Original Article

Access this article online

Quick Response Code:

Website:
www.jorthodsci.org

DOI:
10.4103/jos.jos_29_24

Evaluation of surface roughness, cytotoxicity, and antibacterial effects of silver nanoparticle coating on copper nickel titanium orthodontic arch wires – *in vitro* study

Daphane Anishya and Ravindra K. Jain

Abstract

BACKGROUND: Surface roughness of arch wires directly impacts their corrosion behavior, friction resistance, and plaque accumulation, which may hinder tooth movement and lead to dental caries.

OBJECTIVES: The study aims to synthesize vanillin-mediated silver nanoparticles (AgNPs), characterize them, assess surface roughness and cytotoxicity of arch wires after silver nanoparticle coating, and test their antibacterial properties.

MATERIALS AND METHODS: Nine copper–nickel–titanium arch wires (CuNiTi) were cut into equal pieces. Three were sent for surface roughness assessment, three for cytotoxicity, and three for antibacterial testing. Dip coating of wires was done using the sol–gel thin film method. The surface roughness (Ra) before and after coating was evaluated using scanning electron microscopy and atomic force microscopy. Cytotoxicity testing was done with a (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using gingival fibroblasts. Statistical analysis was done using SPSS software. Antibacterial activity against *S. mutans* was tested using the Agar-well diffusion method.

RESULTS: The CuNiTi wires were coated successfully, and the coating appeared homogeneous. The mean Ra after coating (297.3+/- 30.4 nm) was significantly less than that before coating (339.7+/-49.2 nm). AgNPs showed minimal cytotoxicity against human gingival fibroblasts at different concentrations. Optical microscopy showed over 90% viability between 12.5 and 100 μg/ml. At 100 μg/ml, only 80% of cells remained viable. AgNP coating is biocompatible at concentrations up to 75 μg/ml. There was a significant intergroup difference in the zone of inhibition (antibacterial activity) noted with higher values in noncoated wires. (*P* value <0.007).

CONCLUSION: AgNPs coated on CuNiTi arch wires showed reduced surface roughness and minimal cytotoxic effects on human gingival cells and good antibacterial activity against S. mutans compared to noncoated arch wires.

Keywords:

Arch wires, cytotoxicity, silver nanoparticle, surface roughness

Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

Address for correspondence:
Dr. Daphane Anishya, Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai - 600 077, Tamil Nadu, India.
E-mail: daphaneanishya98@gmail.com

Submitted: 08-Mar-2024
Revised: 17-May-2024
Accepted: 26-Jun-2024
Published: 17-Sep-2024

Introduction

The surface morphology of orthodontic arch wires is a critical attribute that influences properties like friction, bacterial adhesion, and corrosion-related damage.[1] According to earlier research, friction losses from applied force can range from 12% to 74% and can compromise the efficiency of the treatment.[2] Therefore, it is essential to minimize friction contributed by surface roughness of the arch wire and also prevent

How to cite this article: Anishya D, Jain RK. Evaluation of surface roughness, cytotoxicity, and antibacterial effects of silver nanoparticle coating on copper nickel titanium orthodontic arch wires – *in vitro* study. J Orthodont Sci 2024;13:31.

aggregation of cariogenic bacteria. Nitinol wires and improved superelastic Niti wires used during initial leveling and alignment are known to have very high surface roughness and less hardness than stainless-steel wires.[3] A recent paper has reported that the adhesion of S. mutans, surface roughness, and surface free energy were greater in Cu-NiTi when compared to other NiTi arch wires.[4] In the recent past, research on antifriction, antibacterial, and corrosion-resistant coatings on orthodontic arch wires of different materials has been tried and tested.[1] Nanoparticle coatings can reduce static and dynamic friction arising between the archwire and the brackets by filling up the grooves and lubricating the appliance surfaces.[5]

Among the various nanoparticles tested for coating on arch wires, silver nanoparticles (AgNPs) have reported good antibacterial and antiadherent properties against S. mutans and L. acidophilus bacteria.[6,7] Nano silver-coated nickel–titanium arch wires exhibit higher microhardness, reduced friction, and increased surface roughness in contrast to the uncoated ones.[8] Synthesis of AgNPs with plant extracts as reducing agents can be beneficial in controlling the shape, size, and monodispersity of the nanoparticles.[9]

