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IP Indian Journal of Orthodontics and Dentofacial Research

Journal homepage: https://www.ijodr.com/

Original Research Article

Evaluation of surface-modified orthodontic brackets with silver nanoparticles and its influence on bacterial inhibition – An in vitro study

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A R T I C L E I N F O

Article history:
Received 04-06-2023
Accepted 03-08-2023
Available online 08-09-2023

Keywords:
White spot lesion
Antibacterial
Lactobacillus acidophilus:
Streptococcus mutans

A B S T R A C T

Introduction: Compared to other fixed orthodontic appliances, orthodontic brackets have a substantial impact on enamel demineralization. This demineralization is a result of organic acid production by various microorganisms, primarily from Streptococcus mutans and Lactobacillus acidophilus. Preventing white spot lesions and caries during orthodontic treatment presents a considerable challenge for clinicians. Silver exhibits broad-spectrum antimicrobial activity against various microorganisms, including Gram-positive and Gram-negative bacteria, certain viruses, fungi, protozoa, and antibiotic-resistant strains. As such, the purpose of this research is to investigate the antibacterial properties of orthodontic brackets coated with silver nanoparticles against Streptococcus mutans and Lactobacillus acidophilus.

Materials and Methods: The study involved 80 stainless steel orthodontic brackets, which were divided into four groups, each containing 20 brackets. In each group, there were uncoated brackets serving as the control group, and the experimental group consisted of brackets coated with silver dioxide. Coating was done by physical vapor deposition with the help of RF Magnetron sputtering unit. Surface morphology and material composition was analyzed by SEM and EDS unit. Afterward, the brackets underwent microbiological tests to evaluate their antibacterial efficacy against Streptococcus mutans and Lactobacillus acidophilus.

Results: The study found that silver nanoparticle-coated stainless-steel brackets exhibited effective antibacterial properties against both Streptococcus mutans and Lactobacillus acidophilus when compared to the uncoated brackets. Silver nano particle coated SS brackets showed increased anti-bacterial effect towards S. mutans than L acidophilus.

Conclusion: Using silver nanoparticles to coat orthodontic brackets represents an innovative and effective approach to prevent the formation of white spot lesions in patients receiving fixed orthodontic treatment. The antimicrobial properties of silver nanoparticles can combat bacteria like Streptococcus mutans and Lactobacillus acidophilus, responsible for enamel demineralization and the occurrence of white spot lesions. This advancement in bracket technology has the potential to significantly reduce the risk of enamel damage and improve overall oral health outcomes for orthodontic patients.

1. Introduction

The most frequently used method for treating malocclusion is fixed orthodontic treatment which includes brackets, bands, arch wires, ties, etc. Due to their design and

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https://doi.org/10.18231/j.ijodr.2023.032

structure, fixed appliances can create favorable conditions that promote the colonization of cariogenic microorganisms due to difficulty in maintaining adequate oral hygiene. As a consequence, the elevated oral microbial count puts the patient at a greater risk for enamel demineralization.[1]

After fixed orthodontic treatment, the occurrence of enamel demineralization can affect approximately 50% to 70% of patients. Within just one month of treatment, white spot lesions (WSL) around orthodontic brackets have been demonstrated in some cases.[2,3]

Enamel demineralization is significantly influenced by orthodontic brackets as compared to other fixed orthodontic appliances, as brackets are attached to the tooth surface throughout the orthodontic treatment period and the complex design of the bracket impedes proper access to the tooth surface for effective cleaning.[4]

The primary reasons for bacteria's initial affinity to a solid surface are electrostatic and hydrophobic interactions. Stainless steel, characterized by its highest critical surface tension and high free energy, tends to attract bacteria more readily, resulting in a higher capacity for plaque retention.[5–10]

Research has revealed that the use of metallic orthodontic brackets can induce specific changes in the oral environment. These changes include reduced pH levels, increased plaque accumulation, and higher colonization of Streptococcus mutans and Streptococcus sobrinus bacteria.[11,12]

Organic acids from microorganisms, especially Streptococcus mutans and Lactobacillus acidophilus, cause enamel demineralization. Streptococcus mutans makes the tooth environment acidic. At low pH, lactobacilli count increases while the number of Streptococcus mutans decreases.[13] Preventing white spot lesions and caries during orthodontic treatment poses a significant challenge to both the clinician and the patient. These lesions are unesthetic, unhealthy, and potentially irreversible, making their prevention of utmost importance. Several methods such as fluoride varnishes or mousses, different kinds of toothpaste, and mouthwashes have been suggested and developed to reduce this biological effects.[14,15] Woefully only about 15% of orthodontic patients adhere and follow to the instructions.

