In Vitro Adhesion of *Streptococcus sanguinis* to Dentine Root Surface After Treatment with Er:Yag Laser, Ultrasonic System, or Manual Curette

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Abstract

Objective: The purpose of this *in vitro* study was to evaluate the dentine root surface roughness and the adherence of *Streptococcus sanguinis* (ATCC 10556) after treatment with an ultrasonic system, Er:YAG laser, or manual curette. **Background Data:** Bacterial adhesion and formation of dental biofilm after scaling and root planing may be a challenge to the long-term stability of periodontal therapy. **Materials and Methods:** Forty flattened bovine roots were randomly assigned to one of the following groups: ultrasonic system ($n = 10$); Er:YAG laser ($n = 10$); manual curette ($n = 10$); or control untreated roots ($n = 10$). The mean surface roughness (R_a , μm) of the specimens before and after exposure to each treatment was determined using a surface profilometer. In addition, *S. sanguinis* was grown on the treated and untreated specimens and the amounts of retained bacteria on the surfaces were measured by culture method. **Results:** All treatments increased the R_a ; however, the roughest surface was produced by the curettes. In addition, the specimens treated with curettes showed the highest *S. sanguinis* adhesion. There was a significant positive correlation between roughness values and bacterial cells counts. **Conclusion:** *S. sanguinis* adhesion was the highest on the curette-treated dentine root surfaces, which also presented the greatest surface roughness.

Introduction

SCALING AND ROOT PLANING by curettes are the most frequently used procedures for treatment of periodontal diseases, due to their low cost and effectiveness in reducing the clinical signs of inflammation and the levels of pathogens.^{1,2} However, these techniques are considerably time consuming, and skill is required in order to reach successful clinical and microbial outcomes after treatment with conventional hand instruments. Therefore, sonic and ultrasonic systems³ and lasers⁴ have enlarged the armamentarium of therapeutic appliances as alternatives or adjuncts for conventional scaling and root planing.

Ultrasonic systems with smaller diameter tips and longer working lengths have been recently introduced onto the market to better access deep probing sites, root grooves, and furcations.^{5,6} These instruments have been widely used in clinical practice with favorable clinical and microbiological outcomes.^{3,7} Among lasers used in dentistry, the erbium:

yttrium–aluminum–garnet (Er:YAG) laser has also been suggested as an alternative tool to conventional periodontal mechanical therapy.^{7–11} Er:YAG irradiation has exhibited high bactericidal properties¹² and the potential to selectively remove calculus without thermal side effects on root surfaces and periodontal tissues.¹³ Furthermore, this technique has demonstrated clinical and microbiological results that are comparable to hand instruments and sonic and ultrasonic scalers.^{7,10,11}

Previous studies have demonstrated that morphological characteristics and roughness of the root surface after scaling and root planing can influence the attachment and growth of gingival fibroblasts.^{14–16} Whilst fibroblasts attach more easily to rough than to smooth surfaces,¹⁷ there is an undesirable positive correlation between surface roughness and oral bacteria adherence¹⁸ that can represent an important factor in the development of caries and periodontal diseases. Thus, surface roughness after periodontal treatment could have a significant impact on the newly formed biofilm and,

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consequently, on the long-term success of the therapy. Therefore, the purpose of this *in vitro* study was to evaluate the dentine root surface roughness and the adherence of *Streptococcus sanguinis* after treatment with an ultrasonic system, Er:YAG laser, or manual curette.

Materials and Methods

Sample preparation

Forty cleaned bovine roots were used in the present study. Initially, all roots were flattened with 300, 600, and 1200 Al₂O₃ abrasive sand paper (Carborundum Abrasivos, Recife, PE, Brazil), consecutively, by means of a polishing machine under water irrigation. The samples were polished with each sand paper for 5 sec in order to remove the cementum, expose the dentine surface, and obtain standardized surfaces. Subsequently, the roots were cut and fixed (flat side up) in polished polystyrene resin (Cromex, Piracicaba, SP, Brazil) in order to obtain standardized blocks of 5 mm long×5 mm wide×3 mm deep. Subsequently, the specimens were washed in tap water for 15 min to remove the smear layer. An initial surface roughness measurement was performed as described below.

