Original Article

Evaluation of Antibacterial Effects of Silver-Coated Stainless Steel Orthodontic Brackets

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Abstract

Objectives: White spots and enamel demineralization around orthodontic brackets are among the most important complications resulting from orthodontic treatments. Since the antibacterial properties of metals and metallic particles have been well documented, the aim of this study was to assess the antibacterial effect of stainless steel orthodontic brackets coated with silver (Ag) particles.

Materials and Methods: In this study, 40 standard metal brackets were divided into two groups of 20 cases and 20 controls. The brackets in the case group were coated with Ag particles using an electroplating method. Atomic force microscopy and scanning electron microscopy were used to assess the adequacy of the coating process. In addition, antibacterial tests, i.e., disk diffusion and direct contact tests were performed at three, six, 24, and 48 hours, and 15 and 30 days using a Streptococcus mutans strain. The results were analyzed using Student's t-test and repeated measures ANOVA.

Results: Analyses via SEM and AFM confirmed that excellent coatings were obtained by using an electroplating method. The groups exhibited similar behavior when subjected to the disk diffusion test in the agar medium. However, the bacterial counts of the Ag-coated brackets were, in general, significantly lower ($P<0.001$) than those of their non-coated counterparts.

Conclusions: Brackets coated with Ag, via an electroplating method, exhibited antibacterial properties when placed in direct contact with Streptococcus mutans. This antibacterial effect persisted for 30 days after contact with the bacteria.

Keywords: Electroplating; Orthodontic Brackets; Silver; Stainless Steel; Streptococcus mutans

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INTRODUCTION

During orthodontic treatment with fixed appliances, white lesions and dental caries often develop on tooth surfaces. These lesions compromise successful orthodontic outcome and result inpatient discomfort. Streptococcus mutans produces organic acids that attack enamel, and thereby results in dental caries [1]. Fixed orthodontic treatment may lead to ecological changes in the oral environment and increase Streptococcus mutans count in the saliva and dental plaque [2]. Patients with fixed appliances exhibit low pH of the gingival plaque, high carbohydrate content of the plaque, and low calcium and phosphorus contents [3]. These lead to bacterial infection, which plays a key role in enamel demineralization in patients with fixed orthodontic appliances [3]. The initial bacterial adhesion to the bracket-adhesive-enamel complex is the most important step in the sequence of events that lead to enamel decalcification [4]. These adverse effects can be prevented by use of chlorhexidine or antibiotic treatment to directly suppress the cariogenic

microflora [5]. Bacterial adhesion to the surfaces, and hence bacterial colonization, can also be prevented. Silver is one of the oldest antimicrobial agents [6] and possibly the most common metallic antimicrobial used on solid surfaces. Silver ions exhibit a broad-spectrum bactericidal effect [7]. In fact, the antibacterial effects of silver are governed by various mechanisms. Silver is also highly toxic for bacteria and slightly toxic for human cells [8]. Moreover, silver has long been used as a disinfectant for hygienic and medicinal purposes and has been extensively used to prevent wound infections since World War II [9]. Various methods have been used to achieve antibacterial effects by adding silver particles, from ion sources, to textiles and surgical instruments [7]. Studies have shown that among the various brackets, stainless steel brackets have the highest critical surface tension and total adhesion are therefore especially attractive to microorganisms [10]. Use of these brackets may aggravate previous carious lesions. Bacterial infections can be prevented by adding anti-bacterial particles to metal surfaces before affixing the appliance [3].

Therefore, the aim of this study was to examine the antibacterial effects of silver-coated brackets on Streptococcus mutans in patients with fixed orthodontic appliances.