Since ancient times, silver ions or salts have been known to have antimicrobial properties.[16] Silver is currently used to inhibit bacterial growth in a wide range of applications, including dental work, catheters, and burn wound dressings.[17]

In light of the rise in antibiotic-resistant bacterial strains, certain metals, especially in nanoparticle form, have garnered heightened interest. Nanoparticles typically consist of insoluble particles with sizes smaller than 100 nm. Compared to traditional antibiotics, bacteria have a reduced likelihood of developing resistance to metal nanoparticles. Utilizing nanoparticles can help decrease microbial adhesion and prevent caries by incorporating them into dental materials or coating surfaces.[18,19]

Several studies have shown the impact of silver, zinc oxide, and titanium oxide nanoparticles on various organisms. Out of these metals, silver stands out for its well-known antimicrobial activity against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria, certain viruses, fungi, protozoa, and antibiotic-resistant strains.[20–26]

Silver-coated Nickel-Titanium (NiTi) and Stainless Steel (SS) wires have been explored for their antimicrobial properties.[27] Given the limited research on silver nanoparticles coated orthodontic brackets, this study was designed to assess the effectiveness of such brackets in their antibacterial properties against Streptococcus mutans and Lactobacillus acidophilus.

2. Materials and Methods

This study involved 80 stainless-steel orthodontic brackets, which were divided into four test groups, with each group containing 20 specimens.

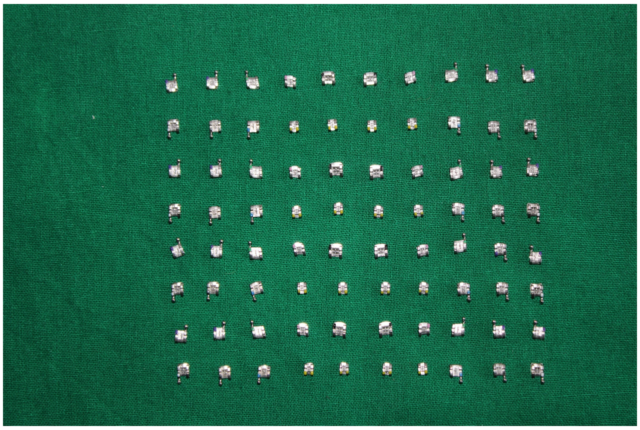

Fig. 1: Uncoated SS brackets.

Grouping [Figures 1 and 2]

1. Group 1A: 20 uncoated SS brackets for S. mutans test.
2. Group 1B: 20 surface-coated SS brackets for S. mutans test.
3. Group 2A: 20 uncoated SS brackets for L. acidophilus test.
4. Group 2B: 20 surface-coated SS brackets for L. acidophilus test.

2.1. Bacterial strains used

1. Lactobacillus acidophilus (MTCC 10307 in MRS broth, 24-hour incubation at 37°C.
2. Streptococcus mutans (MTCC 890 in BHI broth, 24-hour incubation at 37°C.

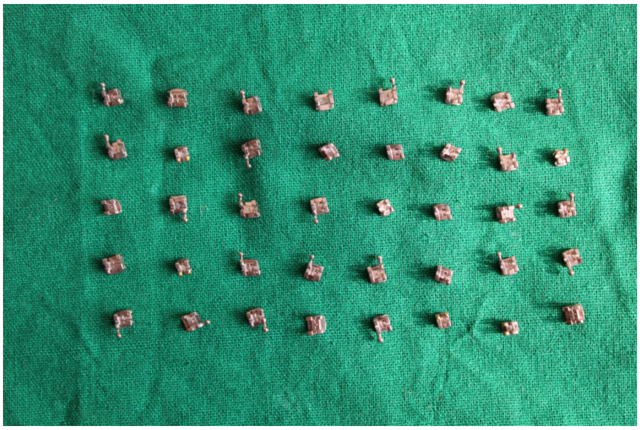

Fig. 2: Coated SS brackets

Orthodontic brackets coateing with silver nanoparticles using RF Magnetron Sputtering.

