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In vitro assessment of stainless steel orthodontic brackets coated with titanium oxide mixed Ag for anti-adherent and antibacterial properties against Streptococcus mutans and Porphyromonas gingivalis

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ABSTRACT

Orthodontic brackets made from stainless steel were introduced in dentistry, though they have less ability in reducing enamel demineralization and are not successful in preventing microbial as well as biofilm growth. In this study, we evaluated the significant role of different brackets in reducing enamel demineralization indirectly. Results from different tests indicate the significant reduction in adhesion, biofilm formation and slow growth of tested bacterial species on brackets coated with Ag + TiO2 and found to be statistically significant lower than control. There was no loss in cell viability in all brackets indicating that the cells are biocompatible with different bracket materials. Scanning electron microscopy showed less bacteria attached with the surface coated with Ag + TiO2 indicated that bacteria were losing adherent nature on coated surface. In conclusion, TiO2+Ag coating on stainless steel brackets possessed anti-adherent properties and also have demonstrable antibacterial properties therefore helps in preventing dental caries and plaque accumulation indirectly. The cell compatibility of TiO2+Ag coated brackets is superior to the uncoated samples therefore can be used in orthodontics as it not only provide suitable antimicrobial activity and resistance to biofilm formation but also sustained the cell viability of human gingival fibroblast (HGF) cell lines.

1. Introduction

Increased plaque accumulation around the dental implants is associated with the two main pathogens namely *Streptococcus mutans* and *Porphyromonas gingivalis* colonizing the fixed appliances in orthodontic therapy [1,2]. These biofilm forming bacteria lead to increased demineralization of the enamel around the brackets in around 50% of the patients forming chalky white spots which are usually irreversible [3]. The organic acids produced by *Streptococcus mutans* leads to demineralization of the enamel and causes dental caries. The placement of the fixed orthodontic brackets increased the number and volume of these bacteria around the tooth resulting in chalky white spots lesions has been reported earlier [4]. These permanent changes in the patients with the fixed appliance are of major concern for the orthodontists since they curb the aesthetic and healthy therapeutic options. Bonding adhesive between the oral brackets and the tooth provide

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predisposing environment for the attachment and growth of oral microbial flora has been observed in previous clinical reports [5].

Bacterial adhesion and colonization to a greater extent depends on the surface free energy and surface roughness of the implant surfaces [6]. Jackfert et al. observed higher plaque retaining capacity in stainless steel material with higher critical tension and also the surfaces with more free energy has high affinity to bacteria especially *S. mutans* [7]. In recent advances, a number of biomaterials have been developed and studied to put in practice in orthodontic treatment in order to increase patient comfort. Despite the risk of enamel demineralization associated with the fixed appliances, the orthodontic brackets made up of different biomaterials gain significance due to their various advantages to the dentists. Among these biomaterials, stainless steel is most commonly used material in brackets [6,7]. Previous reports showed enamel demineralization and plaque accumulation bonded near the bracket-adhesive-enamel junction due to the alterations in the oral environment [8], therefore posing the threat of enamel decalcification by stimulating biofilm formation by bacteria which, therefore can be reduced by adjusting the antimicrobial properties of the orthodontic bracket surface, prior to application either by direct impregnation or by coating in order to overcome this persisting problem. In this regard, Ag based biomaterials has benefitted the orthodontists due to the nontoxicity active Ag + to mammalian cells and also its antimicrobial effect. Nevertheless, these orthodontic appliances may be corroded or abraded by chewing food which can be avoided by increasing the hardness and resistance of Ag + implants by coating with palladium [9]. Moreover for coating purpose, titanium (Ti) is commonly used in orthodontic and orthopedic clinics due to its biocompatibility properties. But the oxide of titanium (TiO2) has received more attention in orthodontics because of its photocatalytic property, chemical stability and benignancy.

In our study, we designed and developed stainless steel orthodontic brackets coated with TiO2 incorporated with Ag+ and also evaluated the antimicrobial, anti-adherent and biocompatibility of the fixed orthodontic brackets. We have also elucidated the significant role of these brackets in reducing enamel demineralization throughout the orthodontic therapy indirectly.

