Instrumentation With Ultrasonic Scalers Facilitates Cleaning of the Sandblasted and Acid-Etched Titanium Implants

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Mechanical instrumentation is widely used to debride dental implants, but this may alter the surface properties of titanium, which in turn may influence bacterial adhesion and make it more difficult to remove the biofilm. This in vitro study was performed (1) to assess the amount of biofilm formation on a sand-blasted and acid-etched titanium fixture treated with ultrasonic scalers with metal, plastic, and carbon tips and (2) to evaluate how this treatment of titanium surfaces affects implant cleaning by brushing with dentifrice. The titanium fixtures were treated with various ultrasonic scaler tips, and surface roughness parameters were measured by confocal microscopy. Biofilm was formed on the treated fixtures by using pooled saliva from 10 subjects, and the quantity of the adherent bacteria was compared with crystal violet assay. The fixture surfaces with biofilm were brushed for total of 30 seconds with a toothbrush with dentifrice. The bacteria remaining on the brushed fixture surfaces were quantified by scanning electron microscopy. Surface changes were evident, and the changes of the surfaces were more discernible when metal tips were used. A statistically significant decrease in roughness value (arithmetic mean height of the surface) was seen in the 2 metal-tip groups and the single plastic-tip group. After brushing with dentifrice, the treated surfaces in all the treatment groups showed significantly fewer bacteria compared with the untreated surfaces in the control group, and the parts of the surfaces left untreated in the test groups. Within the limits of this study, treatment of titanium fixture surfaces with ultrasonic metal, plastic, or carbon tips significantly enhanced the bacterial removal efficacy of brushing. Thorough instrumentation that smooths the whole exposed surface may facilitate maintenance of the implants.

Key Words: bacteria, dental implant, dental scaling, microscopy, electron scanning, surface properties, titanium, toothbrushing

INTRODUCTION

P eri-implant mucositis is the term used to describe reversible inflammatory reactions in the mucosa adjacent to an implant; peri-implantitis is defined as an inflammatory process that affects the tissues around an implant resulting in a loss of supporting alveolar bone.1 As soon as an implant surface is exposed to the oral cavity, it is immediately covered by the salivary pellicle and colonized by the oral microorganisms that form a microbial biofilm.2 Microbial colonization is reported to play a major role in the etiology of peri-implant infections.3 Thus, studies have been performed on the removal of bacteria from implant surfaces.2–4 Mechanical instrumentation is widely used for the debridement of dental implants, but this may damage the fixture surfaces.5,6 Thus, the use of manual plastic scalers, manual titanium scalers, and plastic or carbon composite ultrasonic scaler tips have been suggested for implant maintenance,7–9 as instrumentation with metal curettes and ultrasonic scalers have induced surface alterations on the surface of smooth implants.10–12 In contrast, it has been reported that the roughness value of a surface treated with plastic curettes was not significantly different from that of an untreated surface.7,13 These instruments were designed to reduce or eliminate the possibility of scratching the implant surface, which would make it more plaque retentive.13,14 Various techniques have been used to modify the surface of the machined titanium, including sand-blasting and acid-etching, for better adherence and propagation of osteoblasts compared with smooth machined surfaces.15–17 Titanium
implants with a sand-blasted and acid-etched (SA) surface demonstrated greater bone-to-implant contact\textsuperscript{18} and higher removal torque values compared with smooth-surface implants.\textsuperscript{15} Sand-blasting and acid-etching is one of the most widely used commercial techniques for surface modifications.\textsuperscript{19–21}

Previous reports have shown that bacterial adhesion is influenced by surface roughness.\textsuperscript{22} A higher degree of bacterial adhesion was observed on rougher surfaces compared with smooth surfaces.\textsuperscript{23,24} Moreover, plaque removal from modified roughened surfaces was more difficult than removal from machined surfaces.\textsuperscript{25} Implants exposed to the oral cavity must be cleaned by the patient using daily oral hygiene measures, and a toothbrush is the most common method.

Thus, this in vitro study was performed (1) to assess the biofilm formation on an SA titanium fixture surface pretreated with ultrasonic scalers with metal, plastic, and carbon tips and (2) to evaluate how pretreatment of SA titanium surfaces affects the efficiency of bacterial removal by brushing as assessed with crystal violet assay and scanning electron microscopy (SEM).

