Bacterial Adhesion on Smooth and Rough Titanium Surfaces After Treatment With Different Instruments

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Background: Newly formed biofilm after implant debridement may challenge the long-term stability of peri-implant therapy. This in vitro study aimed to assess the roughness and adherence of *Streptococcus sanguinis* after treatment of smooth and rough titanium surfaces with an erbium-doped: yttrium, aluminum, and garnet (Er:YAG) laser, metal and plastic curets, and an air-powder abrasive system.

Methods: Forty titanium disks with smooth-machined surfaces and 40 with sand-blasted and acid-etched surfaces were divided into the following treatment groups: Er:YAG laser; plastic curet; metal curet, and air-powder abrasive system. The surface roughness (roughness average [Ra]) before and after treatments was determined using a profilometer. *S. sanguinis* (American Type Culture Collection 10556) was grown on treated and untreated specimens, and the amounts of retained bacteria on the surfaces were measured by the culture method. Rough and smooth surfaces with and without a suspension of *S. sanguinis* were also analyzed using scanning electron microscopy (SEM).

Results: For smooth surfaces, the roughest surfaces were produced by metal curets (repeated-measures analysis of variance [ANOVA] and Tukey test; $P < 0.05$). The rough-surface profile was not altered by any of the treatments (repeated-measures ANOVA; $P > 0.05$). Rough surfaces treated with metal curets and air-powder abrasion showed the lowest level of bacterial adhesion (two-way ANOVA and Tukey test; $P < 0.05$). SEM analysis revealed distinct surface profiles produced by all devices.

Conclusions: Metal curets are not recommended for smooth titanium surface debridement due to severe texture alteration. Rough surfaces treated with a metal curet and the air-powder abrasive system were less susceptible to bacterial adhesion, probably due to texture modification and the presence of abrasive deposits. *J Periodontol 2009;80: 1824-1832.*

KEY WORDS

Air-powder abrasive system; Er:YAG laser; metal curets; mucositis; peri-implantitis; plastic curets; SEM; scaling.

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Titanium dental implants have been considered excellent alternatives to conventional prostheses in the oral rehabilitation of partially and totally edentulous subjects. Therefore, various types of implant surfaces, ranging from smooth machined to rough surfaces, are currently present in human oral cavities.[1] Despite the efforts to improve osseointegration by the modification of implant surfaces, evidence has shown that bacterial infection inducing mucositis or peri-implantitis can jeopardize the long-term success of some implant rehabilitations.[2,3] Both peri-implant diseases are infectious disorders associated with pathogenic bacterial species commonly observed in periodontal diseases.[2,3] Therefore, similar to periodontal treatment, the removal of bacterial biofilm and calculus deposits around implants seems to be crucial in the prevention and treatment of peri-implant infections.[4-6]

Various procedures and instruments have been proposed to reduce the number of pathogenic species and, consequently, to improve or preserve periodontal health around titanium implants.[4,5] Besides the mechanical removal of biofilm by plastic curets, air-powder abrasive systems, and the application of chemical agents and local antimicrobials, lasers have been introduced as a potential alternative in reducing pathogens on implant surfaces.[4,6-9] Among lasers used in dentistry,

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the erbium-doped:yttrium, aluminum, and garnet (Er:YAG) laser has been considered a promising therapy for peri-implant disinfection.[10] The Er:YAG laser has demonstrated a high potential for bacterial reduction on implant surfaces[11] and the ability to remove biofilm from both smooth and rough titanium surfaces, leading to significant improvement of peri-implant clinical parameters and new bone-to-implant formation.[12-14]

Several studies[15-20] investigated the effects of different mechanical treatments, such as metal and plastic curets and air-powder abrasive systems, on titanium surfaces with respect to texture changes, cleaning efficacy, and fibroblast attachment. In addition, the effects of the Er:YAG laser on the morphologic characteristics of implant surfaces, effectiveness for removing biofilm, and biocompatibility of osteoblastic and fibroblastic cells were investigated.[7,11,21-23] However, there is limited information about the influence of the titanium surface modification after treatment with different instruments on bacterial adherence. The surface profile and roughness produced by different instruments could have an important impact on the newly formed biofilm and, consequently, can be an important factor in peri-implant health maintenance. Therefore, the aim of this in vitro study was to assess the surface roughness (roughness average [Ra]) and adherence of *Streptococcus sanguinis* (American Type Culture Collection [ATCC] 10556) after treatment of smooth and rough titanium surfaces with an Er:YAG laser, metal and plastic curets, and an air-powder abrasive system.