Experimental design

The specimens were randomly assigned to one of the following groups.

Ultrasonic system ($n=10$). Samples were scaled by an ultrasonic system (Cavitron Select SPC; Dentsply Professional, York, PA, USA), insert TFI-1000 25 kHz, medium power setting. The insert was tangentially applied to the sample without pressure in an apical to coronal direction under profuse water solution irrigation. This insert is composed of a magnetostrictive stack that converts energy provided by the handpiece into mechanical oscillations used to activate the insert tip (Fig. 1).

Er:YAG LASER ($n=10$). Samples were irradiated with the Er:YAG laser (Kavo Key II; Kavo, Biberach, Germany) working at 2940 nm. The energy and repetition rate of this equipment ranged from 60 to 500 mJ and 1 to 15 Hz, respectively. A periodontal handpiece (no. 2056) was used with a prismatically cut glass tip (1.1×0.5 mm). The fluency and repetition rate used for the laser irradiation were 8.4 J/cm² per pulse and 10 Hz, respectively. Laser parameters were set at 214 mJ/pulse and the energy delivered at the end of the tip, taking into account the transmitting factor (0.56) for the selected tip, was 120 mJ/pulse.⁸ The tip was used at an incidence angle of 45° and a continuous water spray was used.

FIG. 1. Ultrasonic scaler tip used (insert TFI-1000 25 kHz).

The application tip was moved in an apical to coronal direction and maintained in slight contact with the root surface.

Curette ($n=10$). Samples were scaled in an apical to coronal direction by a Gracey curette (no. 5–6; Hu-Friedy Co, Chicago, IL, USA). Each side of the curette was used for five specimens and then replaced by a new side.

Control ($n=10$). Flattened specimens were left untreated to be used as controls.

In the treatment groups, the entire surface of the specimens (5 mm×5 mm) was treated for 1 min by the same operator, resulting in approximately 50 strokes for each sample. Once again, the specimens were washed in tap water for 15 min to remove possible smear layer.

Surface roughness analysis

The surface roughness averages (Ra; μ m) of the test specimens before and after treatments and of the control group were determined using a surface profilometer (TR200; Time Group Inc., Beijing, China). The surface roughness of each specimen was scanned with a microneedle using a cutoff of 0.8 mm (λ_c) and a velocity of 0.1 mm/sec (ISO 4228). All measurements were made by the same blinded operator in three longitudinal and three transversal directions, and the scanned area was limited to the specimens' size (5 mm×5 mm).

Morphological analysis

After roughness analysis, three treated roots from each experimental group and three flattened untreated specimens (control) were randomly chosen for observation by scanning electron microscopy (SEM). Samples were fixed in 2.5% glutaraldehyde in 0.05 mol/L cacodylate buffer, pH 7.4. Subsequently, specimens were washed in five changes of tap water for 15 min to remove the smear layer, fixed, post-fixed, dehydrated in ascending acetone concentrations up to 100%, critical point dried (CPD 030; BAL-TEC, Furstentum, Liechtenstein), sputter-coated with gold (MED 010; BAL-TEC), and observed by SEM (LEO 435 VP; LEO Electron Microscopy Ltd., Cambridge, UK). Representative areas were photographed at ×1500 magnification.

Adhesion assay

Saliva coating of the specimens. Unstimulated saliva of healthy donors was collected with their informed consent during 1 h per day from each individual for 7 d. The saliva samples were frozen at −20°C until a total of 500 mL was collected. Subsequently, the saliva sample was pooled and centrifuged (30 min, 4°C, 27,000×g). The supernatant was pasteurized (60°C, 30 min) to inactivate endogenous enzymes, recentrifuged (30 min, 4°C, 27,000×g) in sterile bottles, and stored at −20°C. The pasteurization efficacy was evaluated by plating 100 μ L of saliva onto brain heart infusion (DIFCO™-BHI) agar (Becton-Dickinson & Co., Sparks, MD, USA) and observing the absence of bacterial growth after 72 h. The specimens were autoclaved (127°C/15 min) and placed in a well of a sterile 24-well polystyrene cell culture plate (Costar-Corning Inc., New York, NY, USA) containing 500 μ L of saliva for 4 h to allow salivary pellicle formation.