**Materials and Methods**

**Specimen preparation**

Figure 1 shows the overview of the study design. SA titanium fixtures (TS254010S, Osstem Implant Co Ltd, Seoul, Korea) measuring 4 mm in diameter and 10 mm in length were used in this study. In total, 15 fixtures were used. The fixtures were divided into 5 groups: (1) no treatment, (2) manufacturer A ultrasonic scaler with metal tip (A-M) (PS, Mini Piezon, EMS Piezon Systems, Nyon, Switzerland), (3) manufacturer A ultrasonic scaler with plastic tip (A-P) (PEEK tip, EMS), (4) manufacturer B ultrasonic scaler with metal tip (B-M) (1, Satelec, Suprason, La ciotat, France), and (5) manufacturer B ultrasonic scaler with carbon tip (B-C) (PH1, Satelec) (Figure 2). Three fixtures were used for each group, and measurements were taken from 4 valleys from the top.

**Determination of surface properties**

The roughness parameters (arithmetic mean height of the surface [Sa], maximum height of the surface [Sz], and skewness roughness [Ssk]) were measured using confocal microscopy (LSM5 Pascal, Zeiss, Jena, Germany) before and after instrumentation. All the values were determined at a cutoff length of 0.04 mm in 50 sections; the stack size of z-sections was 0.80 μm. Each scanning covered an area of 460.7 μm × 460.7 μm; a Gaussian filter, a low-pass filter to reduce noise from the signal, was used to determine surface values. The roughness measurements were calculated by proprietary software (Topography package, Zeiss). Three fixtures were used for each group, and measurements were taken from 4 valleys from the top.

**Biofilm formation**

Biofilm formation was performed according to a protocol described by Lee et al.\textsuperscript{26}

1. **Salivary Pellicle Formation**

Pooled saliva of healthy donors was centrifuged at 8000g for 10 minutes at 4°C, and the supernatant was filtrated with a 0.22 μm polyvinylidene fluoride membrane. Filtered saliva was diluted to twofold using phosphate buffered saline (PBS) and added to the plates containing 12 instrumented and 3 nontreated SA titanium fixtures. The fixtures were completely dried at 37°C and sterilized in an ultraviolet sterilizer.

2. **Biofilm Formation and Observation**

Pooled saliva was collected from 10 healthy donors and centrifuged at 1500g for 10 minutes at 4°C to remove debris. The saliva supernatant was transferred into a new tube to which a threefold volume of brain heart infusion (BHI) (BD Biosciences, Franklin Lakes, NJ) broth containing 2% sucrose and 1% mannose was added, and then vortexed for 20 seconds. Pellicle formed fixtures were placed, one in each well of a 24-well plate and fixed to the bottom of the plate. Then, 1 mL of saliva-added BHI was placed in each well. The plate was incubated in an anaerobic chamber in an atmosphere of 85% N₂, 10% H₂, and 5% CO₂ for 48 hours.

**Examination of bacterial adhesion on pretreated SA fixtures using crystal violet assay**

Three fixtures in each group were used for crystal violet assay. The crystal violet assay was performed to evaluate the total amount of

\[ \text{FIGURE 1. Overview of the study design.} \]
bacteria on the pretreated implant fixture surface. After incubation of the titanium implants pretreated with bacteria, fixtures were washed with PBS twice to remove unattached bacteria and debris. Adhering bacteria were stained with 1% crystal violet for 10 minutes at room temperature. The bound dye was extracted using destaining solution consisting of 80% ethanol and 20% acetone. The amount of bacteria was measured at optical density of 570 nm using a microplate reader (BioTek, Winooski, Vt.). The optical density was measured twice.

**Brushing of the pretreated SA fixtures**

The fixtures from groups 1 through 5 were brushed for total of 30 seconds (15 seconds in a horizontal direction and 15

**FIGURE 2.** Metal and nonmetal ultrasonic scaler tips and toothbrush. (a) Manufacturer A metal tip. (b) Manufacturer A plastic tip. (c) Manufacturer B metal tip. (d) Manufacturer B carbon tip. (e) Toothbrush.
seconds in a vertical direction) with a toothbrush (Implant Care, TePe, Malmö, Sweden) and a fluoride dentifrice (Anti-Plaque, Bukwang, Seoul, Korea) containing calcium phosphate, sodium monofluorophosphate, and xylitol. The fixtures were rinsed with tap water for 30 seconds.