MATERIALS AND METHODS

Sample

Smooth and rough disks made out of commercially pure titanium (grade 4) measuring 5 mm in diameter and 3 mm in thickness were used.§ The smooth surfaces were machined, whereas the rough surfaces were sand-blasted with aluminum oxide beads and acid-etched with nitric acid. The disks were removed from the original packaging and stored individually in microtubes before experimental procedures. The disks were handled by the lateral walls to avoid contact with the treated surfaces that could alter the surface profile.

Treatments

The smooth and rough disks were randomly divided into one of the following groups.

Er:YAG laser (n = 10 smooth; n = 10 rough). Samples were irradiated with the Er:YAG laser‖ working at 2,940 nm. The energy and repetition rate of this equipment ranged from 60 to 500 mJ and 1 to 15 Hz, respectively. A periodontal handpiece (#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/cm^2 and 10 Hz, respectively. Laser parameters were set at 120 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 67.2 mJ/pulse. The tip was used at an incidence angle of 45° under continuous water irrigation. The application tip was moved from bottom to top and maintained in slight contact with the disk surface.

Plastic curet (n = 10 smooth; n = 10 rough). Samples were scaled from bottom to top with the plastic curet¶ in which the tip was placed at a contact angle of 70°.

The curet had a steel handle, and tips were produced from a high-grade resin. Each side of the curet was used for five specimens and then replaced by a new side.

Metal curet (n = 10 smooth; n = 10 rough). Samples were scaled from bottom to top using a Gracey curet# in which the blade was placed at a contact angle of 70°. Each side of the curet was used for five disks and then replaced by a new side.

Air-powder abrasive system (n = 10 smooth; n = 10 rough). Samples were treated with an air-powder abrasive system** using medium water and an air-power setting. The insert was perpendicularly applied to the surfaces and worked by directing fine particles of sodium bicarbonate that were propelled by compressed air in a water spray at a distance of 5 mm from the surface.

The entire top surface of the disks was treated for 50 seconds by the same operator (PMD), resulting in ~30 curet strokes for each sample. No attempt was made to standardize the application of the scaling force because the operator applied the curets freely. This protocol was applied in an attempt to simulate approximately five clinical visits for implant decontamination.

Surface Roughness

All disks were rinsed with distilled water and allowed to air dry. The Ra (μm) of the titanium surfaces before and after treatments were determined using a surface profilometer.†† The surface roughness of each specimen was scanned with a diamond microneedle (10 μm diameter) using a cutoff of 0.8 mm (λc) and a speed of 0.1 mm/second (ISO 4228). A masked operator (AFR) made all measurements in two longitudinal and two transversal directions, and the scanned area was limited to the size of the disks (5 mm in diameter).

§ AS Technology, São José dos Campos, SP, Brazil.
‖ KaVo KEY II, Kavo, Biberach, Germany.
¶ Implacare, Hu-Friedy, Chicago, IL.
# 5-6, Hu-Friedy.
** Jet Sonic, Gnatus, Ribeirão Preto, SP, Brazil.
†† TR200, Time Group, Beijing, China.

Adhesion Assay

Besides the above described treated disks, 10 rough and 10 smooth disks did not receive any treatment and were used as controls to the assay.

Saliva coating of the specimens. Unstimulated saliva was collected from each one of four healthy male donors (age range, 18 to 24 years) for 1 hour per day for 7 days (in February 2009). All donors provided written informed consent. This study protocol was approved by the Ethics Committee in Clinical Research of Guarulhos University. The saliva samples were frozen at –20°C until a total of 500 ml was collected. Subsequently, the saliva sample was pooled and centrifuged (27,000 × g for 30 minutes at 4°C). The supernatant was pasteurized (30 minutes at 60°C) to inactivate endogenous enzymes, recentrifuged (27,000 × g for 30 minutes at 4°C) in sterile bottles, and stored at –20°C. The pasteurization efficacy was evaluated by plating 100 μl saliva onto brain-heart infusion (BHI) agar[‡‡] and observing the absence of bacterial growth after 72 hours. The disks were autoclaved (15 minutes at 127°C) and placed in a well of a sterile 24-well polystyrene cell-culture plate[§§] containing 500 μl saliva for 4 hours to allow salivary pellicle formation.