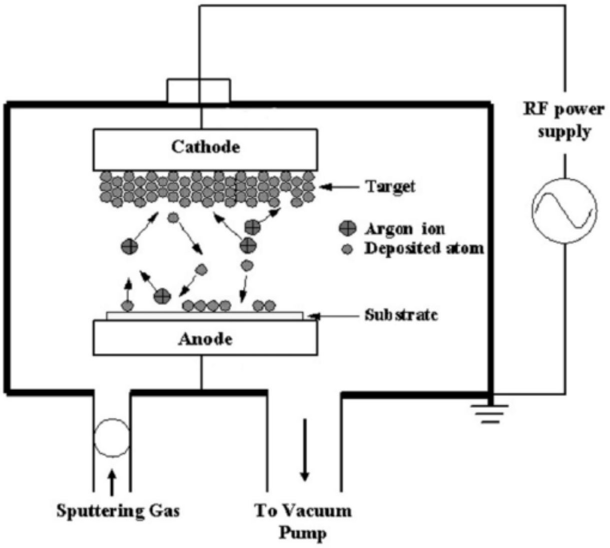

Fig. 3: RF magnetron sputtering machine

Sputter deposition is a vacuum-based process for thin film creation. It involves using RF power to ionize inert gas (usually argon) in the chamber containing the target material, substrate, and RF electrodes [Figure 3]. The ions interact with the target material, forming a coating on the substrate.

In this study, 40 stainless steel orthodontic brackets were coated with silver nanoparticles using sputtering. The process involved a vacuumed chamber with plasma generating silver atom deposition onto the brackets. The distance between substrate and target was 7 cm, and sputtering lasted for 10 minutes, achieving a thin, uniform silver coating [Figure 4]. SEM [Figure 5] and EDS evaluated the silver nanoparticle deposition on the brackets.

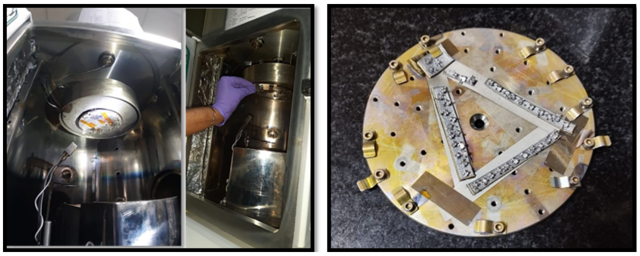

Fig. 4: RF magnetron sputteringbefore coating (Left) & after coating (Right).

Fig. 5: Scanning electron microscope (JOEL SEM S 2200)

RF Sputter Tool Details: [Figure 6]

1. Tool Make: HHV Pumps
2. Base Pressure: 4e-6 Torr
3. RF Gun Frequency: 14.56 MHz
4. Number of Samples: 80
5. Pre-Clean: Ultrasonication in IPA
6. Thin film Deposition rate was 10 A/S
7. Coating Thickness - 1000 A
8. Deposition Pressure: 4e-6 Torr
9. Deposition Temp: Room Temp
10. Sputter Power: 50 to 80 Watts

2.2. *SEM characterization on orthodontic brackets*

The scanning electron microscope was used to examine the surface morphology of the coated orthodontic bracket at Zoom Scales of 500x, 1000x, 1500x, and 2000x. [Figures 12 and 13].

2.3. *Energy dispersive X-ray spectroscopy (EDS) [Figure 7]*

EDS for material composition analysis was done on Rigaku EDS HyPix-3000 to analyze the silver content on the orthodontic bracket after coating.

