

# Assessment of Antibacterial Property of Silver Coated Stainless Steel Orthodontic Brackets Against *Streptococcus Mutans*, *Lactobacillus Acidophilus* and *Porphyromonas Gingivalis*—An In Vitro Study

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## ABSTRACT

**Introduction:** White spot lesions and gingivitis are most common sequelae of fixed orthodontic appliance therapy. Studies have revealed that silver nanoparticles have antimicrobial property against bacteria, fungi and protozoa.

**Aim:** To assess antibacterial property of silver coated stainless steel orthodontic brackets against *S. mutans*, *L. acidophilus*, *P. gingivalis*.

**Material and Method:** This experimental study was carried out in 120 stainless steel orthodontic brackets, out of which 60 brackets were silver coated, 60 brackets were uncoated. These brackets were tested for its antimicrobial property against *S. mutans*, *L. acidophilus* and *P. gingivalis*. Antibacterial property of silver coated stainless steel brackets measured by counting colony forming unit (CFU). Antibacterial property of silver coated brackets against *P. gingivalis* demonstrated by reduction in optical density.

**Results and Conclusion:** These data were statistically analyzed and it was concluded that Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *S. mutans*, *L. acidophilus* and *P. gingivalis* compared to the control group.

Silver coating of stainless steel orthodontic brackets can be used to prevent development of dental plaque, there by controlling the dental caries and periodontal disease.

**Key words:** White spot lesions, Nanoparticles, Antimicrobial.

## INTRODUCTION

Fixed orthodontic appliances provide conducive condition for the growth of microorganisms. Patients have difficulty in maintaining adequate oral hygiene and the appliance provides additional sites for microorganisms to bind and colonize.<sup>1</sup> In addition, enamel demineralization is also caused by fixed orthodontic appliances. Eliades et al, in their study have suggested that stainless steel brackets, due to their high critical surface tension and energy, are expected to have high plaque retaining capacity.<sup>2,5</sup>

Different pathogenic contribute to formation of plaque. *Streptococcus mutans* play an important role in making the tooth environment acidic. At low pH, lactobacilli count increases while the number of *S. mutans* decreases.<sup>1,3,4,6,7</sup> This causes demineralization of the teeth once the lesions are

established. *Porphyromonas gingivalis* plays causes onset and progression of periodontal disease and it is implicated as an indicator of periodontal disease.<sup>5-9,12-16</sup> Preventing these lesions is an important concern as these lesions are unesthetic, unhealthy and potentially irreversible.

Certain metals in nanoparticle form have been found to be active against even the antibiotic-resistant strains of bacteria. Nanoparticles are insoluble when size smaller than 100 nm.

Various studies have suggested the effect of silver, titanium oxide and zinc oxide nanoparticles on multiple organisms<sup>10-16,18-24</sup> Silver has antimicrobial activity against Gram-positive and -negative bacteria, fungi, protozoa, and certain viruses, including antibiotic-resistant strains.

Silver nanoparticles are now being incorporated in composites, denture base resins etc. for their anti microbial prop-

erty.<sup>17,18,25,26</sup> Silver coated NiTi and SS wires have also been tried for their anti microbial property.<sup>19,27</sup> However, silver coated orthodontic brackets have not been evaluated so far. Hence this study was planned to evaluate the efficiency of silver nanoparticle coated orthodontic brackets for their anti bacterial property against *S. mutans*, *L. acidophilus*, *P. gingivalis*. The study also assesses and compares the antibacterial activity of silver coated stainless steel brackets against, these micro-organisms by counting their Colony Forming Units (CFU).

## MATERIALS AND METHODS

### Materials

#### Orthodontic Materials

- Stainless Steel MBT .022" Slot pre adjusted edgewise appliance brackets (3M Gemini).

#### Nano Laboratory Materials

- Planar magnetron sputtering unit (Nano sensor laboratory- PSG institute of advanced studies, Coimbatore)
- Scanning electron microscope (Mechanical Department, Anna University, Chennai).

#### Microbiological Laboratory Materials

- Bacterial strains (Hi tech lab, Chennai)
- MRS broth (Hi tech lab, Chennai)
- BHI broth (Hi tech lab, Chennai)
- Petri dishes (Hi tech lab, Chennai)
- MRS agar plates (Hi tech lab, Chennai)
- BHI agar plates (Hi tech lab, Chennai)
- Anaerobic chamber (Hi tech lab, Chennai)
- Manual colony counter (Hi tech lab, Chennai)
- Incubator (Hi tech lab, Chennai)
- Spectrophotometer (Hi tech lab, Chennai)

### Method

This study was done on 120 specimens of stainless steel orthodontic brackets. The specimens were categorized into six test groups. Each group consisted of 20 specimens.

Study was allocated into 6 groups, one control and one experimental group each for culture media of all the three micro-organisms.