Adhesion assay. Saliva was aspirated from each well and replaced with 500 μl BHI broth (double concentrated) and 500 μl saliva. Inoculums were prepared by harvesting the standard reference strain *S. sanguinis* (ATCC 10556) cells from BHI agar plates previously inoculated and incubated under microaerofilic conditions for 24 hours (candle jar; 37°C). The bacterial cells were suspended in sterile saline solution, adjusting the turbidity to optical density $(OD)_{630}$ 0.15 (~10^6 colony forming units (CFUs)/ml), and each well was inoculated with 100 μl of this inoculum suspension. Plates were incubated for 16 hours under microaerofilic conditions. Afterwards, the specimens were washed in sterile saline solution to remove unattached cells and inserted in microtubes containing 1,000 μl sterile peptone water. The microtubes were vigorously vortexed for 2 minutes to free the bacteria attached on the surface of each specimen and sonificated to disperse bacterial cells. Serial dilutions (10^{-4} to 10^{-8}) of these suspensions were made and inoculated in BHI agar plates for 48 hours. Tests were performed in triplicate, and the CFUs were determined using a stereomicroscope by an examiner (COT) who was masked to the experimental groups.

Scanning Electron Microscopy (SEM)

Smooth and rough disks from each treatment group were observed using SEM. In addition, smooth and rough untreated disks were examined to observe any preexisting surface defects. Specimens were attached to stubs, placed into the vacuum chamber of a scanning electron microscope,[‖‖] and the central areas of the disks were photographed at magnifications ×200 and ×1,000. The microscopic appearance of *S. sanguinis* on treated and control titanium surfaces was also observed by SEM. Samples were fixed in 2.5% glutaraldehyde in 0.05 mol/l cacodylate buffer, pH 7.4. Subsequently, they were fixed, post-fixed, dehydrated in ascending ethanol concentrations up to 100%, sputter-coated with gold,[¶¶] and observed by SEM. Representative areas of treated and untreated smooth and rough surfaces after bacterial incubation were photographed at a magnification ×10,000.

Statistical Analyses

Statistical analyses were performed using software.[##] Comparisons were made among untreated and treated smooth and rough surfaces and among the four types of treatment modalities. The Ra was registered for each scanned position and averaged for each disk and, subsequently, for each group before and after treatments. Repeated-measures analysis of variance (ANOVA) was used to compare the surface roughness among groups before and after treatments. CFUs for disks 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. Two-way ANOVA was used for comparisons among CFU formed on untreated (control) and treated rough and smooth surfaces. When significant differences were detected by repeated-measures ANOVA or two-way ANOVA, a pairwise comparison was performed using the Tukey test. The significance level established for all analyses was 5%.

RESULTS

Surface Roughness

The Ra for smooth and rough titanium surfaces before and after treatments are shown in Table 1. Statistical analysis revealed no significant differences in Ra within smooth or rough surfaces before treatments (P>0.05), indicating a similar profile within each type of surface. For smooth surfaces, there was an increase in Ra values after treatment with a metal curet (P<0.05), whereas no significant differences were observed after treatment with the Er:YAG laser, plastic curet, and air-powder abrasive system (P >0.05). The smooth specimens treated by the metal curet presented the roughest surface compared to the other treatments (P <0.05). No significant changes in Ra values were registered after treatment of rough surfaces with any instrument (P>0.05).

‡‡ Difco, Sparks, NE.
§§ Costar Corning, New York, NY.
‖‖ LEO 435 VP, LEO Electron Microscopy, Cambridge, U.K.
¶¶ MED 010, BAL-TEC, Fürstentum, Liechtenstein.
SANEST, Minas Gerais Agriculture Research Institute, Belo Horizonte, MG, Brazil.

Table 1.

Ra (mean ± SD; μm) of Smooth and Rough Surfaces Before and After Treatments

	Smooth (n = 10)		Rough (n = 10)	
Treatment	Pretreatment	Post-Treatment	Pretreatment	Post-Treatment
Er:YAG laser	0.18 ± 0.02	0.23 ± 0.06	0.70 ± 0.07	0.68 ± 0.06
Plastic curets	0.19 ± 0.02	0.24 ± 0.02	0.70 ± 0.03	0.70 ± 0.08
Metal curets	0.20 ± 0.02	0.38 ± 0.08*†	0.71 ± 0.03	0.73 ± 0.27
Air-powder system	0.18 ± 0.02	0.20 ± 0.06	0.70 ± 0.07	0.69 ± 0.08

* Differences between pre- and post-treatment (repeated-measures ANOVA and Tukey test; $P<0.05$).
† Differences among groups after treatments (repeated-measures ANOVA and Tukey test; $P<0.05$).

Adhesion Assay

Figure 1 presents the adherence of *S. sanguinis* CFU (log^{10}) to control and treated rough and smooth surfaces. There were no differences in the levels of *S. sanguinis* adhesion among control and treated smooth surfaces. With regard to rough surfaces, control and Er:YAG laser–treated disks showed the highest levels of *S. sanguinis* adhesion, followed by those treated with plastic curets, metal curets, and the air-powder abrasive system, respectively ($P<0.05$). Control, laser-treated and plastic curet-treated rough surfaces presented a higher number of bacterial adhesion than the corresponding smooth ones ($P<0.05$). Differences between rough and smooth surfaces treated with metal curets and the air-powder abrasive system were not significant ($P>0.05$).

