shamri B, Aabed KF. Antibacterial and Cytotoxic Potential of Biosynthesized Silver Nanoparticles by Some Plant Extracts. Nanomaterials (Basel). 2018;8(6):382.
26. Masoud AI, Alshams FA. The use of decorative braces in Jeddah, Saudi Arabia. J Orthod Sci. 2020;9:18.
27. Karakuş S. Preparation and rheological characterization of Chitosan-Gelatine@ZnO-Si nanoparticles. Int J Biol Macromol. 2019;137:821-28.
28. Facchinatto WM, Fiamingo A, Dos Santos DM, Campana-Filho SP. Characterization and physical-chemistry of methoxypoly(ethylene glycol)-g-chitosan. Int J Biol Macromol. 2019;124:828-37.
29. Bombaldi de Souza RF, Bombaldi de Souza FC, Thorpe A, Mantovani D, Popat KC, Moraes ÂM. Phosphorylation of chitosan to improve osteoinduction of chitosan/xanthan-based scaffolds for periosteal tissue engineering. Int J Biol Macromol. 2020;143:619-32.
30. Yin IX, Zhao IS, Mei ML, Li Q, Yu OY, Chu CH. Use of Silver Nanomaterials for Caries Prevention: A Concise Review. Int J Nanomedicine. 2020;15:3181-3191.
31. Urzedo AL, Gonçalves MC, Nascimento MHM, Lombello CB, Nakazato G, Seabra AB. Multifunctional alginate nanoparticles containing nitric oxide donor and silver nanoparticles for biomedical applications. Mater Sci Eng C Mater Biol Appl. 2020;112:110933.
32. Fakhruddin KS, Egusa H, Ngo HC, Panduwawala C, Pesee S, Samaranayake LP. Clinical efficacy and the antimicrobial potential of silver formulations in arresting dental caries: a systematic review. BMC Oral Health. 2020;20(1):160.
33. Scarpelli BB, Punhagui MF, Hoeppner MG, Almeida RSC, Juliani FA, Guiraldo RD, Berger SB. In Vitro Evaluation of the Remineralizing Potential and Antimicrobial Activity of a Cariostatic Agent with Silver Nanoparticles. Braz Dent J. 2017;28(6):738-43.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

CORRESPONDING AUTHOR

Dr. José Guilherme Neves
Department of Restorative Dentistry- Dental Materials Area, Piracicaba Dental School, Unicamp, Piracicaba, SP, Brazil. Av. Limeira, 901 - Areião, Piracicaba - SP, 13414-903 Telephone:(19) 2106-5200,
e-mail: nevesjoseguilherme@gmail.com

Received 12/04/2022
Accepted 15/06/2022