The bacterial **Strep. Mutans** (MTCC 890) were inoculated in 5 mL of a BHI and incubated for 24 hours at 37°C. Strains

of **L. acidophilus** (MTCC 447) Lactobacilli were inoculated into 5 mL of MRS broth and were incubated for 24 hours at 37°C while strains of **P. gingivalis** (ATCC 33277) were cultivated in BHI broth containing 0.1% vitamin K1 and 1% hemin at 37°C; in an anaerobic chamber with 85% nitrogen, 10% hydrogen, and 5% carbon dioxide (CO<sub>2</sub>) mixed gas.

### Preparation of Silver-coated Orthodontic Brackets

Surface modification of stainless steel orthodontic brackets with silver oxide was carried out by Magnetron sputtering method. Sputtering was carried out on stainless steel orthodontic brackets (substrate) using silver (Ag) as the target. A plasma generated inside the vacuum chamber ejected surface atoms from the silver target, which were sputtered onto the stainless steel brackets (substrate). A constant distance of 7 cm was kept between the substrate and the target. Sputtering was conducted for a period of 10 minutes. All brackets were sputtered to achieve a thin and uniform coating of silver at the same time.

The surface morphology of the silver thin film was investigated with a scanning electron microscope.

Antibacterial activity assay of orthodontic brackets for **S. mutans** was done by diluting the bacterial culture with BHI broth to achieve an optical density of 1.0 at 660 nm. The **Lactobacilli** culture broth was diluted with MRS broth to achieve an optical density of 1.0 at 660 nm. **P. Gingivalis** culture broth was diluted with BHI broth to make an optical density of 1.0 at 660 nm. Around 10 micro litre of the diluted bacterial suspension was transferred onto test tubes containing silver coated and uncoated stainless steel brackets. These tubes were incubated inside the laminar air flow chamber. After incubation, 100 ml of the bacterial suspension was serially diluted and plated onto BHI agar plates. Antibacterial activity was described as the survival rate by colony-forming units (CFUs) using manual colony counter. 10 micro litre of bacterial suspension in diluted form was transferred onto test tubes containing either the uncoated stainless steel brackets or silver coated stainless steel brackets. These tubes were incubated inside the anaerobic chamber.

*P. gingivalis* is difficult to culture, Hence Antibacterial activity of the surface modified orthodontic brackets was demonstrated by spectrophotometry. Reduction in optical density was measured.

The mechanism of this method is based on the turbidity of the culture media for the evaluation of antibacterial properties of the materials containing antibacterial particles.

## RESULTS

The collected data was subjected to statistical analysis using SPSS version 17. The data was assessed for normality by Shapiro-Wilks test. Based on the distribution of data, the appropriate statistical test was used. Descriptive statistics were obtained for each group. The mean CFU were compared between uncoated and coated bracket group using unpaired t-test. Mann-Whitney U Test was used to compare reduction in optical density between groups.

The antimicrobial activity of silver coated stainless steel brackets and uncoated brackets against *S. mutans*, *L. acidophilus*, *P. gingivalis* and the antimicrobial activity of silver coated stainless steel brackets against *S. mutans* and *L. acidophilus* were assessed and compared.

The group containing surface-modified brackets showed statistically significant decrease in the survival rate of *S. mutans* when compared to uncoated group.

Survival rate of the bacterial cells is calculated in terms of CFUs. The survival rate of *S. mutans* was  $77.85 \pm 12.893$  in control group. The survival rate of *S. mutans* in experimental group was  $53.60 \pm 10.990$ . P value was 0.000 ( $<0.005$ ). Log of colony count of uncoated brackets was  $1.8854 \pm 0.07347$ . Log of colony count of silver coated brackets was  $1.7203 \pm 0.090$ . P value for log of colony count was 0.000 ( $<0.005$ ). Thus, the group containing surface-modified brackets showed statistically significant decrease in the survival rate of *S. mutans* when compared to uncoated group.

Survival rate of the bacterial cells is calculated in terms of CFUs. The survival rate of *L. acidophilus* was  $45.35 \pm 12.304$  in control group. The survival rate of *L. acidophilus* in experimental group was  $36.65 \pm 11.717$ . P value was 0.028 ( $<0.005$ ) Log

of colony count of un coated brackets was  $1.6382 \pm 0.13663$ . Log of colony count of silver coated brackets was  $1.5355 \pm 0.1777$ . P value for log of colony count was 0.030 ( $<0.005$ ). Thus, the groups containing surface-modified brackets showed statistically significant decrease in the survival rate of *L. acidophilus* when compared to groups containing uncoated stainless steel brackets.