Fig. 6: RF magnetron sputtering unit HHV

Fig. 7: Energy dispersive X-ray spectroscopy EDS (Rigaku EDS HyPix-3000)

2.4. *Antimicrobial activity assay of orthodontic brackets*

2.5. *Disk agar diffusion test (DAD)*

The antimicrobial activity test for orthodontic brackets for S. mutans and L. acidophilus were tested by Disk agar diffusion method on B H I Agar plates and M R S Agar Plates respectively. About 100μl of pre-cultured test organisms 1x10^8 CFUs were spread onto the agar plates using a sterilized swap [Figure 8]. Four coated & uncoated sterile orthodontic brackets were located on each agar plate in equidistance. The bacterial plates were duplicated and then incubated at 37°C for 24 hours. Subsequently, the zone of inhibition was measured in millimeters and recorded in a table. [Figures 9 and 10]

Fig. 8: Disk agar diffusion test

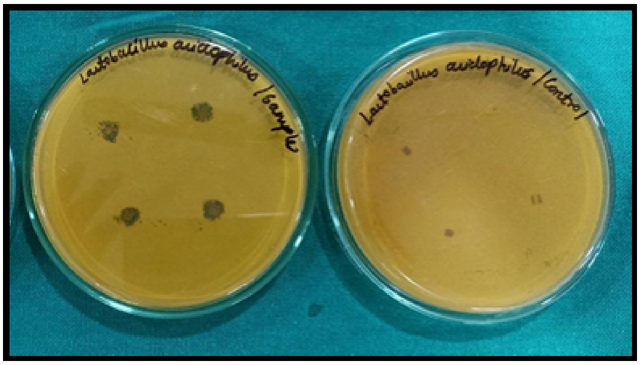

Fig. 9: Petri dish showing ZOI of L. acidophilus.

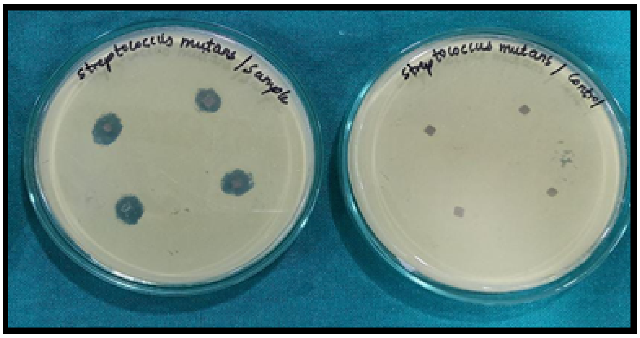

Fig. 10: Petri dish showing ZOI of S. mutans.

3. Results

3.1. *Data analysis*

The data was entered into a Microsoft Excel spreadsheet and thoroughly reviewed for discrepancies. Summarized information was presented using tables and graphs. The analysis was conducted using SPSS version 21.0. The Shapiro-Wilk test was utilized to determine if the variables followed a normal distribution. Since the data was normally distributed, bivariate analyses were conducted using parametric tests, specifically independent t-tests. The level of statistical significance was set at a p-value less than 0.05.

3.2. Surface morphology and material composition of coated and uncoated SS orthodontic brackets

SEM examination of SS orthodontic bracket showed that the silver coating was done uniformly on the surface and the thickness was about 1000A^0 [Figure 11]. The morphology examined at 500x,1000x,1500x and 2000x showed some pores on the uncoated SS bracket and the coated bracket had smooth surface without any pores. [Figures 12 and 13].