**Quantitative evaluation of bacterial removal efficiency after brushing using SEM**

After brushing the pretreated titanium implant fixtures, the surfaces were washed with PBS twice to remove debris. The surfaces were evaluated using SEM to determine the efficiency of bacterial removal after brushing. Each implant fixture was fixed with 2.5% glutaraldehyde in PBS overnight. The fixtures were then washed in PBS 3 times and post-fixed in 1% osmium tetroxide for 1.5 hours. After dehydration through a graded series of ethanol (70%, 80%, 90%, and 95% for 15 minutes each and in 100% for 15 minutes twice), the samples were mounted on stubs. These were then air-dried by evaporation of hexamethyldisilazane on a clean bench and sputter-coated with gold palladium. The samples were observed using SEM (S-4700, Hitachi, Tokyo, Japan) at 15 kV at magnification of 5000×.

Three fixtures were used for each group. After 40 strokes of instrumentation, there were untreated surfaces left on all fixtures. As areas that had not been instrumented could clearly be distinguished from treated areas in the SEM view, surfaces were divided into instrumented and noninstrumented areas for bacteria quantification. Ten images per fixture were captured from the no-treatment group. Twenty images per fixture were captured for treatment groups. Ten images each were obtained from instrumented and noninstrumented surfaces (A-M, A-P, B-M, and B-C groups). The images were saved as tiff files, and bacteria remaining on the fixtures were manually counted. If less than half of the bacterium was apparent in the image, it was not counted.

**Statistical methods**

Data were represented as means ± standard deviations. One-way analysis of variance was used to test for statistical differences among treatment groups with commercially available statistical software (SPSS 12 for Windows, SPSS Inc, Chicago, Ill). Statistically significant differences were evaluated with significance set at *P* < .05.

**RESULTS**

The gross morphology of the surface of the titanium fixtures after different treatments, including ultrasonic scaler tips, is shown in Figure 3. Surface changes could be discriminated by inspection alone. The change of the surfaces was more obvious when the surfaces were treated with metal tips (A-M and B-M groups).

The surface characteristics of the examined area observed with confocal microscopy are listed in Figure 4. Ultrasonic metal tips from manufacturer A and manufacturer B produced smoother surfaces than the other groups. The qualitative analyses of 3-dimensional roughness parameters are seen in the Table. The Sa values of SA fixture surfaces were reduced after all treatments. A statistically significant decrease of the Sa value was seen in the A-M (6.70 ± 1.22 μm), A-P (6.80 ± 0.55 μm), and B-M (6.84 ± 1.61 μm) groups compared with the untreated group (8.56 ± 3.59 μm). Treatment of the SA surface resulted in decrease of Sz similar to decreases in the Sa value. The Sz values of the A-M and B-M groups, 23.69 ± 3.85 and 24.50 ± 3.44 μm, respectively, were statistically less than the Sz values of the no-treatment group (28.80 ± 3.64). The Ssk values of the no-treatment, A-M, A-P, B-M, and B-C groups were −0.54 ± 0.27, −0.72 ± 0.19, −0.56 ± 0.22, −0.71 ± 0.17, and −0.63 ± 0.18, respectively. No significant differences were noticed between the groups. Collectively, the A-M and B-M groups were the only groups that showed a significant decrease in Sa and Sz values compared with the untreated group (*P* < .05).

The gross morphology of the surface of the pretreated titanium fixtures after biofilm formation and application of crystal violet is shown in Figure 5. The A-M, A-P, B-M, and B-C groups had 108.4% ± 38.1%, 105.0% ± 11.7%, 117.9% ± 20.1%, and 145.9% ± 39.9% of bacteria, compared with the amount of bacteria in the no-treatment group (*P* > .05).

