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ORIGINAL ARTICLE

Effect of nanosilver incorporation on antibacterial properties and bracket bond strength of composite resin

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Abstract:

Background and aim: Present in-vitro study was designed to evaluate the effect of nanosilver incorporation on antibacterial properties and Bracket Bond Strength (BBS) of orthodontic composite resin.

Materials and methods: A light curing composite resin was mixed with metallic nanosilver to obtain final concentrations of 0%, 0.5%, 1% and 2.5% (wt/wt). Scanning electron microscopy (TESCAN, VEGA II, XMU, Czech Republic) was used to confirm the uniform distribution of nanoparticles in resin matrix. Thirty disk type specimens (4.0 × 1.0mm) were prepared for each group. Antibacterial activity was determined by evaluation of bacterial growth in suspension media versus growth in direct contact with specimens. BBS and bond failure interface (ARI) were evaluated and compared between the specimens. Bacterial activity of control group compared with the group with maximum antibacterial activity.

Results: There were no significant differences in bacterial growth in any specimens in suspension media (P=0.623). The results of second method (direct contact) showed significant differences between all groups (P<0.001). Specimens with maximum antibacterial activity (containing 1% nanosilver) and control group had no significant difference in the BBS (P=0.455). ARI was completely identical in these two groups.

Conclusions: Nanosilver containing composite could confer surface antibacterial activity without significant difference on BBS and ARI.

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INTRODUCTION

Far too often a less-than-optimal esthetic result occurs after orthodontic treatment due to demineralization of enamel adjacent to fixed orthodontic appliances in patients with inadequate oral hygiene.[1] As oral hygiene becomes more difficult in patients with fixed orthodontic appliances, the decalcification of the enamel surface adjacent to these appliances is prevalent.[2] S. mutans growth not only is one of the main etiologic factors of demineralization of enamel surface but also increases surface roughness of resin composite which can accelerate biofilm accumulation.[3] Generally, adhesion of cariogenic streptococci was significantly higher for bonding adhesives than for bracket materials.[4] Findings show that the most common sites for demineralization are at the junctions of the bonding adhesives and the enamel.[5] Suboptimal oral hygiene, long intervals between appointments and potentially poor patient cooperation necessitate compliance free means of prevention.

One method to prevent enamel demineralization is to use orthodontic adhesives resistant to bacterial accumulation.[6-8]Various antimicrobial agents have been incorporated into orthodontic products and approved for intraoral use. Fluoride and chlorhexidine are the most common preventive additives for orthodontic use.[9-11] The released amounts of fluoride and chlorhexidine do not last for long periods although initially strong.[12,13] In addition, incorporation of fluoride and chlorhexidine in bonding agents could affect their mechanical properties and cause higher bond failure rate.[14-16]

Silver has a long history of use in medicine as an antimicrobial agent.[17] Materials that release silver ions have been found to have antibacterial effects on oral streptococci.[18] Recently, dental nano-filled resin composites were introduced in the market. The advantages of nano- composite materials include excellent optical properties, easy handling characteristics, and superior polish ability. In addition, nano-fillers can decrease surface roughness (SR) of orthodontic adhesives, which is one of the most significant factors for bacterial adhesion.[19] Some study evaluated the anti-bacterial and anti-fungal effects of dental cements incorporated nanosilver but they didn't report its physical properties.[20-21]

This study was designed to evaluate the antibacterial activity and shear bond strength of composites with different concentration of incorporated nanosilver.

MATERIAL AND METHODS

Part 1: For this in vitro experimental study, Opallis flow light curing composite resin (DENTSCARE LTDA, Lisbon, Portugal, filler size between 0.05 and 5.0 microns) was mixed with metallic nanosilver particles (Top Nano Tech Co.,Ltd, Taipei, Taiwan) in a dark room by a high speed mixer (SIMENS, DAC, 150FVZ-K, Germany, 3500 rpm for 5 min) to obtain final nanosilver concentrations of 0%, 0.5%, 1% and 2.5% (wt/wt). Particles had dimensions between 10 to 20nn.. Disk type specimens (4.0 × 1.0mm) were prepared from different nanosilver concentrations of composite resin. The specimens were soaked in normal saline solution at 37˚C for a period of 7 days to release the free monomers [22, 23] then sterilized by ethylene oxide.

Antibacterial activity was evaluated using Streptococcus mutans PTCC 1683. Antibacterial properties of the nanosilver-resins were measured with two methods. In the first method (A) the growth of S. mutans in the bacterial suspension containing sterile adhesives was evaluated. In the second method (B) specimens after a short period of direct contact to S. mutans dropped to a sterile suspension of culture medium and the growth of the S. mutans was evaluated (Direct Contact Test). The numbers of bacteria were determined using Colony Count Method.

In method A, 150 pipets containing 0.5mL Brain Heart Infusion (BHI) medium with approximately 1×10^3 bacterial cells were divided in 5 equal groups. Group 1 as control, anaerobically was placed in 37 ºC for 12h. Group 2 received adhesive disks without nanosilver. Group 3, 4 and 5 received adhesive disks containing 0.5%, 1% and 2.5% nanosilver respectively. All specimens were incubated for 12h, anaerobically at 37 ºC. The bacterial growth was then determined with Colony Counting Method (CCM).