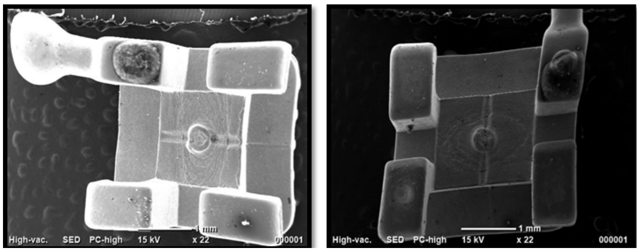

Fig. 11: SEM Pictures of coated and uncoated brackets

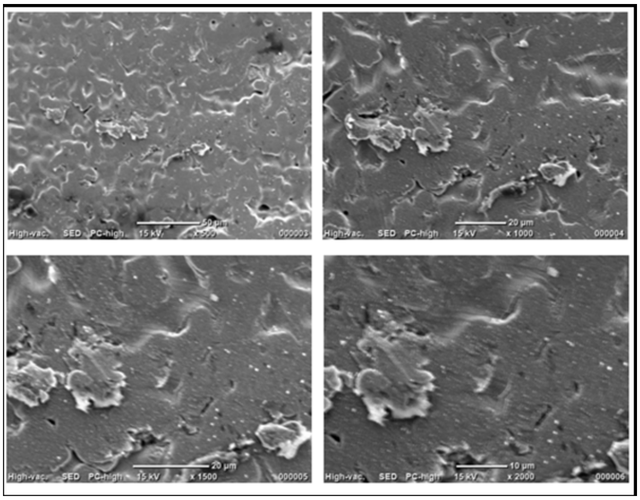

Fig. 12: SEM Morphology before coating showig 500X, 1000X, 1500X, 2000X

The material composition analysis done by Energy dispersive X-ray spectroscopy (EDS) revealed the high silver content in the coated SS bracket. The Mass % of Ag in uncoated SS bracket is 5.27 and the coated SS bracket has Mass% value of 44.64. [Figures 14 and 15].

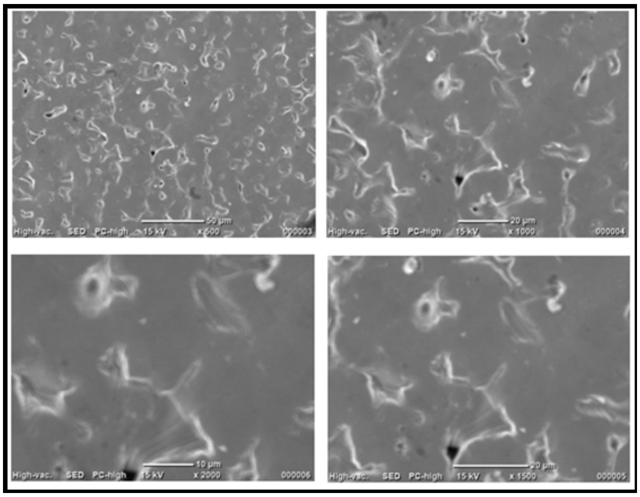

Fig. 13: SEM Morphology after coating 500X, 1000X, 1500X, 2000X

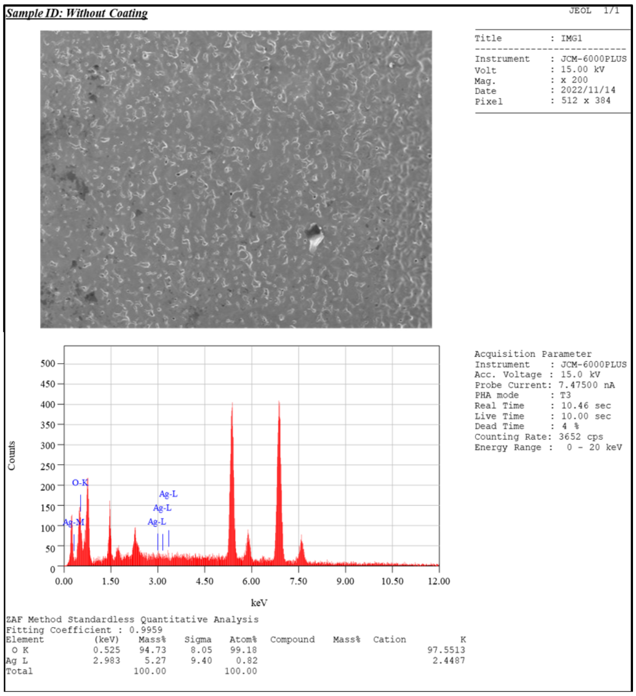

Fig. 14: EDS analysis before coating.

3.3. Antimicrobial activity assay of orthodontic brackets

The study analyzed:

1. Antimicrobial activity of silver nanoparticle-coated stainless steel orthodontic brackets compared to uncoated brackets against Streptococcus mutans.
2. Antimicrobial activity of silver nanoparticle-coated stainless steel orthodontic brackets compared to uncoated brackets against Lactobacillus acidophilus.