Vanillin is an active component of Vanilla Planiflora plant extract and has strong antibacterial qualities against many Gram-positive, Gram-negative, and multi-drug-resistant bacteria.[10] In a previous study, vanillin was used successfully as a reducing and capping agent for the production of gold nanoparticles.[11] Vanillin has an aromatic ring with different functional groups, which include hydroxyl, aldehyde, and ether. In the production of gold nanoparticles, the carbonyl moiety of vanillin interacted with the Au ion and reduced it to form VAuNPs.[11] Vanillin has also been used as a reducing agent for synthesizing AgNPs.[8,12] A previous study reported the production of titanium dioxide NPs using vanillin as a reducing agent.[13]

Copper NiTi wires are presently used widely with self-ligation systems, and the higher surface roughness makes them more amenable to dental plaque biofilm formation. The present study aimed at modifying the surface of copper NiTi (CuNiTi) wires by coating with vanillin-mediated AgNPs using the sol–gel dip coating method, followed by an assessment of antibacterial activity against S. mutans bacteria, cytotoxicity, and comparison of the surface roughness before and after coating.

Materials and Methods

The present study was an *in vitro* study conducted in the Department of Orthodontics. The Scientific Review Board of the institute had given approval to conduct the present study.

Sample size calculation

Sample size calculation was not performed in this study as it was a pilot *in vitro* assessment.

Sample preparation

Prefabricated CuNiTi arch wires were cut into nine equal pieces, each measuring four centimeters. Three were sent for surface roughness assessment, three for cytotoxicity, and three for antibacterial testing.

The production of solid and crystalline AgNPs requires several sequential steps using vanillin as a reducing/capping agent.[11] First, a solution containing vanillin, silver nitrate (AgNO3), distilled water, and sodium hydroxide (NaOH) is prepared. The prepared solution is then left to stir overnight in a magnetic stirrer, allowing the vanillin to dissolve into silver ions (Ag+) and silver nanoparticles, and at the same time acts as a stabilizing agent.

After complete mixing of the solutions, the solution undergoes a 48-hour heating system in a hot air oven to promote the formation of stable, crystalline silver nanoparticles. The drying process results in the evaporation of excess water and leaving the wire samples coated with the newly synthesized AgNPs using a dip coating technique, wherein the wire samples were immersed within the solution containing the AgNPs. The dip coating was done at ambient room temperature. To ensure that the wire samples were evenly coated with AgNPs, the dip coating procedure was carried out a second time. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) investigations were used to evaluate the homogeneity of the coating.

Scanning electron microscopy analysis (SEM and SEM EDAX)

The SEM analysis is done to visualize the surface topography or surface texture of solid objects. A high-resolution JSM-IT800 Schottky Field Emission Scanning Electron Microscope (HR-SEM; Model: JEOL, JSM IT-800 EDA-OXFORD machinery, USA) was used to study the surface topography. The surface topography of the commercially available uncoated rectangular CuNiTi wires and AgNP-coated rectangular CuNiTi wires were examined using SEM. The wire surface was studied using a field of view of 51.2 × 38.4 μm.

Atomic force microscopy

AFM was used to acquire three-dimensional images of the surface topography of uncoated and AgNP-coated samples. Surface profiling was done using a Digital Instruments DimensionTM 3100 Atomic Force

Microscope. The wire samples were mounted on a metallic holder with cyanoacrylate adhesive. One portion from the center and two from each side of the arch wire were selected. The surface topography of the same three sections of the wire was examined both before and after coating. The Gwyddion program was used to process the 3D images of the arch wires. The Ra value is a measure of surface roughness; a higher Ra value shows a rougher surface, and a lower Ra signifies a smoother surface. This metric is critical for assessing the surface topography of the materials. The Ra values of the three sections were subjected to descriptive statistics to obtain the final Ra value of the arch wires before and after coating.