Adhesion assay. Saliva was aspirated from each well and replaced with 500 μL of BHI broth (double concentrated) and 500 μL of saliva. Inoculums were prepared by harvesting the standard reference strain *S. sanguinis* (ATCC 10556) cells from BHI agar plates previously inoculated and incubated under microaerophilic conditions for 24 h (candle jar, 37°C). The bacterial cells were suspended in sterile saline solution, adjusting the turbidity to OD₆₃₀ 0.15 ($\sim 10^6$ colony-forming units [CFU]/mL) and, each well was inoculated with 100 μL of this inoculum suspension. Plates were then incubated for 16 h under microaerophilic conditions. Afterwards, the specimens were washed in sterile saline solution to remove unattached cells and inserted in microtubes containing 1000 μL sterile peptone water. The microtubes were vortexed vigorously for 2 min to free the bacteria attached on the surface of each specimen and sonicated to disperse the bacterial cells. Serial dilutions (10^{-4} to 10^{-8}) of these suspensions were made and incubated on BHI agar plates for 48 h. The tests were performed in triplicate and the CFUs were determined using a stereomicroscope by an examiner who was blinded for the experimental groups.

Statistical analysis

Statistical analysis was performed using the SANEST (Sistema de Análise Estatística, Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG, Belco Horizonte MG, Brazil) software. The roughness average (Ra) was registered for each scanned position and averaged for each specimen and, subsequently, for test groups before and after treatments and for the control group. Repeated measures ANOVA was used to compare the surface roughness among test groups before and after treatments, and one-way ANOVA was used for comparisons among treated and untreated surfaces (control specimens). CFUs for discs inoculated in triplicate were averaged and submitted to logarithmic transformation. Normal distributions of $\log_{10}$ CFU values per specimen were confirmed by the Kolmogorov-Smirnov test. Subsequently, one-way ANOVA was used for comparison of CFU counts among treated and untreated specimens. When significant differences were detected by one-way ANOVA, a pairwise comparison was performed using Tukey test. Pearson's analysis was used to test the possible correlations between surface roughness and bacterial adhesion. The significance level established for all analyses was 5% ($p < 0.05$).

Results

Surface roughness

There were no significant differences in the Ra values among the flattened specimens before treatments ($p > 0.05$). After 1 min of instrumentation, all treatments demonstrated an increased Ra ($p < 0.05$). The roughest surface was produced by the curettes, followed by the Er:YAG laser, while the smoothest surface was produced by the ultrasonic system (Table 1, repeated measures ANOVA). The Ra value observed for the control specimens used in the adhesion assay was 0.08 ± 0.01 . These untreated specimens were compared to the treated specimens by one-way ANOVA in parallel with the adhesion results. The results demonstrated that specimens treated by curettes presented the roughest surface, followed by Er:YAG laser, ultrasonic system, and untreated specimens, respectively.

TABLE 1. MEAN VALUES AND STANDARD DEVIATIONS OF MEAN SURFACE ROUGHNESS FOR TEST GROUPS BEFORE AND AFTER TREATMENTS

	Pretreatment		Posttreatment	
	Ra (μm)	SD	Ra (μm)	SD
Ultrasonic system	0.07 Aa	0.01	0.13 Ba	0.04
Er:YAG laser	0.06 Aa	0.01	0.20 Bb	0.04
Curette	0.07 Aa	0.02	0.53 Bc	0.08

Values followed by different letters (uppercase, row; lowercase, column) are significantly different by the Tukey test at the 5% confidence level.

Ra, mean surface roughness.