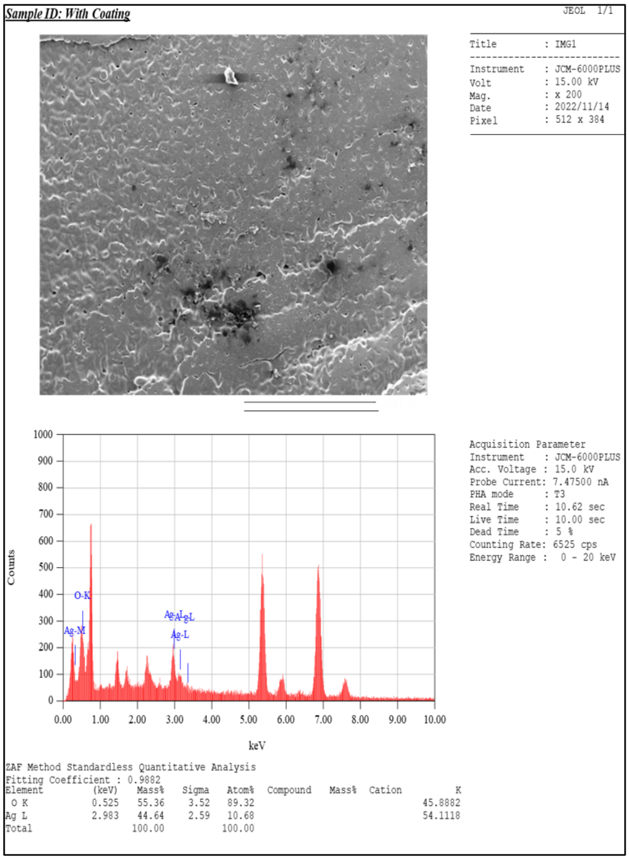

Fig. 15: EDS analysis after coating.

3. Antimicrobial activity of silver nanoparticle-coated stainless steel orthodontic brackets against both Streptococcus mutans and Lactobacillus acidophilus.

Based on the research finding, it appears that the uncoated brackets had significantly more bacterial adhesion compared to the silver (Ag) coated brackets. This was demonstrated through the use of a zone of inhibition test. In this study the zone of inhibition formed for Ag-coated stainless-steel brackets for S. mutans is 6.85mm (mean) and for L. acidophilus is 4.50mm(mean), and for the uncoated stainless-steel bracket, there was no zone formed, [Table 1].

The antimicrobial effectiveness of silver-coated stainless steel orthodontic brackets was found to be higher against Streptococcus mutans compared to Lactobacillus acidophilus. This difference was statistically significant when comparing the two groups (P value < 0.001). [Table 2]

Overall, these results suggest that the silver coating on the brackets may have some antibacterial properties, which may help to reduce the amount of bacterial adhesion on these surfaces.

4. Discussion

Studies have demonstrated that fixed orthodontic appliances lead to a rapid increase in dental plaque accumulation, and this plaque tends to have a lower pH compared to non-orthodontic cases.[28] Following the insertion of orthodontic appliances, there is a rapid change in the composition of the bacterial flora of the dental plaque. Furthermore, in orthodontic patients, the presence of acidogenic bacteria, particularly Streptococcus mutans, significantly rises. When these bacteria are exposed to fermentable carbohydrates, they produce acid by-products, leading to a decrease in plaque pH. If the pH drops below the remineralization threshold, carious decalcification occurs.[29]

As caries progress, the number of streptococci decreases while the presence of lactobacillus increases. Lactobacillus acidophilus is responsible for the advancement of caries.[29] In the vicinity of orthodontic appliances or under loose bands, where the environment is highly cariogenic, these lesions can advance swiftly. If left untreated, they may result in carious cavitations requiring appropriate restoration. Hence, it is essential to prevent white spot lesions (WSLs) to avoid tooth decay and preserve the smile's aesthetics.

Silver possesses antimicrobial properties and acts by binding to critical functional clusters of enzymes. Furthermore, it induces the release of K+ ions from bacterial plasma or cytoplasmic membrane, which are sites associated with various essential bacterial enzymes. This makes the K+ ion release a potent target for silver's antimicrobial action.[30]

In the study by Gilani RA et al[31] the silver-coated brackets exhibited anti-adherent and anti-bacterial properties when tested against S. mutans.

In another study by Irania Jasso et al[32] surface modified brackets with AgNPs were tested for antibacterial property. This study utilized a radioactive marker (3H) to label the bacteria, resulting in positive findings for S. mutans and S. sobrinus. However, the antibacterial property of silver-coated stainless-steel brackets against S. mutans and L. acidophilus remains unclear, as reported in previous studies.

Hence, this study was done to examine the antibacterial effectiveness of silver-coated stainless-steel brackets against S. mutans and L. acidophilus.

