Streptococcus mutans counts in patients wearing removable retainers with silver nanoparticles vs those wearing conventional retainers: A randomized clinical trial

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Introduction: The rough surface of Hawley removable appliances provides an environment for plaque accumulation, leading to enamel demineralization. The aim of this study was to evaluate the effect of silver nanoparticles incorporated into acrylic baseplates of orthodontic retainers on *Streptococcus mutans* colony-forming units. **Methods:** Sixty-six orthodontic patients at the debonding stage were randomly assigned to 2 sex-matched groups with stratified block randomization: group 1 received conventional removable retainers; group 2 received removable retainers containing silver nanoparticles (about 40 nm in size and 500 ppm in concentration). After comprehensive orthodontic treatment, patients who revealed no clinical evidence of dental caries, periodontal pockets, or systemic disease were considered eligible for this study. Swab samples were taken from the maxillary palatal side from the patient in the dental chair at retainer placement (T1, 1 week after debonding the fixed orthodontic appliance) and T2, 7 weeks later. The main outcome was to compare the number of *S mutans* colony-forming units between the 2 groups 7 weeks after retainer delivery. The results were analyzed by using analysis of covariance. The participants and the assessors were blinded to the allocation groups. **Results:** Twenty-nine patients in the control group and 32 in the intervention group were analyzed. At T1, the intervention group had higher *S mutans* colony counts relative to the control group. The analysis of covariance test showed a significant reduction of colonies in the intervention group after 7 weeks. The mean difference of colony counts between the 2 groups was 40.31 (95% confidence interval, 24.83-55.79; $P < 0.001$). **Conclusions:** Adding silver nanoparticles to the acrylic plate of retainers had a strong antimicrobial effect against *S mutans* under clinical conditions. **Registration:** This study was registered as a clinical trial at the Iranian Clinical Trial Center under the code number IRCT201309239086N2. **Funding:** This trial was supported by Hamadan Dental Research Centre, Hamadan University of Medical Sciences, Hamadan, Iran. (Am J Orthod Dentofacial Orthop 2016;149:155-60)

Conventional Hawley removable appliances are popular in orthodontics beacause of their acceptable esthetics, short chairside time, low cost, and ease of oral hygiene. On the other hand, their rough surface provides an environment for plaque accumulation, changing the oral microbial flora and preventing the flushing effect of saliva on dental and mucous tissues, finally leading to enamel demineralization and gingival inflammation.[1] It has been shown that microorganisms can penetrate into the acrylic base of these appliances as deep as 1 to 2 mm, making disinfection difficult.[2]

Oral hygiene control in patients wearing acrylic plates as dentures or orthodontic removable appliances is challenging. In spite of improvements in dental materials and techniques, caries and white spots on tooth surfaces are common. The use of antimicrobial agents has been advised for patients to aid in the control of bacterial biofilm formation because toothbrushes cannot completely remove microorganisms from critical retentive sites of

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Submitted, August 2014; revised and accepted, July 2015.
0889-5406/$36.00

http://dx.doi.org/10.1016/j.ajodo.2015.07.031

the appliances.[3] Frequency and skill of brushing the teeth and the appliances are the main factors in oral hygiene and are totally related to patient awareness and cooperation. Disinfection methods with antimicrobial agents are also useful but need patient compliance, which is unreliable.[4] Professional compliance-free methods are preferred to overcome these problems.[5]

Silver particles have been introduced as antimicrobial agents used in wound dressing agents, bone prostheses, cardiac prostheses, and dental composite resins. Silver zeolite (porous crystalline material of hydrated aluminosilicate) has been incorporated into tissue conditioners, acrylic resins, and mouth rinses. Nanosilver particles, in the form of Ag+, destroy bacterial membranes through direct contact. Orthodontic adhesives with nanosilver particles have their antibacterial capacity raised without compromising their physical properties.[6,7]

Important issues regarding the use of nanosilver are its biocompatibility and toxicity. Several studies have been carried out in this area, and most have reported that factors such as particle size, particle shape, and, most importantly, the concentration of silver ions released are the major factors that influence the toxicity of silver.[8] The cytotoxicity of silver nanoparticles smaller than 20 nm significantly increases for human periodontal fibroblasts.[9] Metallic silver appears to pose a minimal risk to health, whereas soluble silver compounds are more readily absorbed and can produce adverse effects. Ingestion is the primary route for entry for silver compounds and colloidal silver proteins. Dietary intake of silver is estimated at 70 to 90 mg per day. American Biotech Labs has reported that oral exposure of a commercial 10-ppm silver nanoparticle solution over 14 days did not cause clinically important changes in metabolic and hematologic parameters and in urinalysis.[10] The United States Environment Protection Agency permits 5 μg per kilogram per day of silver intake for humans.

Specific objectives or hypothesis

In this in-vivo study, we tested the hypothesis that silver nanoparticles incorporated into the acrylic baseplates of orthodontic removable retainers during the retention period could change the *Streptococcus mutans* counts around the palatal surfaces of the teeth.

MATERIAL AND METHODS

Trial design

This was a 1:1 parallel 2-arm sex-matched randomized trial with no changes after trial commencement.

This randomized clinical trial was conducted at the School of Dentistry, Hamadan University of Medical Sciences. The study protocol was approved by the research ethics committee of Hamadan University of Medical Sciences.

Participants, eligibility criteria, and setting

Patients who completed their fixed orthodontic treatment in the orthodontics department of the Hamadan dental faculty in Iran and needed removable appliances as retainers were included. Subjects with smoking habits, active caries or periodontal pockets, systemic diseases, age greater than 25 years, or a history of antibiotic therapy during the last 3 months were excluded. A swab was rubbed over the palatal surfaces of the permanent teeth, put in a closed container, and sent to the laboratory for immediate culture of *S mutans*.

INTERVENTIONS

In the first stage, acrylic resin with silver nanoparticles was produced. The methylmethacrylate polymer powder (self-curing acrylic resin; Acropars, Tehran, Iran) was coated with metallic silver nanoparticles with an average size of 40 nm (Top Nano Tech, Taipei, Taiwan) at a concentration of 500 ppm using helium-driven chemical-vapor transport and the sputtering method. The final acrylic powder had a gray color. It was important to obtain an insoluble compound that was stable in saliva. To produce it, the atomic absorption test was carried out to ensure the insolubility of this product. Therefore, 10 plates made of acrylic resin coated with silver nanoparticles, measuring 10 × 20 × 2 mm, were prepared. The plates were soaked in a bottle containing 10 mL of artificial saliva (Hypozalix; Biocodex, Gentilly, France) and then sealed. The samples underwent 30 days of thermocycling procedures consisting of 5000 cycles. Each thermal cycle consisted of 30 seconds of immersion in hot water (55°C), 30 seconds in cold water (6°C), and 30 seconds for transfer of each plate (Delta Thermocycler, Mashhad, Iran). Then the atomic absorption test was performed (Atomic Absorption Spectrometer; Aurora Biomed, Vancouver, Canada) to determine the presence of silver or silver ions in the artificial saliva (solubility of silver). The release of silver in any form was not reported in any sample.

Then the antibacterial properties of this new compound were investigated in the laboratory. Ten disks of conventional acrylic resin and 10 with silver nanoparticles, measuring 1 cm in diameter, were prepared and sterilized in an autoclave; 0.5 McFarland suspension (a concentration of 1.5×10^8 colony-forming units/mL) was placed on each disk so that the bacteria came in direct contact with the disk. At certain intervals (1, 2, 3, 4, and 5 hours), the suspensions were cultured on blood agar culture medium. The plates were incubated (Incubator