Morphological analysis

Under SEM, untreated root surfaces showed a smear layer and parallel lines produced by the abrasive sandpaper. In addition, some exposed dentine tubules were also observed in these control specimens, demonstrating that the cementum was removed and the dentine surface was exposed after the flattening procedures (Fig. 2A). Dentine root surface treated with the ultrasonic system demonstrated random areas with scratches probably produced by the scaler tip (Fig. 2B). The Er:YAG laser treatment produced discontinuous crater-like defects, with ablation of the intertubular dentin (Fig. 2C). In addition, the specimens treated with both the ultrasonic system and the Er:YAG laser exhibited some regions with the surface similar to the untreated group, indicating that some areas of the specimens were not reached by these devices during the treatment phase (1 min). The curette instrumentation produced a wide exposition of dentine tubules in the entire surface of the specimens (Fig. 2D).

Adhesion assay

With regard to the levels of *S. sanguinis* adhesion to the different surfaces, the specimens treated with curettes showed the highest bacterial adhesion, followed by the ultrasonic system, Er:YAG laser, and control, which did not differ from each other ($p < 0.05$; Fig. 3). In addition, there was a moderate, but significant, positive correlation between roughness values and the *S. sanguinis* cell counts ($p = 0.001$; $r = 0.65$, $r^2 = 0.42$).

Discussion

Although considerable effort has been made to establish the role of surface roughness following periodontal treatment in the attachment of reparative cells and blood components,¹⁴⁻¹⁶ evidence is scarce regarding its role in bacterial adhesion after treatment with the available commercial periodontal instruments.¹⁹ Following periodontal treatment, newly formed biofilm may cause inflammatory reactions in soft and hard periodontal tissues and may sometimes put the long-term success of the therapy at risk. Therefore, the aim of this *in vitro* study was to evaluate the dentine root surface roughness and the adhesion of *S. sanguinis* after treatment with Er:YAG laser, an ultrasonic system, or manual curette.

In agreement with our previous study,²⁰ all treatments increased the roughness of dentine root surfaces; however, the curettes produced rougher surfaces when compared to the

FIG. 2. Scanning electron photomicrograph ($\times 1500$) showing (A) control (untreated specimens): parallel lines induced by the abrasive sandpaper, presence of smear layer and some exposed dentine tubules; (B) ultrasonic system: scratch induced by the ultrasonic tip and adjacent areas not reached by the ultrasonic system presenting aspect similar to the untreated specimens; (C) Er:YAG laser: irradiated areas with ablation of the intertubular dentin and adjacent areas not reached by the irradiation; (D) curette: exposed dentine tubules.

Er:YAG laser and the ultrasonic system. The increased exposure of dentine tubules after curette treatment could be a possible explanation for the higher Ra, since exposed dentine tubules are considered to constitute major surface irregularities.²¹ In addition, during instrumentation, the tip of the curettes appear to reach a wider area of the root surface than the ultrasonic and Er:YAG laser tips. As a result, a more ample area for roughness detection by the profilometer is produced in curette-treated surfaces, when compared to the other instruments. In contrast to the present findings, in which Er:YAG yielded a rougher surface than the ultrasonic scaler, our previous study²⁰ demonstrated no significant differences in surface roughness between laser and ultrasonic treatments, despite the morphological differences between the two appliances. This contradictory finding may be due to differences in the ultrasonic systems used in the studies that utilized different tip designs and power settings. In fact, the effects of different periodontal instruments on the root surface roughness have often been investigated over the years and conflicting results have been found. Some investigations

demonstrated no difference in the roughness of surfaces treated with either curettes or ultrasonic instruments²²; others demonstrated that the smoothest surfaces were produced by hand instruments^{23,24} or by ultrasonic instruments.²⁵ With regard to the use of the Er:YAG laser, a previous investigation¹² found no differences in root surface roughness following Er:YAG laser irradiation and treatment with curettes. These results could be attributed to differences in experimental designs of these studies (i.e., methods for roughness evaluation, standardization of the surfaces to be treated, etc.) and also to the characteristics of the instruments (i.e., shape and size of tips, materials, force of application) and their different effects on the root surface morphology.^{16,26}