Cytotoxicity testing

The current study employed the MTT assay to examine the cytotoxic effects of different concentrations of AgNPs. The MTT assay is a colorimetric test used to determine the metabolic activity of cells as a marker of proliferation, cell viability, and cytotoxicity. The NAD (P) H-dependent oxidoreductase enzymes present in the live/viable cells convert MTT to formazan. The insoluble formazan crystals are broken down using a solubilization solution, and the absorbance of the colored solution that results is determined at 500–600 nm using a multiwell spectrophotometer. The more metabolically active, living/viable cells exist, the darker is the solution.[14,15]

Procedure

1×10^5 cells per well were introduced in 24-well plates and incubated at 37°C with 5% CO2. The AgNPs were incubated with human gingival cells for 24 h. On alternate days, fresh media were added. The used media-containing samples were removed from the well shortly after incubation and rinsed with phosphate-buffered saline (PBS 7.4 pH). MTT (4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl—tetrazolium bromide) was mixed into 50 ul of wells (5 mg/ml) and incubated for 4 hours. Post incubation, 50 µl of DMSO was introduced to each well for dissolution of formazan crystals. Cells that had received no treatment at all had been selected for use as a negative control. The absorbance at 570 nm was determined using an ELIZA plate reader. Measurements were taken, and a graphical representation of the concentration necessary to inhibit an organism by 50% (IC 50) was established.[14,15]

Antibacterial testing – Agar well diffusion method

The Agar-well diffusion method was used to study the antibacterial effect of both coated and uncoated wire samples against *S. mutans*. Under sterile conditions, 100 microliters of *S. mutans* were uniformly placed on the nutrient agar plates. The Gram-positive bacteria were tested against amoxicillin. The agar plate was incubated for 24 hours at 37°C to promote bacterial growth. Following the incubation period, the test was regarded as effective if a zone of inhibition formed around the well, indicating the existence of an antimicrobial property.

Statistical analysis

Data obtained from AFM tests, cytotoxicity, and antibacterial assessment were tabulated using a Microsoft Excel 365 spreadsheet. The data were then imported into the statistical package for social sciences (SPSS) software version 26 (SPSS Inc.) for statistical analysis. The Shapiro–Wilk test was performed to ensure that the data were normally distributed. The intergroup differences in surface roughness, cytotoxicity, and zone of inhibition were evaluated using a paired *t*-test. *P* values less than 0.05 were considered statistically significant.

Results

Scanning electron microscopy

The wires were studied within a field of view of 51.2 × 38.4 µm at 2.50x magnification. The uncoated CuNiTi wires have irregular and rough surfaces, while the AgNP-coated CuNiTi wires have a smooth and homogeneous coating layer [Figures 1 and 2].

Scanning electron microscopy with EDAX (SEM)

CuNiTi wires coated with AgNPs were subjected to SEM EDAX at an accelerating voltage of 15 KeV. 2.6 weight percent silver, 34 weight percent carbon, and 61.5 weight percent oxygen were found. This validates the AgNP coating on CuNiTi wires [Figure 3].

Atomic force microscopy

Comparing the 3D images of the coated and uncoated CuNiTi wires, the coated wires appeared to have a smoother and more even surface topography and texture. This indicates that the coating altered the surface topography of the wires, which can affect the functional properties of the wires [Figure 4 and Table 1]. The Ra value after coating was significantly decreased after coating. (*P* value <0.009).

Cytotoxicity testing

The AgNPs showed minimal cytotoxicity against human gingival fibroblasts at different concentrations. Optical microscopy was used to visualize the cells. Between