MATERIALS AND METHODS

Electroplating process

Forty standard maxillary first premolar metal brackets (Dentaurum, Turnstraße, Ispringen, Germany) were divided into two groups, namely cases and controls. Twenty brackets in the case group were coated with Ag, via electroplating, as in the case of a previous study [11]; 15 brackets of the case group were used for antibacterial tests and the remaining five were used for morphological analysis. Prior to electroplating, the specimen surfaces were all polished by barrel polishing and cleaned ultrasonically with isopropyl alcohol for 15 minutes. The cleaned brackets were then electroplated with Ag [12]. Stainless steel brackets are electrically conducting and were therefore easily coated by electroplating. During this process, electrochemical reactions resulted in the galvanic deposition of Ag on the brackets. The electrolyte (supplier of Ag ions) used in this process composed of silver nitrate, sodium phosphate, and ammonium phosphate and had a pH of ~8.5. The positively charged Ag ions (i.e., the anode) moved into the electrolyte solution and over time, precipitated on the negatively charged bracket surfaces (i.e., the cathode).

Surface morphology and atomic composition

The surface morphology of the specimens was examined under scanning electron microscope (SEM) (Seron-AIS2010, Gyeonggi-Do, Korea) and the atomic composition was determined by means of AFM (Easy Scan 2–flex AFM, Liestal, Switzerland). Five coated brackets from each group were used for this purpose.

Antibacterial test

The antibacterial properties of the samples were evaluated using standard strains of Gram-positive Streptococcus mutans (PTCC1683). Two antibacterial tests were performed in this study namely the disk diffusion test and the direct contact test.

Disc Diffusion test

Ten coated brackets and 10 uncoated brackets were subjected to this test, which was performed in accordance with the 2010 CLSI standard [13]. During the procedure, Streptococcus mutans suspension was prepared in 0.5 McFarland standard concentration (10^8 bacteria per mL), and transferred to a Hinton blood agar culture medium. The coated and uncoated brackets were placed in the culture at specific millimeter intervals, and the plates were all incubated for 24-48 hours at 37°C.Furthermore, the bacterial growth zone was measured in millimeters.

Direct contact test

Five coated brackets and five uncoated brackets were used for the direct contact test (which is

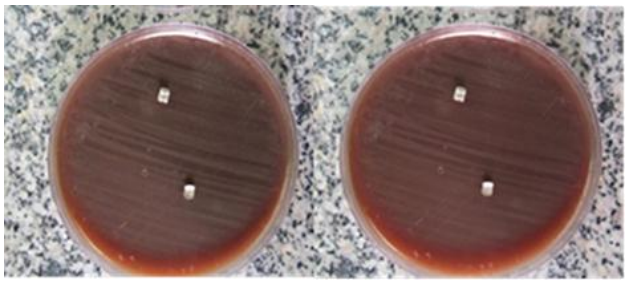

Fig. 1: Disk diffusion test performed on the non-coated bracket (left) and silver-coated bracket (right)

more accurate than the disk diffusion test).

Each of the 10 brackets was placed in a separate micro-tube containing 1 mL of brain heart infusion broth culture medium. The Streptococcus mutans strains (1683PTCC) reached the standard 0.5 McFarland concentration, and were then diluted in 1:10 ratio; 5 µL of this suspension were then poured into each tube containing 1 mL of the culture. The microtubes were incubated at 37°C and after periods of three, six, 24 and 48 hours and 15 and 30 days, 10 µL of the suspension were taken from each microtube (five coated, and five uncoated) and cultured on a separate blood agar culture medium. These cultures were incubated at 37°C for 24–48 hours and the colonies, each composed of a single set of cultures, were counted after each period. Bacterial counts of lower than 500 in the culture medium of the diluted 0.5 McFarland standard concentration were attributed to the antibacterial effect of Ag. The mean and standard deviation of bacterial count in each of the 60 cultures were determined after each period.

Statistical analysis

The statistical significance was assessed using SPSS version 18 (SPSS Inc., Chicago, IL, USA) and Student's t-test for comparing groups in each period of time and repeated measures ANOVA for comparing bacterial counts at different time points in each group. Significance was set at $P < 0.05$.

RESULTS

Surface morphology and atomic composition

The surface morphology of the specimens was examined under SEM and AFM. The SEM micrographs showed that the Ag coatings had a uniform thickness. Moreover, the AFM analysis revealed that the coatings were homogeneous, and had an average particle size and surface roughness of 370nm and 181nm, respectively.