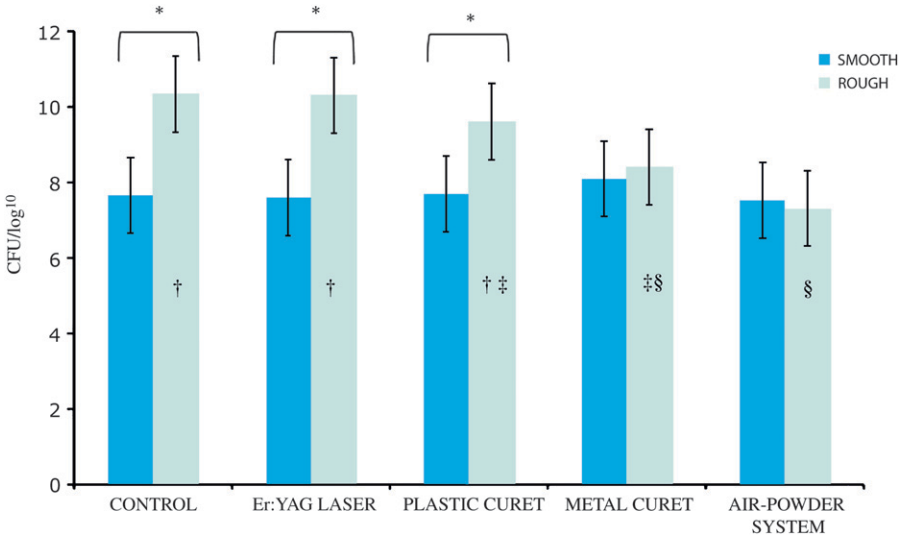

Figure 1.
Adherence of S. sanguinis *(mean CFU/log^{10}) to untreated and treated smooth and rough surfaces. *Differences between smooth and rough surfaces within each treatment group (two-way ANOVA and Tukey test; P <0.05); †‡§differences among treated and control rough surfaces (two-way ANOVA and Tukey test; P <0.05).*

SEM

Figure 2 presents the SEM photomicrographs of the control and treated smooth and rough titanium surfaces. The smooth control surfaces exhibited pronounced circumferential machining marks, whereas the rough control specimens showed irregular topography due to surface porosities. Surface imperfections that appeared as metal scratches or tags were commonly observed in the smooth control disks and were irregular in size, shape, and distribution. The Er:YAG laser produced slight alterations on smooth and rough titanium surfaces. The machining marks were still observed on smooth surfaces treated with the Er:YAG laser; however, these marks were less evident than the control ones. Some thin scratch lines over the original surfaces, probably produced by the tip of the laser device, were also observed on smooth and rough specimens treated with the laser. Plastic curets did not appear to significantly affect either of the titanium surfaces, retaining surface characteristics similar to the control ones. Only minor grooves produced by the tips of the curet and slight flattening of the ridges of the porosities were noted for smooth and rough surfaces treated with plastic curets, respectively. The surfaces treated with metal curets displayed an evident modification on smooth and rough surfaces. Many scratches and disruptions of the original machined surfaces were observed in the smooth specimens treated with metal curets. In addition, the irregularities of the rough specimens appeared to have smoothed out by flattening of the ridges after treatment with metal curets. The air-powder abrasive