In the present study, the data regarding *S. sanguinis* adhesion suggested a negative influence of the mechanical modification of the root surface by the curette on bacterial colonization. *Streptococci*, especially *S. sanguinis*, are considered early colonizers that organize the environment for secondary colonizers, which require more challenging growth conditions.^{27,28} Many secondary colonizers, which adhere to

FIG. 3. Adherence of *S. sanguinis* colony-forming unit (CFU/log₁₀) to control and test groups. Different lowercase letters signify differences among treated and untreated (control) specimens by one-way ANOVA.

bacteria already in the biofilm mass, are well recognized to be involved in periodontal infection (e.g. *Fusobacterium*, *Capnocytophaga*, and *Prevotella* species).²⁹ As discussed in the surface roughness analysis, a possible explanation for the higher levels of *S. sanguinis* on curette-treated surfaces could also be the exposure of dentine tubules. It has been demonstrated that bacterial cells are able to invade the root dentine tubules, which could act as bacterial reservoirs for recolonization of the periodontal pocket after debridement.^{30–33} It is important to note two major limitations of this *in vitro* study that may have contributed to the widely opened dentine tubules (Fig. 2D) and, consequently, to the results of roughness and bacterial adhesion in the curette group. The first one was the use of bovine roots that present larger dentine tubules when compared to the human roots. The second is that the specimens were washed in tap water that may have removed the smear layer. This fact would not represent the clinical situation immediately after scaling and root planning, since previous investigations have demonstrated that a smear layer covers the root surface following mechanical instrumentation.³³ However, we speculate that, over time, the smear layer could be almost completely removed from the root surfaces exposed to mechanical forces like brushing and chemical factors like acidic foods and beverages. Once again, another explanation for such an increase in bacterial adhesion on curette-treated dentine surface may be the wider extent of root surface produced by curettes, which also make a larger area available for bacterial adherence.

The data of the present study also demonstrate that root surface roughness was positively correlated with *S. sanguinis* adhesion, highlighting the significance of posttreatment surface profile in bacterial recolonization. Such findings have already been reported for oral implants and restorative materials in many *in vitro* and *in vivo* studies.³⁴ In support of our data, in general, the results of these investigations showed that a high surface roughness increased the bacterial adhesion and, as a result, the levels of mature biofilm formation and periodontal signs of inflammation.^{35,36} The Ra value of 0.2 μ m has been previously suggested as a threshold surface roughness value, below which no further significant changes in the

total amount of adhering bacteria can be observed.^{18,37,38} On the other hand, Ra values higher than 0.2 μ m are positively correlated with an increase in the levels of biofilm accumulation.³⁵ Interestingly, in the present study, no differences were observed in bacterial adhesion between specimens treated with the ultrasonic system, Er:YAG laser, or control groups, all of which presented Ra values of below or equal to 0.2 μ m. There are various possible mechanisms by which surface roughness can influence bacterial adhesion in the oral cavity. First, surface irregularities are able to protect bacteria against shear forces during their initial reversible binding to allow them a stronger attachment. Secondly, rough surfaces increase the area available for adhesion and are more difficult to clean.³⁴

It is important to note that the surfaces treated in this study were free of calculus and cementum, because they were flattened at the beginning of the experiment. This yielded a surface that reflects common clinical situations, in which dentine is exposed due to cementum removal and gingival recession after scaling and root planing procedures, especially in cases of over-instrumentation and repetitive instrumentation throughout supportive periodontal therapies. Based on the results of the present study, it could be suggested that specific instruments might be required for each phase of periodontal treatment. Although many studies have shown superior results for curette instrumentation, with regard to calculus and contaminated cementum removal,^{39,40} when the clinical aim is to remove biofilm or newly formatted calculus on a previously scaled surface, ultrasonic and laser devices, for example, could be better choices.

In conclusion, *S. sanguinis* adhesion was higher on the curette-treated dentine root surfaces, which also presented the highest surface roughness. Therefore, it can be assumed that different instruments may exert a strong influence on both surface roughness and bacterial adhesion.

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Disclosure Statement

No competing financial interests exist.

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