Antibacterial effect

Disk Diffusion Test

The inhibition halo associated with the Streptococcus mutans culture was absent in both the test and control groups (Fig. 1).

Direct contact test

The mean number of bacteria grown after three, six, 24 and 48 hours, and 15 and 30 days is shown in Fig. 2 and Table 1.

The bacterial count in the control group increased over time but it increased slightly after six hours and subsequently decreased in the test group ($P<0.001$, Fig. 2 and 3).

Table 1: The mean and standard deviation (SD) of bacterial colonies subjected to the direct contact test for coated and uncoated orthodontic brackets (n = 5)

			Growth Period						P-value
			3 h	6 h	24 h	48 h	15 d	30 d	
Groups	Case	Mean	379	537.6	491.6	402.6	348.8	248	0.02
		SD	31.3	55.3	172.5	21.7	45.8	87.5	
	Control	Mean	644	1316	2226	1101	12664.2	14397.8	0.001
		SD	123.9	90.6	39.3	118.9	49.3	91.1	
	P-value		0.007	<0.001	<0.001	<0.001	0.002	0.07	

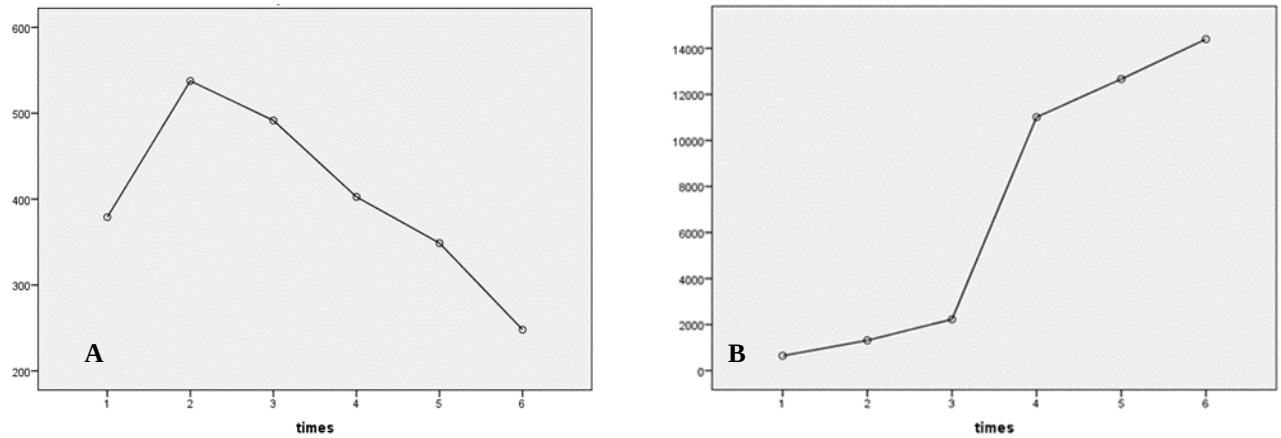

Fig. 2: Average growth of bacteria over time in the case **(A)** and control **(B)** groups

DISCUSSION

Unless a patient practices meticulous oral health care [14,15], fixed orthodontic appliances lead to plaque accumulation and subsequent development of dental caries and periodontal problems. The two main bacterial species involved in these processes are Aggregatibacter actinomycetemcomitans and Gram-positive Streptococcus mutans, which result in enamel decalcification and periodontitis [16,17]. Patients can reduce bacterial plaque by regular tooth brushing and using products containing fluoride, chlorhexidine, or antibiotics [18-20]. However, many researchers are trying to produce biomaterials that exert an antibacterial effect on the oral cavity. Bacterial colonization in the oral cavity can be reduced by applying a silver coating on orthodontic appliances. Therefore, in this study, metallic surfaces were coated with Ag by electroplating, and the physical and antibacterial properties of the Ag coating were evaluated with the aim of developing antibacterial orthodontic appliances. Various methods can be used to apply a silver coating, including physical vapor deposition [4] and electroplating (which was used as the coating method in the current study). The electroplating method used in the current study has not been reported elsewhere. Electroplating was selected due to its affordability and the ability to retain positive characteristics of the brackets after coating.