Antibacterial activity of silver coated stainless steel brackets against *P. gingivalis* was demonstrated by spectrophotometry. Reduction in optical density was measured. Initial optical density of standard medium was 0.3. Optical density for uncoated orthodontic brackets against *P. gingivalis* is  $1.06 \pm .027$ . Optical density for silver coated orthodontic brackets against *P. gingivalis* is  $0.75 \pm .029$ . Optical density was reduced in coated group compared to uncoated group. Mann-Whitney U Test was used to compare reduction in optical density between groups. P value was  $< 0.05$ . As the bacterial count decreases optical density also decreases. Thus Antibacterial activity of surface-modified orthodontic brackets on *P. gingivalis* is statistically significant than uncoated brackets.

Unpaired student *t* test was used to compare the silver coated stainless steel orthodontic brackets against *S. mutans* and *L. acidophilus*. The survival rate of *S. mutans* in experimental group was  $53.60 \pm 10.990$ . The survival rate of *L. acidophilus* in experimental group was  $36.65 \pm 11.717$ . P value was 0.000 ( $<0.005$ ).

Thus antibacterial activity of silver coated stainless steel orthodontic brackets is more against *S. mutans* than *L. acidophilus* and this difference was statistically significant (Table 1).

**Table 1**

Unpaired student *t*-Test (Parametric Test) for comparing antibacterial property of coated and uncoated brackets

Bracket Group		N	Mean	Std. Deviation	Std. Error Mean
S.mutans (CFU)	1A	20	77.85	12.893	2.883
	1B	20	53.60	10.990	2.457
L.acidophilus (CFU)	2A	20	45.35	12.304	2.751
	2B	20	36.65	11.717	2.620
P.gingivalis (Reduction in Optical density)	3A	20	1.06	0.119	0.027
	3B	20	0.75	0.128	0.029

## DISCUSSION

Enamel surface decalcification adjacent to fixed orthodontic appliances is an important and prevalent iatrogenic effect of orthodontic therapy. Due to plaque-retention by fixed appliances patients are at an increased cariogenic risk. There occurs a shift in the composition of the bacterial flora of the plaque over time. More specifically, the levels of acidogenic bacteria, such as *S. mutans*, become significantly elevated in orthodontic patients. If these bacteria have an adequate supply of fermentable carbohydrates, acid by-products will be produced, lowering the pH of the plaque.

As the pH drops below the threshold for remineralization, enamel decalcification starts.<sup>20</sup> With caries progression, the number of streptococcus (Aerobic bacteria) decreases and that of lactobacillus (Anaerobic bacteria) increases. *L. acidophilus* is responsible for the progression of caries.<sup>21,22</sup>

According to Zachrisson, within 1-2 months after fixed appliance placement patients usually experience mild to moderate gingivitis. In 10% of adolescents, there occurs considerable irreversible periodontal attachment apparatus destruction.<sup>23,24,28,29</sup> *Porphyromonas gingivalis* (gram negative anaerobic bacilli) is a major pathogenic bacterium causing periodontitis.

The interaction of silver with thiol groups in enzymes and proteins plays an essential role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved. Mi-Jin Chun,<sup>25</sup> Choi<sup>26</sup> Shaha<sup>27</sup> reported positive results in surface modification of stainless steel orthodontic wires and brackets with photocatalytic TiO<sub>2</sub> and TiAg (titanium silver). But there was discolouration of wires and brackets after TiO<sub>2</sub> coating and also there was loss of properties of NiTi wires on heating at 500°C for 5 hours.

For the effect of silver, reduction in size in nanoparticle form is an important requirement. Greater surface-to-volume ratio because of smaller sizes leads to more close interaction with microbial membrane and larger surface area for antimicrobial activity. According to Yamamoto<sup>28</sup> among all, physical vapor deposition exhibits a strong antimicrobial effect. So in this study, silver coating of stainless steel orthodontic brackets was carried out by magnetron sputtering method which is one of the physical vapor deposition methods. However silver coated brackets may be prone to wear as silver coating is purely surface based process. Thus durability and sustainability of silver coated brackets is critical in oral environment.

Anti-bacterial property of silver coated stainless steel bracket against *S. mutans*, *L. acidophilus* measured by counting colony forming unit (CFU).

Anti-bacterial property of silver coated stainless steel brackets against *P. gingivalis* was demonstrated by Spectropho-

tometry. Reduction in optical density was used to assess the antibacterial activity. As the bacterial count decreases, optical density also decreases.

The use of silver must be undertaken with caution, as it has been demonstrated that there is concentration dependent toxicity. Silver has not been mentioned in the list of the hazardous heavy metals to public health. However accumulation in the environment should be considered.<sup>29</sup> It was also proved that silver did not have cytotoxic or genotoxic effect.

It is essential to determine maximum lethal dose and the amount of silver necessary to carry out antibacterial properties before applying nanotechnology in orthodontics.

## CONCLUSION

- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *S. mutans* with respect to control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *L. acidophilus* compared to the control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *P. gingivalis* compared to the control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *S. mutans* than *L. acidophilus* and this difference was statistically significant.
- Silver coating of stainless steel orthodontic brackets can be used to prevent Formation of dental plaque, which helps in controlling the dental caries and periodontal disease.

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