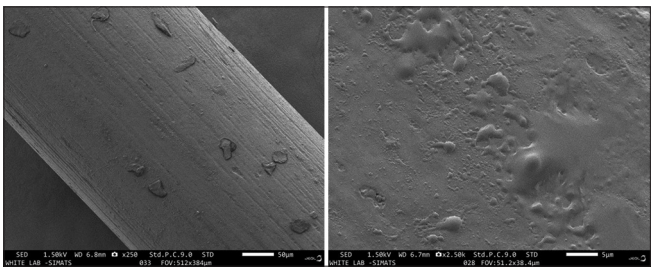

Figure 1: Uncoated CuNiTi – SEM images – rough and course surface evident

12.5 and 100 µg/ml, more than 90% of the cells were considered viable as they did not show any clustering or change in their morphology. At 100 µg/ml, however, only 80% of the cells were still viable. Hence, it can be inferred that AgNP coating is biocompatible when applied at concentrations up to 75 µg/ml. Optical microscopy images of cell morphology at 75 µg/ml (highest % cell viability when compared with other concentrations and hence we displayed the cell morphology at 75 µg/ml) showed proper cell attachment with AgNPs. A similar kind of cell morphology is seen when compared with the control [Table 2]. A graphical representation of the concentration necessary to inhibit an organism by 50% (IC 50) was established [Figure 5]. Based on the statistical analysis, there was a significant increase in cytotoxicity at 100 µg/ml concentration of AgNPs when compared to 12.5 and 75 µg/ml concentration of AgNPs (*P* value <0.003) [Table 2].

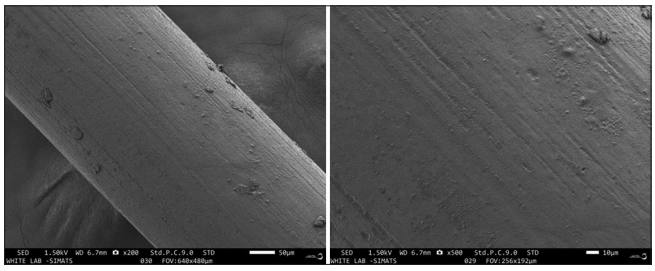

Figure 2: Coated CuNiTi – SEM images, smooth surface evident

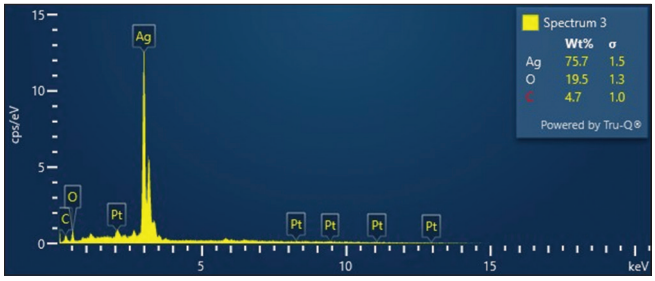

Figure 3: Coated CuNiTi – SEM EDAX. Presence of AgNPs is confirmed

Antibacterial testing

The mean and standard deviation of the zone of inhibition against *S. mutans* for uncoated and coated wire samples and control antibiotic are mentioned in Table 3. AgNP-coated wires demonstrated a good antibacterial action against *S. mutans* [Figures 6 and 7]. A significant intergroup difference in the zone of inhibition was noted with higher values in noncoated wires (*P* value <0.007) [Table 3].

Discussion

Biocompatibility, corrosion resistance, and frictional characteristics of orthodontic arch wires are all influenced by their surface roughness (SR), which in turn affects orthodontic tooth movement. Despite the manufacturers' claims, orthodontic arch wires have irregular, uneven, and rough surfaces as noted in the present study. Increased surface roughness of orthodontic arch wires can lead to an increase in bacterial adherence.[16] On intraoral use, arch wire surfaces become rough over a while, leading to an increase in bacterial buildup and friction, which

Table 1: Mean, standard deviation, and paired *t*-tests *P* of average R_a

Surface roughness	Uncoated wire	Coated wire	*P*
R_a	339.7±49.2 nm	297.3±30.4 nm	0.009

Table 2: Mean and standard deviations of cytotoxicity values for both samples of coated wires at different concentrations

Concentration of AgNps	1 2.5-75 µg/ml	100 µg/ml	*P*
	90±5.7	80±5.2	0.003

Table 3: Zone of inhibition

Microorganism	Antibiotic	Uncoated wire	Coated wire	*P*
S.mutans	14±1.9	15±1.2	10±2.0	0.007