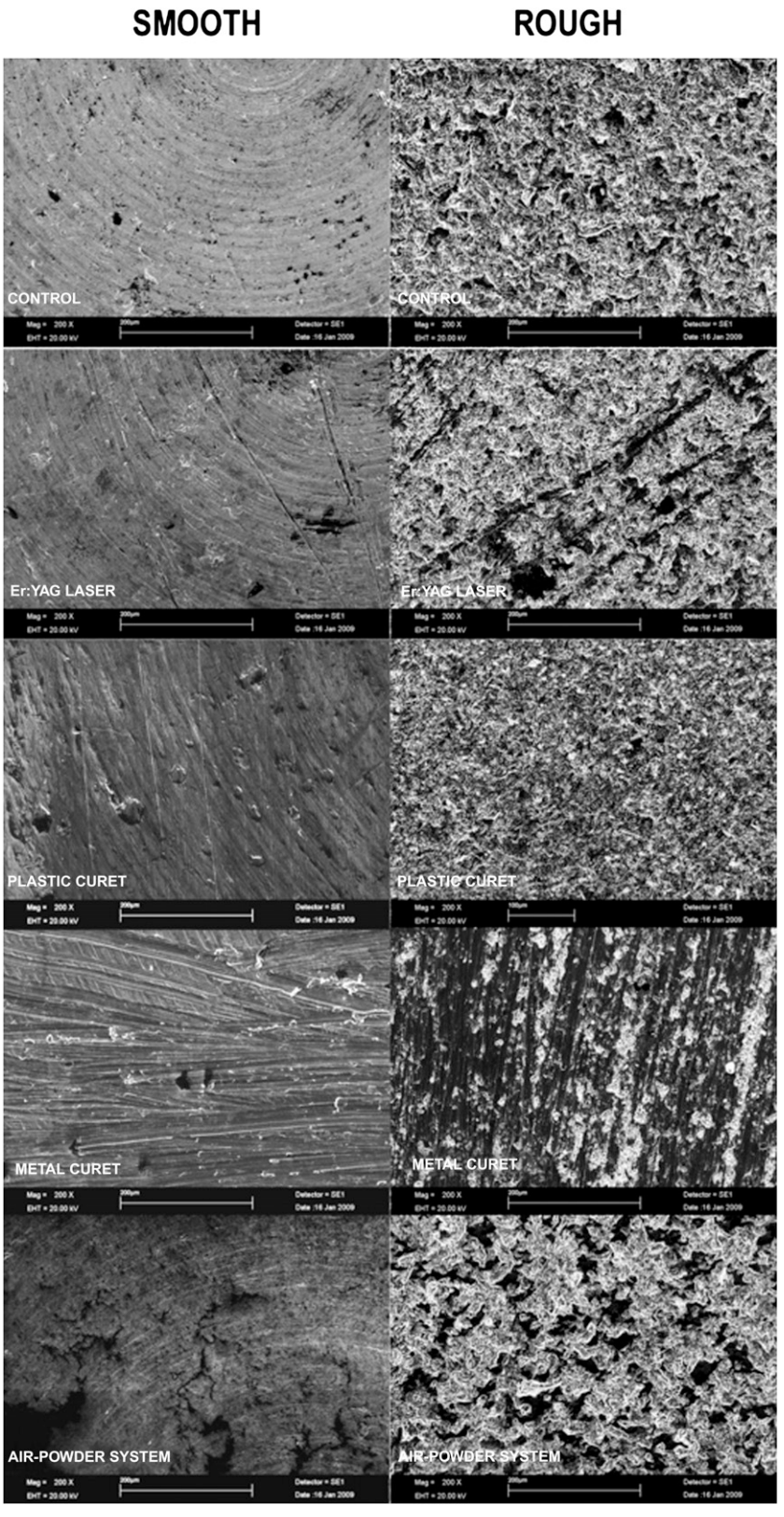

Figure 2.
Scanning electron photomicrographs of untreated (control) and treated smooth and rough surfaces (original magnification ×200).

system caused some sand-blasting aspect and irregular crater-like defects on the smooth surfaces. In addition, treatment with the air-powder abrasive system leveled down the edges of the elevations of the rough surfaces. Powder deposits were observed on rough surfaces treated with the air-powder abrasive system at a higher magnification (×1,000) (Fig. 3).

Figure 4 presents the SEM photomicrographs of the appearance of *S. sanguinis* adhered to control and treated smooth and rough titanium surfaces. In general, there was a trend toward the formation of chains of bacterial cells on the titanium surfaces. A moderate colonization was noted on the control and treated smooth surfaces, with the attached bacteria predominantly forming a monolayer compared to rough surfaces. A more intense colonization of *S. sanguinis* was observed on the rough surfaces, which exhibited multilayers of bacterial cells. A more sparse distribution of cells was detected on the rough surfaces treated with metal curets and the air-powder abrasive system.

DISCUSSION

Several methods including chemical (i.e., chlorhexidine or metronidazole application) and mechanical cleaning (i.e., air-powered abrasive, plastic curets, and laser therapies) have been proposed for implant debridement. It was demonstrated that the treatment of implant surfaces with chlorhexidine rinse, for example, did not cause damage on different types of implant surfaces, but it also did not remove already existing biofilm from such surfaces.[20] Therefore, chemical methods have been proposed to be used in association with mechanical cleaning methods.[7,13] Due to differences in material composition and application methods, the available mechanical cleaning instruments may differently affect the surface of titanium abutments and implants and, consequently, may have a direct effect on *de novo* biofilm formation after supportive implant therapy and/or treatment of peri-implant infections. Therefore, the first aim of this study was to examine the effects of