Figure 6 shows the images obtained from no-treatment group and from noninstrumented surfaces from the treated groups after brushing with dentifrice. Remnants of dentifrice were observed from images of all groups, even after rinsing with tap water. All the SEM images showed bacteria in the pits of SA titanium surfaces (images of surfaces from the no-treatment group and noninstrumented surfaces from the treated groups) after brushing the surfaces with dentifrice. The adhering bacteria in the SEM images from each group were counted and compared (Figure 7). The no-treatment, A-M, A-P, B-M, and B-C groups had 46.4% ± 59.4%, 23.1% ± 24.2%, 18.0% ± 25.1%, 26.7% ± 30.6%, and 28.5% ± 32.8% of bacteria.

Figure 8 shows the images obtained from the no-treatment group and from the instrumented surfaces of the treatment groups after brushing with dentifrice. Brushing the instrumented surfaces significantly decreased the amount of bacteria adhering on the surfaces in all the groups (A-M, A-P, B-M, and B-C) (Figure 9). The A-M, A-P, B-M, and B-C groups had 2.3% ± 6.8%, 0.8% ± 1.9%, 9.7% ± 16.5%, and 2.7% ± 5.1% of bacteria, respectively, when the number of bacteria of the no-treatment group was considered to be 100%. The results clearly showed that it was easier to remove bacteria from instrumented surfaces, regardless of treatment method (*P* < .05). The amount of bacteria left on the treated surfaces was not significantly different among the treatment groups (A-M, A-P, B-M, and B-C) (*P* > .05).

**DISCUSSION**

This in vitro study evaluated the effect of instrumentation on the adherence of bacteria on the SA titanium fixtures and on the efficacy of bacteria removal from the fixtures after brushing with dentifrice.

Roughness of the surface may be evaluated by various values. Average roughness (Ra) is one of the most widely used surface parameters, and this value has been used for the profilometric analysis of the implant fixtures. Although Ra can be used to provide a general impression of the characteristics of surfaces, surfaces with sharp spikes and surfaces with deep pits may all yield the same average roughness values because...
Ra makes no distinction between peaks and valleys.\textsuperscript{28} The Rz value (the arithmetic mean of the positive predominant crest and the analog absolute value of the negative crests) is one of the most frequently used roughness parameters in the industry.\textsuperscript{29,30} Both Ra and Rz provide information regarding the profile and do not include information about spatial structure. Moreover, the measurement direction may influence the results of Ra and Rz.\textsuperscript{30,31} Thus, Sa and Sz values were used to evaluate the fixture surfaces because these values give information about the given surface area and provide more
reliable and consistent information. The Ssk value is used because this represents the symmetry of the surface but no significant changes were achieved after the treatment.32

Different methods have been used to evaluate surface changes, including SEM33,34 and confocal microscopy.35,36 Although SEM is widely used to evaluate surface topography, this method may be limited to the qualitative analysis; furthermore, surface preparation is needed for this procedure.37 However, confocal microscopy can give quantitative roughness parameters by reconstructing optical sections by reflection or fluorescence imaging.38,39

Treatment with ultrasonic metal, plastic, and carbon tips all reduced the roughness of the fixture surfaces. However, only the A-M and B-M groups showed statistically significant decreases in Sa and Sz values compared with the untreated group. The lowest mean value was from the A-M group, which indicates that treatment with the ultrasonic scaler from manufacturer A produced the smoothest surfaces by reducing the irregularities.

The bacteria implicated in peri-implantitis are similar to those in periodontal disease, and include such anaerobic bacteria as Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans (formerly known as Actinobacillus actinomycetemcomitans), and Treponema denticola.40,41 The P gingivalis counts were higher in cases with progressive peri-implantitis,42 and P gingivalis has been used to evaluate the bacterial removal efficiency in our group.43 In other reports, specimens were fixed to individual removable acrylic upper jaw splints for in vivo biofilm formation.28,29 The splints were worn for 12 hours to 10 days.28 This would be a very good method to investigate the removal efficiency of biofilm. However, volunteers are needed, prostheses need to be fabricated, and there may be individual and tooth-site differences on the rate of biofilm formation.45-47 In this experiment, early biofilm formation was examined by in vitro methods incorporating methods reported previously with salivary bacteria.26

Crystal violet assay was used to evaluate bacterial adhesion on pretreated SA fixtures.48,49 Adherence of bacteria was evaluated after treating the SA titanium surface up to 40 seconds with various instruments. Instrumentation of the titanium surface may influence early adhesion of bacteria, but at 48 hours, colonization of the bacteria on the instrumented fixtures did not differ from that of the untreated SA fixture surfaces. Comparable growths of the biofilms were observed on all treatment groups.