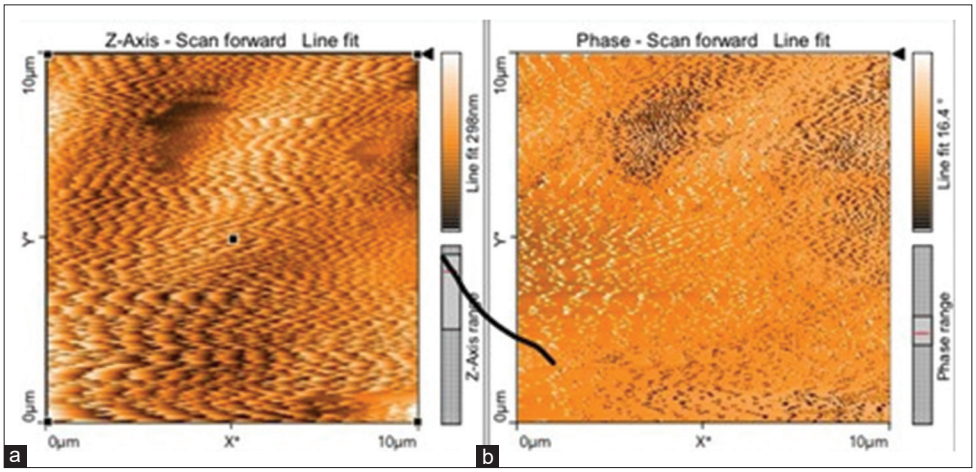

Figure 4: AFM images of uncoated and AgNP-coated CuNiTi wires: (a) Uncoated – rougher and uneven surface; (b) coated – smooth surface

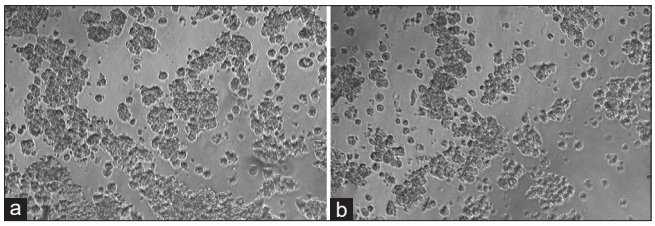

Figure 5: Optical microscopy viable cells before (a) coating and (b) after coating

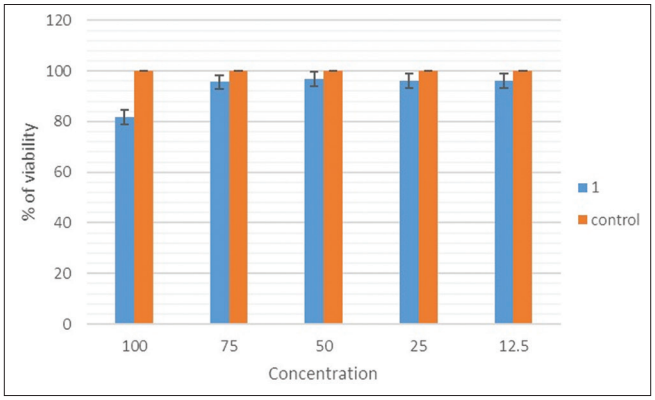

Figure 6: Graphical representation of the cell viability in % for different concentrations of the AgNPs

Figure 7: Zone of inhibition – evident antibacterial activity seen around coated arch wires

impairs effective sliding.[17] Hence, coating with metal NPs can be beneficial in improving the surface morphology.

In the present study, the dip coating method was used for coating the CuNiTi rectangular wires with vanillin-mediated AgNPs. The coating was analyzed with SEM and SEM EDX analysis. The surfaces of noncoated samples compared to that of coated samples were rough. The study also found that the NP coating was biocompatible and the coated wires showed excellent antibacterial action against *S. mutans*.

Surface roughness assessment

The surface topography and roughness can be influenced by different types of coatings.[18] Silver coating on NiTi wires has been reported with reduced surface roughness.[19] In the present study, vanillin-mediated silver NPs were coated on Cu NiTi wires and the surface roughness was significantly less than that of noncoated arch wires. A previous study concluded reduced surface roughness after AgNP coating of stainless-steel orthodontic brackets.[20] On the contrary, a study showed that nanosilver-coated wires had no statistically significant differences between the coated and uncoated wires in terms of microscopic surface roughness and microhardness.[8]