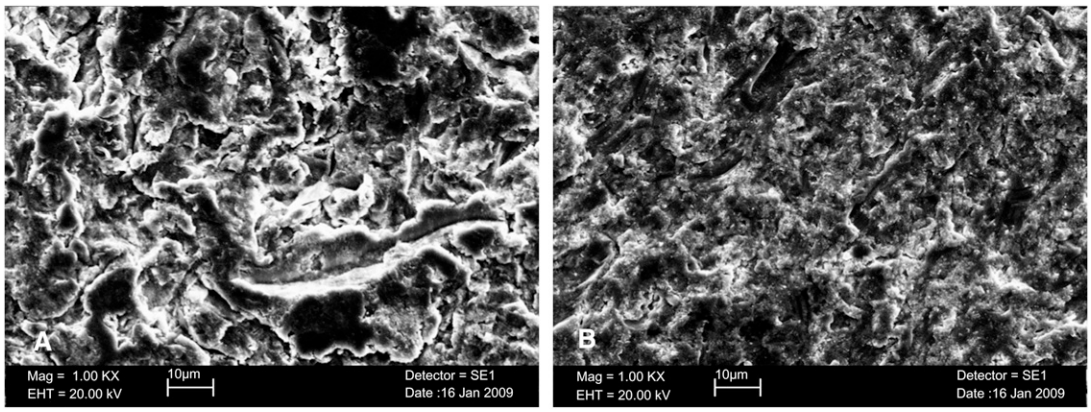

Figure 3.
Scanning electron photomicrographs of an untreated rough surface (control) ***(A)*** *and a rough surface treated with the air-powder abrasive system* ***(B)*** *(original magnification ×1,000). Note the presence of sodium carbonate deposits in the treated rough surface compared to the control.*

different instruments used for implant and abutment decontamination on the morphologic characteristics and roughness of smooth and rough titanium surfaces. The second aim was to evaluate if the adhesion of *S. sanguinis* on such surfaces could be changed after treatment with these different methods. The length of treatment period (50 seconds) was meant to simulate multiple patient recalls, because some damage is frequently not observed until after various applications. The standardization of the force of scaling did not seem practical because the treatments proposed vary widely in application methods. Thus, this study proposed to simulate how the instruments would perform on the titanium surfaces when a human hand applies them freely.

Under the protocol used in the present study, the Er:YAG laser did not significantly alter the roughness (Table 1) and morphology (Fig. 2) of smooth and rough titanium surfaces, except for minor mechanical damage caused by the contact of the tip. The clinical implication of these findings is that the Er:YAG laser may be used for the removal of biofilm from implants without significantly injuring their surfaces. To date, there are only a few studies[11,21-24] describing the effects of the Er:YAG laser on rough and smooth titanium surfaces. Differences on irradiation parameters (contact mode, water irrigation, and angle and time of irradiation) make it difficult to compare the outcomes of the different studies.[11,21-24] In agreement with our results, Schwarz et al.[22] did not find alterations in machine-polished and sand-blasted and acid-etched implant surfaces after treatment with an Er:YAG laser (85 mJ/pulse and 12.7 J/ cm^2). Also, Kreisler et al.[11,23] observed that laser treatment at 60 or 120 mJ/pulse of sand-blasted and acid-etched surfaces did not result in any visible microscopic changes, even without water cooling. Visible alterations in different types of implant surfaces were reported by Kreisler et al.[24] using Er:YAG laser irradiation at pulse energies over 120 mJ and by Matsuyama et al.[21] when titanium surfaces were irradiated with an Er:YAG laser at 100 and 200 mJ/pulse, corresponding to an output energy density of 35.4 and 70.8 J/cm^2, respectively.

Similar to Er:YAG laser treatment, plastic curet instrumentation caused no or minimal changes on the smooth and rough titanium surfaces. These data are in accordance with previous studies[18,20,25,26] that also showed no marked alterations after treatment of smooth and rough surfaces with plastic curets. If the preservation of surface integrity is the primary objective, the plastic curet may be one of the instruments of choice for implant biofilm debridement; nevertheless, the ability of this tool to effectively remove calculus and biofilm from smooth and rough surfaces has been widely questioned.[20]

The results of the present study demonstrate that the instrumentation with metal curets produced rougher textures compared to the other instruments (Table 1) and yielded definite damage on machined titanium surfaces (Fig. 2). The scratching and improper modification of the smooth surfaces of abutments and implants by metal instruments were also observed in previous studies.[16,25-27] Concerning rough surfaces, the roughness analysis did not reveal any difference between pre- and post-treatment with metal curets (Table 1). However, SEM pictures showed an evident difference between metal curet-treated and control surfaces (Fig. 2). Metal curets flattened out the rough surfaces, removed the edges of the irregularities, and formed surfaces with much lower visible levels of porosity, as previously demonstrated by Rühling et al.[18]