2. Materials and methods

For evaluating the anti-adherent and antimicrobial properties of Ag + TiO2 coated brackets of stainless steel, we designed a study comprising of four groups each with 35 samples. The samples with uncoated brackets were considered as control groups for the respective groups with coated brackets.

2.1. Photo-catalytic TiO2-coated stainless steel brackets

Stainless steel orthodontic brackets (Unitek™ Gemini MBT.022" slot size) were cleaned with alcohol and polished by using barrel polishing. Radio frequency magnetron sputtering was done with Ag and Ti as targets and stainless steel as substrate in which plasma generated vacuumized chamber ejected the ions from the target and coating was done on the substrate. A thin uniform coating of Ag, TiO2 and Ag + TiO2 was achieved by maintaining constant distance and time. The solution was recurrently stirred for 2.0 h and then aged for 24 h at ambient temperature in room air. The thickness of the TiO_2 films could be adjusted by repeating the dipping cycle. After coating, the coating were dried at 100 °C for 15 min.

2.2. Bacteria sampling and culture procedures

Samples were collected from the wedges of the plaques at the interproximal area of the patients with orthodontic infection (gingival). The samples were sent for microbiological evaluation within 2 h. The diluted samples were inoculated in Todd Hewit broth and Trypticase yeast cysteine sucrose Bacto agar for isolation of *Streptococcus mutans* and *Porphyromonas gingivalis,* agar for *P. gingivalis* isolation. The presumptive identification based upon the colony morphology was done and these organisms were further characterized by biochemical activity and API Strep 20 system (Biomerieux SA, Montalieu-Vercieu, France). The isolated bacteria were stored at −70 °C in storage vials.

2.3. Adhesion assay of bacteria to orthodontic brackets

The frozen stock cultures were revived and three each of Streptococcus *mutans* and *Porphyromonas gingivalis,* were used for adhesion assay. Sub culture was done on blood agar plates and the bacteria were adjusted for optical density measurements in Phosphate-buffered saline (PBS) for adhesion assays to get 0.5 (1×10^7 cfu/mL). Orthodontic brackets coated (Ag, TiO2, Ag + TiO2) and uncoated brackets were incubated in 16 well titer plates containing 2 ml of PBS inoculated with bacteria with continuous agitation for upto 8 h at 37 °C in Co2 incubator and also anaerobically. Negative control without inoculum was maintained simultaneously. Following incubation the brackets were washed and sonicated in 10 ml of PBS in an ultrasonic water bath (TPC-120; Telsonic AG, Bronschhofen, Switzerland) for 10 s at 30 kHz with a power output of 100 W. A culture was done before and after sonication and the average colony forming units were calculated with corresponding SD values.

2.4. Biofilm formation assay on the orthodontic brackets

The revived bacterial culture same as earlier assay was grown in Lysogeny broth (LB) as per requirements and 1:100 dilution of the overnight culture was used for biofilm formation assay. 500 μL of the diluted inoculum broth with added supplements to promote the growth of biofilm was added to each bracket placed on the microtitre plate and incubated for 24 h at 37 °C. Initially, after incubation the less adherent cells were removed from the brackets by submerging in water two to three times, followed by staining with 250 μL of 0.1% crystal violet and the absorbance was recorded as previously described [10].

2.5. Antimicrobial assay of orthodontic brackets

The optical density of test organism (three each of Streptococcus *mutans* and *Porphyromonas gingivalis*) was made suspension into the inoculated LB broth and was adjusted by dilutions to 1.0 at 590 nm. 10 μL of the bacterial suspension was added to a 15 ml tube containing LB broth of uncoated and coated stainless steel brackets with Ag, TiO2 and Ag + TiO2. Presence of growth was observed in CO_2 incubator and in anaerobic condition. Absorbance was measured at A590 nm using spectrophotometer.

2.6. Cell cytotoxicity

We also studied the effect of individual metals used in brackets for their effect on human gingival fibroblast (HGF) growth. HGF cells are an ideal cell source to evaluate their interaction with fabricated material surfaces. The cell line was grown in 16 wells plate as per described methods earlier in literature. The conditioned media contained 10% Fetal Bovine Serum (FBS) (Gibco Laboratories), penicillin (100 units/mL), and streptomycin (100 mg/ml). The wells were incubated at 37 °C with 5% CO2 and 95% relative humidity for 72 h, to get complete proliferation. After tripsinization