Antibacterial activity

In a previous systematic review, it was reported that modifying the surfaces of orthodontic arch wires by coating with NPs appears to offer some level of and antiadherence activity.[21] In the current study, the antibacterial property was tested against *S. mutans* as it is the most common strain of bacteria responsible for white spot lesions during or after fixed orthodontic treatment. The results of the current study have confirmed that AgNP-coated CuNiTi arch wires exhibited excellent antibacterial activity. In a study by Gil FJ *et al.*,[22] it was observed that electrodeposition of silver NPs on NiTi orthodontic arch wires can prevent the growth of oral bacteria including *S. mutans* while retaining their characteristics and nickel release levels. Mhaske *et al.*[7] in their study had examined the antiadherent and antibacterial qualities of NiTi and stainless-steel orthodontic wires coated with AgNPs against L. acidophilus and concluded that coated wires showed significant antiadherent and antibacterial properties against *L. acidophilus.*

Cytotoxicity assessment

For coating of orthodontic arch wires with a homogeneous and smooth NP layer utilizing nanochemicals, the sol–gel method can be used.[18] AgNPs have both beneficial and detrimental properties, posing as a paradoxical weapon that can both eliminate bacteria and induce cytotoxicity.[23] The results of the cytotoxicity tests indicated that the AgNP coating was biocompatible at concentrations below 75 µg/mm where over 90% of human gingival cells were found to be viable, but only 80% of the cells were viable at concentrations over 100 µg/ml, making it less biocompatible compared to 75 µg/mm. According to experiments on cell culture, AgNPs elicit cytotoxicity in a dose-, size-, and time-dependent state in an array of human cell lines, especially those with diameters ≤10 nm. Tests on *in vivo* animal models have demonstrated that AgNPs may cross the mice's blood-brain barrier and cause neurotoxicity and

neuronal death. A systematic review concluded that the cytotoxicity of AgNPs is determined by several parameters, including the size, shape, concentration, and aggregation of the nanoparticles; smaller particles exhibit increased toxicity. They also stated that cytotoxicity is also dependent on the method used for its assessment.[23]

Limitations

In the present study, coating thickness and the coating stability especially in intraoral conditions were not evaluated. The antibacterial property against only one bacterium was evaluated. Further studies addressing the limitations of the present study must be planned and executed.

Conclusion

In the present study, CuNiTi arch wires were successfully coated with vanillin-mediated AgNPs using a dip coating method. The arch wire surface topography was altered, and a significant reduction in the surface roughness due to the NP coating was noted. The AgNPs were biocompatible, and the coated samples had good antibacterial properties against *Streptococcus mutans*, a bacterium frequently related to dental caries. Consequently, CuNiTi arch wires coated with AgNPs have benefits that make them advantageous in orthodontic treatment.

Ethical approval

The Institutional Ethics Committee of Chennai, Tamil Nadu, India granted ethical approval. The Scientific Review Board number for the study is **SRB/SDC/ORTHO-2201/23/110** and Instituitional Human ethical Committee number is **IHEC/SDC/ORTHO-2201/23/261.**