Although the SEM photomicrographs demonstrated some titanium surface changes after treatment

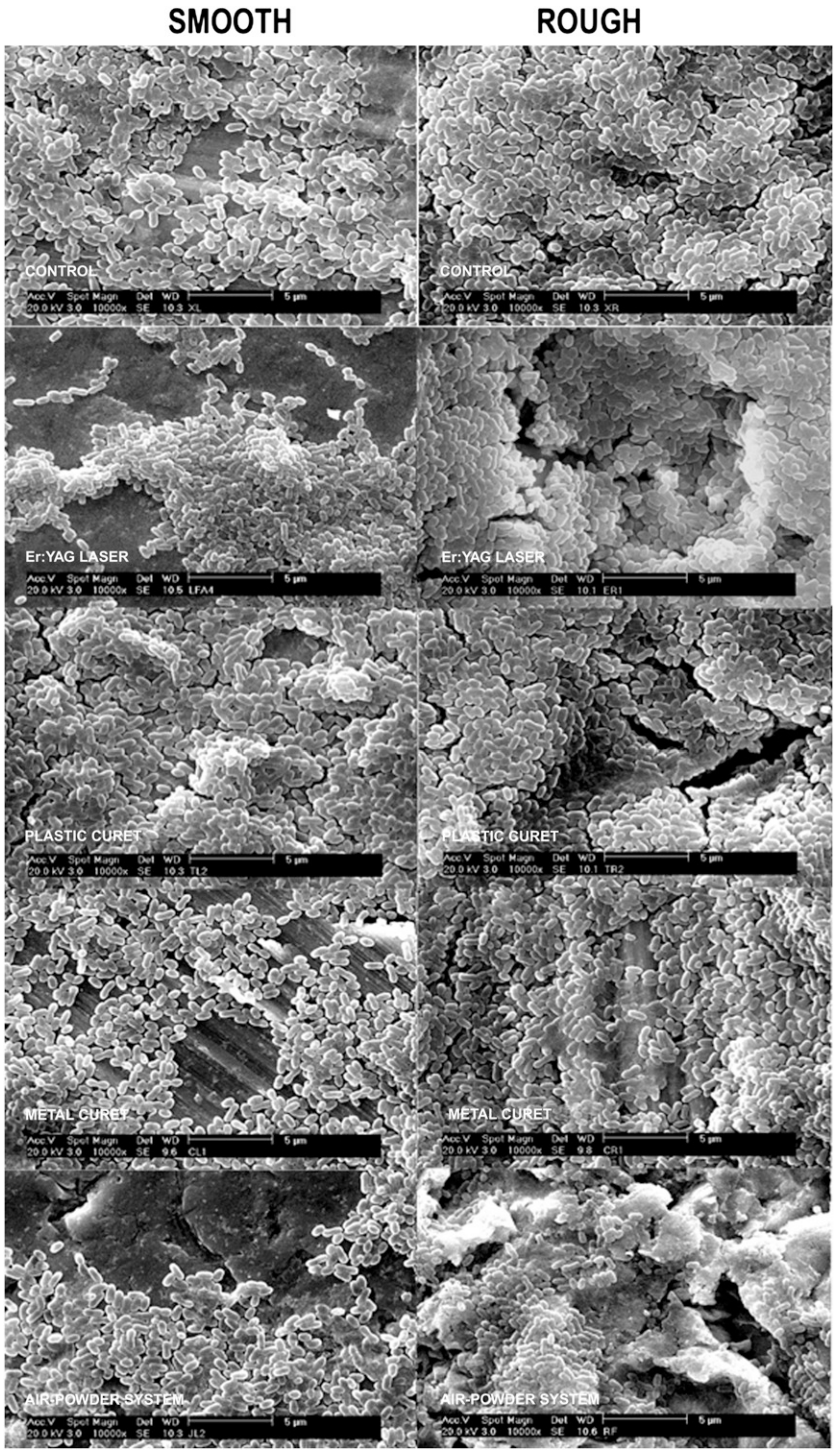

Figure 4.
Scanning electron photomicrographs of untreated (control) and treated smooth and rough surfaces after incubation of a suspension of S. sanguinis *(original magnification ×10,000).*