In method B thirty specimens of each 4 adhesive groups (0%, 0.5%, 1%, 2.5% nanosilver concentration) were incubated with 0.01mL of bacterial suspension in sterile water with approximately 1×10^3 cells for 1h at 37 ºC temperature until the surface of specimens dried and the

bacteria reached to direct contact with specimens. Prepared specimens of each adhesive were then incubated in 0.5mL of sterile BHI broth for 12h anaerobically at 37 ºC. The bacterial growth was then estimated with CCM.

Part 2: For bracket bond strength evaluation, according to the result of part 1 , composite resin containing nanoparticles with maximum antibacterial effect was selected as test group and composite resin without nanosilver as control group. Sixty intact human premolars extracted for orthodontic reasons from patients under the age of 18 were randomly divided into 2 equal groups. The criteria for tooth selection were: undamaged buccal enamel, no caries, no restorations, and no pretreatment with any chemicals agents. The enamel surfaces were polished for 5 seconds with oil and fluoride-free fine pumice. The bonding surface was etched with 37% phosphoric acid gel for 20s, rinsed with water for 30s, and dried with oil-free and moisture-free air until the enamel presented a faintly white appearance. Standard edgewise premolar brackets (Dentaurum, Ispringen, Germany) with a base area of 10.23mm^2 were bonded using Opallis flow in control group and Opallis flow containing 1% nanosilver in test group (DENTSCARE LTDA, Lisbon, Portugal). The roots were held in a vise while the recommended 300-g force was applied to the brackets.[24] Excess adhesive was removed, and the adhesive was light-cured (Optilux 501, Kerr, Orange, Calif; approximately 820 mW/cm2) with a total of 40 seconds (10 seconds from each side of the bracket). The specimens were prepared in wire loop design demonstrated by Mojtahedzadeh and coworkers [24] and stored in deionized water for 24h at 37 ºC prior to the debonding test. The bond strength was tested with a testing machine (SANTAM, STM-20, Tehran, Iran), at a crosshead speed of 1 mm per minute. After debonding process the surfaces of the teeth were examined with a reflecting microscope to determine the amount of adhesive remaining on the enamel surface, which was recorded by using the adhesive remnant index (ARI). [15]

Statistical analysis

Statistical analysis was performed with SPSS 13 software (SPSS, Chicago, Ill). According to Kolmogrov-Smirnov and Shapiro-Wilk tests, the data were not normally distributed (P<0.001), Therefore the Kruskal-Wallis and Mann-Whitney U Test were used for data analysis. The level of significance in this present study was set at P= 0.01.

RESULTS

Scanning electron microscopy revealed relatively uniform distribution of nanosilver in the resins matrix in all groups except group 5 (Figures1, 2).

Table I shows the means, standard deviations, mean ranks, minimum and maximum levels of bacterial number in liquid media containing *St.mutans* with sterile adhesives (groups 1-5). There were no significant differences among groups (P=0.625).

The results of direct contact test (DCT) revealed significant differences between group 4 (1% nanosilver) and all other groups (P<0.001). The means, standard deviations, mean ranks, minimum and maximum levels of bacterial number for DCT were showed in Table II.

There was no significant difference in the bracket bond strength (P.value=0.455), between control and group 4. Table III shows the means, standard deviations, minimum and maximum levels of shear bond strength in groups.

The ARI for all teeth in test and control groups were #3 indicating all adhesive left on the tooth, with a distinct impression of the bracket base and therefore no statistical analysis was needed.

Table I: The means, standard deviations, minimum and maximum levels, and mean ranks of bacterial colony count in liquid media containing *St.mutans* with sterile adhesives

Group	N	Minimum	Maximum	Mean	Std. Deviation
1	30	350	400	384	17.14
2	30	350	400	382.66	18.74
3	30	350	400	386	16.93
4	30	350	400	378	19.72
5	30	350	400	382.33	16.95

P=0.62

Table II: The means, standard deviations, mean ranks, minimum and maximum levels of bacterial number for DCT

Group	N	Minimum	Maximum	Mean	Std. Deviation
2	30	470	540	512	24.04
3	30	170	230	215	17.64
4	30	.00	7.0	3.5	1.45
5	30	120	160	150	12.28

P<0.001

Table III: the means, standard deviations, minimum and maximum levels of shear bond strength in groups.