Even after instrumentation, untreated surfaces were left on all the fixtures. This in part pertains to the fact that it was difficult to maneuver the instrument tips between the threads. Only a small number of bacteria were left on the smooth treated surfaces after brushing with dentifrice; however, SEM images showed bacteria were still left inside the pits and grooves of the untreated or unscratched titanium surfaces even after brushing. This clearly shows that it is more difficult to remove the bacteria from these deep niches by brushing.50 It would be easier to maintain smooth surfaces free of bacteria than try to remove bacteria from the pits and grooves of rough surfaces.

Several methods have been used to evaluate changes in surface roughness after treatment with different instruments.12,51,52 In some reports, double pan balance was used to give standardized horizontal movement with a constant force.53 No standardization was done for this study because the objective of this study was to simulate clinical situations.53 The applied force of scalers in different studies also varies greatly, from 40 g to 300 g.12,51,54 In this study, care was taken to place minimal lateral pressure—approximately 30 g of pressure for
each stroke. However, there were some difficulties in treating the surface evenly because of the threads of fixture. Previous studies showed great variations in application time of scalers, from 5 seconds to 30 seconds, or even up to 5 minutes. In one study, 25 to 250 strokes were used, other studies applied as many as 350 400 strokes to the specimens. Forty strokes were applied within 40 seconds in this experiment, a number chosen to simulate a single maintenance visit. Longer times with greater numbers of

**Figure 5.** Gross morphology of the surface of the pretreated titanium fixtures after biofilm formation and application of crystal violet. (a) No treatment. (b) Manufacturer A metal tip. (c) Manufacturer A plastic tip. (d) Manufacturer B metal tip. (e) Manufacturer B carbon tip.
strokes may be applied to reflect multiple visits or an extended period of implant maintenance.

Results of this study show that the surface changes that occur after various mechanical instrumentations did not influence the adherence of bacteria. However, removing bacteria from the treated surface, irrespective of the instrument used (ultrasonic metal, plastic, or carbon tips), was significantly easier. Mechanical therapy with various instruments may

FIGURES 6 AND 7. FIGURE 6. The bacteria were grown on pretreated surfaces and all surfaces were brushed with dentifrice. The figures were achieved from the no-treatment group and nonscratched surfaces from the treated groups. (a) No treatment. (b) Manufacturer A metal tip. (c) Manufacturer A plastic tip. (d) Manufacturer B metal tip. (e) Manufacturer B carbon tip. FIGURE 7. Average number of bacteria in each group (no treatment surface or nonscratched surface from treated groups) seen from scanning electron microscopy images.

FIGURES 8 AND 9. FIGURE 8. The images were obtained after the no-treatment surface or scratched surfaces from the treated groups were brushed with dentifrice. (a) No treatment. (b) Manufacturer A metal tip. (c) Manufacturer A plastic tip. (d) Manufacturer B metal tip. (e) Manufacturer B carbon tip. FIGURE 9. Average number of bacteria was calculated after the no-treatment surface or scratched surfaces from the treated groups were brushed with dentifrice.
change the surface, but in situations where the fixture surface is exposed to the oral cavity and oral hygiene measures are performed by the patient, careful instrumentation that leaves the surface of the exposed fixture completely smooth may be more efficacious for bacterial removal.

**Conclusion**

This in vitro study assessed the amount of biofilm formation on an SA titanium fixture treated with ultrasonic scalers with metal, plastic, and carbon tips, and evaluated how this treatment of titanium surfaces affects the cleaning of the implant by brushing with dentifrice. Within the limits of this study, treatment of titanium fixture surfaces with ultrasonic metal, plastic, or carbon tip significantly enhanced bacterial removal efficacy of brushing. Thorough instrumentation that can smooth the whole exposed surface may facilitate maintenance of the implants.

**Abbreviations**

BHI: brain heart infusion
PBS: phosphate buffered saline
Ra: average roughness
Rz: the arithmetic mean of the positive predominant crest and the analog absolute value of the negative crests
Sa: arithmetic mean height of the surface
SA: sand-blasted and acid-etched
SEM: scanning electron microscopy
Ssk: skewness roughness
Sz: maximum height of the surface

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