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhang R, Han B, Liu X. Functional surface coatings on orthodontic appliances: Reviews of friction reduction, antibacterial properties, and corrosion resistance. Int J Mol Sci 2023;24:6919. doi: 10.3390/ijms24086919.
2. El-Bialy T, Alobeid A, Dirk C, Jäger A, Keilig L, Bourauel C. Comparison of force loss due to friction of different wire sizes and materials in conventional and new self-ligating orthodontic brackets during simulated canine retraction. J Orofac Orthop 2019;80:68-78.
3. Yu J-H, Wu LC, Hsu JT, Chang YY, Huang HH, Huang HL. Surface roughness and topography of four commonly used types of orthodontic archwire. J Med Biol Eng 2011;31:367-70.
4. Abraham KS, Jagdish N, Kailasam V, Padmanabhan S. Streptococcus mutans adhesion on nickel titanium (NiTi) and copper-NiTi arch wires: A comparative prospective clinical study. Angle Orthod 2017;87:448-54.
5. Iijima M, Muguruma T, Brantley W, Choe HC, Nakagaki S, Alapati SB, *et al*. Effect of coating on properties of esthetic orthodontic nickel-titanium wires. Angle Orthod 2012;82:319-325.
6. Espinosa-Cristóbal LF, López-Ruiz N, Cabada-Tarín D, Reyes-López SY, Zaragoza-Contreras A, Constandse-Cortéz D, *et al*. Antiadherence and antimicrobial properties of silver nanoparticles against *Streptococcus mutans* on brackets and wires used for orthodontic treatments. J Nanomater 2018;2018:9248527. doi: 10.1155/2018/9248527.
7. Mhaske AR, Shetty PC, Bhat NS, Ramachandra CS, Laxmikanth SM, Nagarahalli K, *et al*. Antiadherent and antibacterial properties of stainless steel and NiTi orthodontic wires coated with silver against Lactobacillus acidophilus—An *in vitro* study. Prog Orthod 2015;16:1-6. doi: 10.1186/s40510-015-0110-0.
8. Rattan Khurana DM, Urala AS, Gautam U, Abraham A. Evaluation Of Frictional Resistance, Microscopic Surface Roughness and Microhardness Of Nano-Silver Coated Nickel-Titanium Archwires: An In-Vitro Study. Journal of Pharmaceutical Negative Results. 2022 Dec 31:5912-8.
9. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, *et al*. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surf B Biointerfaces 2009;74:328-35.
10. Maisch NA, Bereswill S, Heimesaat MM. Antibacterial effects of vanilla ingredients provide novel treatment options for infections with multidrug-resistant bacteria-A recent literature review. Eur J Microbiol Immunol 2022;12:53-62.
11. Vanillin mediated green synthesis and application of gold nanoparticles for reversal of antimicrobial resistance in Pseudomonas aeruginosa clinical isolates. Heliyon 2019;5:e02021.
12. Microwave assisted synthesis and UV-Vis spectroscopic studies of silver nanoparticles synthesized using vanillin as a reducing agent. J Mol Liq 2011;159:165-9.
13. García-Castañeda MC, Castellanos-Águila JE, Maury-Cuna GHI, Olea-Amezcua MA, Escalante-Gomez VM, Fuentes-Ramirez R. A novel route to obtain TiO2 nanoparticles using green synthesis with vanillin and Bougainvillea glabra Choisy extract. Appl Nanosci 2021;11:887-94.
14. Liao C, Li Y, Tjong SC. Bactericidal and cytotoxic properties of silver nanoparticles. Int J Mol Sci 2019;20:449.
15. Vistica DT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd MR. Tetrazolium-based assays for cellular viability: A critical examination of selected parameters affecting formazan production. Cancer Res 1991;51:2515-20.
16. Tawfik M, Maaly T, EL-Nagar R. Evaluation of surface roughness and microbial biofilm adhesion of different orthodontic arch-wires. Egypt Dent J 2020;66:727-36.
17. Sapata DM, Ramos AL, Sábio S, Normando D, Pascotto RC. Evaluation of biofilm accumulation on and deactivation force of orthodontic Ni-Ti arch wires before and after exposure to an oral medium: A prospective clinical study. J Dent Res Dent Clin Dent Prospects 2020;14:41-7.
18. Bącela J, Łabowska MB, Detyna J, Zięty A, Michalak I. Functional coatings for orthodontic archwires-A review. Materials 2020;13:3257. doi: 10.3390/ma13153257.
19. Krishnan M, Seema S, Tiwari B, Sharma HS, Londhe S, Arora V. Surface characterization of nickel titanium orthodontic arch wires. Armed Forces Med J India 2015;71:S340-5.e5.
20. Ghasemi T, Arash V, Rabiee SM, Rajabnia R, Pourzare A, Rakhshan V. Antimicrobial effect, frictional resistance, and surface roughness of stainless steel orthodontic brackets coated

with nanofilms of silver and titanium oxide: A preliminary study. Microsc Res Tech 2017;80:599-607.

21. Maliael MT, Varghese RM, Subramanian AK. Antibacterial activity of nanoparticle-coated orthodontic archwires: A systematic review. J Int Oral Health 2023;15:1.
22. Gil FJ, Espinar-Escalona E, Clusellas N, Fernandez-Bozal J, Artes-Ribas M, Puigdollers A. New Bactericide orthodonthic archwire: NiTi with silver nanoparticles. Metals 2020;10:702.
23. Akter M, Sikder MT, Rahman MM, Ullah AKMA, Hossain KFB, Banik S, *et al*. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. J Advert Res 2018;9:1-16. doi: 10.1016/j.jare.2017.10.008.