Group	N	Minimum	Maximum	Mean	Std.Deviation
Control	30	10.00	15.30	12.4467	1.92815
Test	30	10.10	15.00	12.8200	1.56412

P=0.455

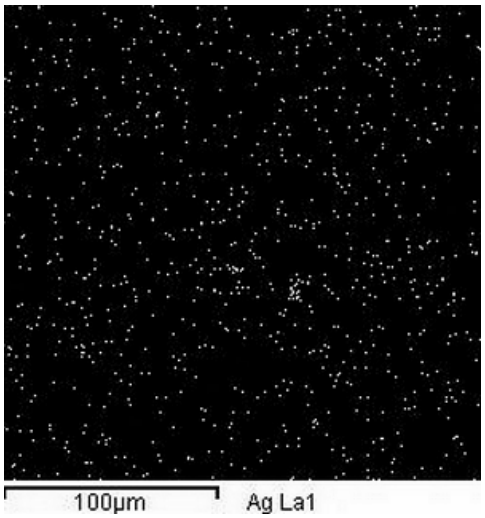

Fig.1: uniform distribution of nanoparticles

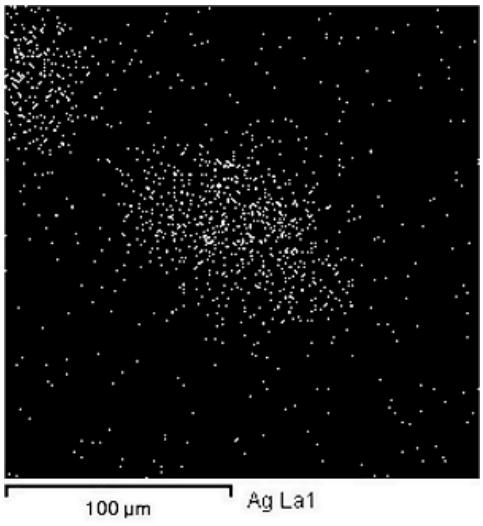

Fig. 2: distribution of nanoparticles in group 5

DISCUSSION

An essential event in the initiation of enamel demineralization is microbial adhesion to the teeth and/or orthodontic appliances. Once adhesion has occurred, cell proliferation can lead to the development of pathogenic plaque, which is the main cause of enamel demineralization. Therefore, the quality and quantity control of cariogenic streptococci adhesion to the tooth surface and orthodontic appliances are important for the success of orthodontic treatment. In particular, orthodontic adhesives are reported to have a higher retaining capacity of cariogenic streptococci than bracket materials.[4]

Although the detailed mechanism of the antimicrobial effect of silver has not been determined, it has been suggested that oxygen is changed into active oxygen (including hydroxyl radicals) by the action of light energy in the air or water as a result of catalytic action of silver, because silver ion can facilitate electron displacement from a molecule. The active oxygen causes structural damage in bacteria. As a result, silver ion can denature proteins and enzymes of bacteria by binding to reactive groups resulting in their inactivation.[25]

Reducing the adhesion of cariogenic streptococci to orthodontic adhesives can be obtained by incorporating silver nanoparticles to composite resins.[8] Surface roughness is important factor in bacterial adhesion. Nanosized fillers show the smoothest surfaces when compared with the conventional adhesives containing micro-sized fillers.[19]

Eventually the strength of material must be adequate and studies showed that uniform distribution of highly separated nanoparticles into dental resins/composites could significantly improve the mechanical properties of the resins/composites.[26]

For these reasons we decided to incorporating silver nanoparticles in to composite resin and evaluating the antibacterial effects and physical properties of this material.

The relatively uniform pattern of distribution of silver nanoparticles were seen in concentrations of 0.5%, 1% but not in 2.5% showing that our method for mixing was not successful in the last group.

Bacterial growth in BHI broth containing S. mutans with sterile adhesives showed no significant differences among groups. This finding may be related to lack or low diffusion of Ag^+ to surrounding environment. This finding is in agreement by Ahn and coworkers who tested disk diffusion test on nanosilver-resins and they had not found significant antibacterial effect. On the contrary they found significant antibacterial effect in liquid media for their material and concluded that this effect was due to decreased adhesion of bacteria to adhesive surface not because the diffusion of Ag^+ ions.[8]

DCT showed significant differences between all groups. This could be mainly due to better expression of surface antibacterial effect of metallic silver when come in direct contact with streptococcus mutants. The composite-resin with 1% of nanosilver content revealed highest antibacterial effect even more than adhesive with 2.5% concentration .We supposed that the particles aggregations in composite resin with 2.5% concentration due to inefficient mixing, have lowered the surface to volume ratio of nanoparticles and lend to decreased antibacterial property. This level of concentration has another major drawback that is deep dark color and will limit its use clinically.

In shear bond tests we preferred to test only one group, which had shown the highest antibacterial property (composite resin with 1% nanosilver) and to compare it with composite resin without these particles. The results showed no significant difference between groups. ARI scores for all teeth in control and test groups are identical (score 3) and no differences exist between groups. Both of recent tests indicating incorporation of silver nanoparticles in 1% concentration had no deleterious effects on bracket bond strength and debonding pattern of composite resin.

Further studies are required to prepare and evaluate higher concentrations of nanosilver containing composite resin, long-term antibacterial and physical properties, and cytotoxicity of this kind of composite resin materials.

CONCLUSIONS

The orthodontists can benefit from adhesive materials containing silver nanoparticles to overcome problems creating by bacterial adhesion around brackets such as white spots. These adhesives confer antibacterial properties without detrimental effect on bracket bond strength.

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