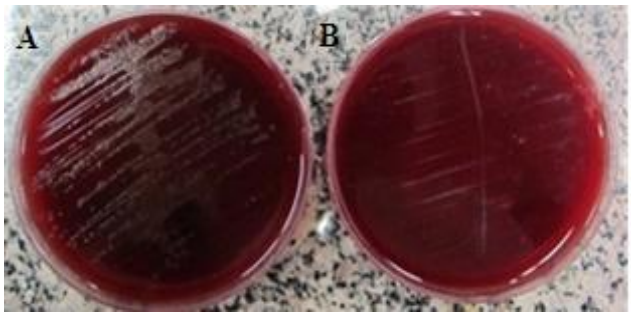

Fig. 3: Direct contact test performed for seven days on the non-coated (A) and coated brackets (B)

The SEM micrographs revealed uniform thickness (8-10 µm) of the coating, as shown previously [11]. The coating was analyzed by AFM in order to visualize the nanoscale irregularities that were not detected under SEM. An average particle size and slight roughness of 370 nm and 181 nm, respectively, confirmed that excellent coatings were produced by this method. Owing to its antibacterial effects, various studies have incorporated Ag in tissue conditioners [21], gargles [22], and resin materials [23].

Some studies have reported that Ag generates significant antibacterial action against Actinobacillus actinomycetemcomitans and Streptococcus mutans. However, molecules of living organisms that contain calcium or zinc interact with Ag and hence, this antibacterial effect might be toxic for human cells. Assessment of the toxicity of Ag for human cells is therefore essential. Ryu et al, in 2012 used human gingival fibroblasts to assess the cytotoxicity of Ag

coatings obtained via physical vapor deposition and found that the toxicity was negligible [4]. In addition, Ag-coated samples have exhibited higher cell compatibility compared with their non-coated counterparts [24]. The Ag coating assessed by Ryu et al, [4] in 2012 exhibited significant antibacterial action against Streptococcus mutans, suggesting that coated brackets would exhibit suitable antibacterial activity against Streptococcus mutan. The disk diffusion test and the count test are two of the most commonly performed antimicrobial tests. The first test is typically used to qualitatively determine the antimicrobial performance by measuring the diameter of the growth inhibition zone. This test can also be performed directly and produces more clear results than the count test, but it is unsuitable for the present set of experiments. The growth inhibition zone around the brackets was only slightly smaller than that in the control group. This resulted possibly from the smaller physical contact between the antimicrobial particles in the bracket and the surrounding culture medium (agar), which resulted in a smaller halo, than that formed during the diffusion test. A count of the colonies revealed that the coated brackets significantly inhibited the growth of Streptococcus mutans, as shown in Fig.1. Bacterial growth in the control group increased, in general, with time (three, six, 24, and 48 hours, and 15 and 30 days). However, the number of bacteria decreased to zero in two out of five repetitions of the 30-day interval. This phenomenon is described by bacterial growth charts, which stipulate that there are four phases of delayed increase, rest, and death. In contrast, the mean bacterial growth in the coated bracket group increased with time by up to six hours and decreased thereafter. The bacterial growth rates in the coated bracket group were, after six hours, significantly lower (Fig. 2) than those in the control group. In addition to the antibacterial effect of Ag coatings, favorable features of orthodontic brackets such as low friction resistance between the brackets and wire should also be investigated.

CONCLUSION

The Ag-coated stainless steel bracket surfaces, evaluated in this study, exhibited adequate antibacterial effect. This indicates that Ag-coated brackets or arch-wire scan significantly reduce the rate of plaque accumulation, especially in orthodontic patients susceptible to caries.

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