with the air-powder system, a quantitative roughness alteration was not detected by the profilometer for smooth and rough surfaces. In agreement with our SEM results, Meschenmoser et al.[27] also observed some small craters caused by salt crystals hitting the smooth titanium surface after treatment with an air-powder abrasive system. In addition, Parham et al.[15] demonstrated that the use of an air-powder abrasive system resulted in rounding of the angles and edges of rough titanium surfaces and occasional surface pitting. More recently, Kreisler et al.[23] noted that an air-powder abrasive system led to changes of rough implant surfaces, consisting in the reduction of the edges of the elevations. Considering that this device has been widely recommended for biofilm removal around dental implants, it is important to note that the surface changes may be affected by the hardness of the titanium, time exposure, air pressure, size and hardness of the abrasive particles, and distance and angulation of the tip.[28] In addition, improper use of an air-powder abrasive system may result in subcutaneous emphysema due to the presence of air in the interstices of connective tissue.[29,30]

As earlier demonstrated by in vivo[31,32] and in vitro studies,[33] in the present study, untreated rough surfaces presented higher levels of *S. sanguinis* adhesion than untreated smooth ones. The microbial adhesion to biomaterials has been related to surface-free energy, chemical composition, and surface roughness,[31-34] which enhance the microbial retention within surface irregularities. An Ra ~0.2 μm has been suggested as a threshold surface roughness value below which no further significant changes in the total amount of adhering bacteria can be observed due to the larger size of most bacteria.[31,35] Therefore, instruments used to decontaminate implants and abutments should not change the surface to make it more biofilm retentive, but they should attempt to minimize *de novo* biofilm formation. In the present study, smooth surfaces treated with all of the tested instruments demonstrated the same level of *S. sanguinis* adhesion as untreated control surfaces, besides the presence of different surface profiles at the SEM level (Fig. 2) and increased roughness after treatment with metal curets (Table 1). In agreement with these results, a previous in vivo study[17] demonstrated that the plaque accumulation on abutments was very

similar after treatment with plastic curets and the air-powder abrasive system, despite some differences in the surface texture between groups. Based on our results, one could speculate that, clinically, the type of instrument used does not play an important role on *de novo* biofilm formation on smooth surfaces. However, at this stage, caution should be used because the comparison of the level of biofilm formation on smooth titanium surfaces modified by these mechanical treatments has not been clinically evaluated.

Even though roughness was not different among all of the treated rough surfaces, the levels of *S. sanguinis* adhesion were lower on rough surfaces treated with metal curets and the air-powder abrasive system, achieving a level similar to those presented by the smooth surfaces treated with these instruments. One possible explanation for the reduced bacterial adhesion on the rough surfaces treated with a metal curet may be the texture produced by this instrument, characterized by flattening of the edges of the surface elevations (Fig. 2). The unexpected finding regarding the low bacterial adhesion rate on the surfaces treated with the air-powder abrasive system could be explained by the presence of deposits of sodium carbonate in the irregularities as observed at a higher magnification (Fig. 3). Therefore, it appears that the level of bacterial adhesion in the present study was not only an effect of the surface roughness but also the presence and nature of surface contaminants after treatment. However, caution must be used in the interpretation of this finding as an advantage because the presence of sodium carbonate deposits in the surface irregularities might have other unknown biologic implications, such as cytotoxicity and impairment of fibroblast and osteoblast adhesion.

One can argue that this study only observed the effect of titanium surface treatments on the adhesion of one bacterial species, which is associated with healthy implant sites. However, *Streptococci*, especially *S. sanguinis*, are considered significant early colonizers that facilitate the attachment of organisms normally incapable of binding to host surfaces and can ultimately lead to the development of a biofilm community.[36-38] Many secondary colonizers, which adhere to bacteria already in the biofilm mass, are well recognized to be involved in peri-implant diseases (e.g., *Fusobacterium*, *Capnocytophaga*, *Porphyromonas*, and *Prevotella* species).[8]

CONCLUSIONS

Metal curets produced higher levels of damage on smooth titanium surfaces, whereas all instruments produced the same quantitative level of roughness on rough surfaces. Therefore, considering surface roughness, metal curets are not recommended for the treatment of smooth surfaces, whereas all tested instruments are suitable for the debridement of rough surfaces. *S. sanguinis* adhesion was lower on rough surfaces treated with metal curets and the air-powder abrasive system, probably because of texture modifications and the presence of abrasive deposits. However, before the clinical recommendation of these modalities, further studies are necessary. This investigation was performed strictly in vitro, and the effect of surface changes on biofilm accumulation in vivo needs to be further studied. In addition, treatments that produce little surface damage also need to be tested according to their cleaning efficacy and impact on attachment of fibroblastic and osteoblast cells during the tissue repairing process.

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