Silver surface coating can be accomplished using different techniques, such as physical vapor deposition, electrodeposition, electroless, and metallurgical methods.[33]

In the study by Yamamoto[24] the surface of stainless-steel orthodontic brackets was coated with silver using the RF magnetron sputtering method, which falls under physical vapor deposition. The thickness of the silver coating was less than 1000 Angstrom [A^0]. Physical vapor deposition, specifically RF magnetron sputtering, is known for its strong antimicrobial effect among the various coating methods.

The antibacterial efficacy of silver-coated stainless-steel brackets against S. mutans and L. acidophilus was assessed

Table 1: Intergroup comparison of ZOI of S mutans andLactobacillus acidophilus

Zone of inhibition		N	Mean	Std. Deviation	Std. Error Mean	P value
S. mutans	Coated sample	20	6.85	0.745	0.167	<0.001
	Uncoated sample	20	.00	0.000	0.000	
Lactobacillus acidophilus	Coated sample	20	4.50	0.761	0.170	<0.001
	Uncoated sample	20	.00	0.000	0.000	

Table 2: Comparison of ZOI, of S. mutans and Lactobacillus acidophilus on coated samples

		Mean	Std. Deviation	Std. Error Mean	P Value
ZOI	S. mutans	6.85	.745	0.167	<0.001
	Lactobacillus acidophilus	4.50	.761	0.170	

by measuring the zone of inhibition in millimeters.

Arash et al[34] assessed the antibacterial effect of silver-coated brackets (8 - 10 μm) against S. mutans using a disk agar diffusion assay. The results demonstrated the absence of an inhibition halo around the silver-coated bracket. Bindu et al[35] evaluated the anti-bacterial effect of AgNPs coated stainless steel band material using the zone of inhibition method. The study found no significant difference in the effectiveness of AgNPs against both S. mutans and L. acidophilus. In our study the zone of inhibition formed for Ag-coated stainless-steel brackets for S. mutans is 6.85mm (mean) and for L. acidophilus is 4.50mm(mean), and for the uncoated stainless-steel bracket, there was no zone formed, this significant finding confirms the anti-bacterial property of Ag-coated stainless steel brackets, showing that silver nanoparticles are more effective against S. mutans compared to L. acidophilus.

Our research findings indicate that silver-coated brackets effectively possess antibacterial properties against both S. mutans and L. acidophilus. Moreover, the antibacterial activity of silver-coated stainless steel orthodontic brackets was observed to be significantly greater against S. mutans than against L. acidophilus, as evidenced by the results of intergroup comparison.

Overall, these results suggest that the silver coating on the brackets may have some antibacterial properties, which may help to reduce the amount of bacterial adhesion on these bracket surfaces and prevent the development of WSL and dental caries to greater extent.

5. Limitations

1. The study was conducted in the microbiological laboratory where the exact oral condition cannot be mimicked.
2. The durability and sustainability of the coating in oral environment was not studied.
3. The amount of silver concentration needed to have effective anti-bacterial effect was not clear.
4. Concentration dependent toxicity of silver was not considered in the study.

6. Scope for the Study

1. Additional research would be needed to confirm the above findings and to determine the underlying mechanisms responsible for the observed differences in bacterial adhesion and anti-bacterial effect between the two types of brackets.
2. The durability and sustainability of the AgNPs coating on SS brackets, an in vivo study is needed under clinical situations in the oral environment.
3. The acceptable dose of silver needs to be studied so that concentration dependent toxicity does not occur.

7. Conclusion

The study's data leads to the following conclusions:

1. The surface-modified orthodontic brackets with silver nanoparticles prevent the adhesion of both S. mutans and L. acidophilus to the brackets, demonstrating the effective antibacterial property of AgNPs.
2. Orthodontic brackets coated with AgNPs can effectively reduce the occurrence and prevalence of white spot lesions due to their antibacterial effects. However, further in vivo research on orthodontically treated patients is needed to validate these findings.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Cite this article: Kadammanavar J, Patil VS, Patil BC, Faseehuddin M, Aniketh, Jajee M. Evaluation of surface-modified orthodontic brackets with silver nanoparticles and its influence on bacterial inhibition – An in vitro study. *IP Indian J Orthod Dentofacial Res* 2023;9(3):175-182.