Resolution of peri-implant inflammation and re-osseointegration of peri-implantitis affected dental implants seem to be dependent on bacterial decontamination. The aims of the study were to evaluate the antimicrobial effects of 3 different instrumentations on a micro-textured dental implant surface contaminated with an avirulent or a virulent Porphyromonas gingivalis strain and to determine alterations to the implant surface following instrumentation. Forty-five dental implants (Straumann SLA) were allocated to 3 treatment groups: Er:YAG laser, chitosan brush, and titanium curette (10 implants each) and a positive (10 implants) and a negative (5 implants) control. Each treatment group and the positive control were split into subgroups of 5 implants subsequently contaminated with either the avirulent or virulent P. gingivalis strain. The antimicrobial effect of instrumentation was evaluated using checkerboard DNA–DNA hybridization. Implant surface alterations were determined using a light interferometer. Instrumentation significantly reduced the number of attached P. gingivalis (P < .001) with no significant differences among groups (P = .310). A significant overall higher median score was found for virulent compared with avirulent P. gingivalis strains (P = .007); the Er:YAG laser uniquely effective removing both bacterial strains. The titanium curette significantly altered the implant surface micro-texture. Neither the Er:YAG laser nor the chitosan brush significantly altered the implant surface. The 3 instrumentations appear to have a similar potential to remove P. gingivalis. The titanium curette significantly altered the microstructure of the implant surface.

Key Words: decontamination, dental implant, laser, P. gingivalis, microstructure, surface alteration

INTRODUCTION

The use of dental implants to replace missing teeth has in many cases been shown to be a successful treatment option to meet functional and esthetic demands. Although considered a safe and predictable treatment commanding a high survival rate, biological and technical complications and failures do occur.1 One of the most prevalent long-term complications is peri-implantitis.2 Pending diagnostic criteria, its prevalence has been estimated at approximately 20% at the patient level and 10% at the implant level.3

Despite diverging opinions,4 peri-implantitis generally is considered an infectious inflammatory condition around an osseointegrated dental implant associated with bleeding, suppuration, and loss of supporting bone.5 The literature provides convincing evidence of a microbiological etiology with a microbiota similar to that of chronic periodontitis including high levels of Gram-negative anaerobic bacteria.6 Porphyromonas gingivalis represents one such anaerobic microorganism associated with peri-implantitis.7 This species harbors an arsenal of virulence factors, including fimbriae. The fimbriae, long or short, seem to participate in nearly all interactions between the bacteria and the host, as well as other surfaces it might contaminate. The long fimbriae are considered directly responsible for many of the adhesive properties of the microorganism. Based on the variation of nucleotide sequence of the encoding gene (fimA), a possible relationship among different fimA genotypes, virulence, and disease has been investigated. Both clinical and experimental studies strongly indicate that fimA genotype II is a determinant of virulence (periodontitis, cardiovascular disease, etc), whereas genotype I is associated with health.8,9 Further, P. gingivalis has the ability to invade and live inside host cells where it can induce inflammatory responses that may lead to tissue breakdown.10 Moreover, P. gingivalis, even in low numbers, may have the ability to orchestrate an inflammatory response by altering a normal, benign microbiota into a dysbiotic one,11 strongly supporting its pathogenic potential.

Contemporary dental implants are manufactured from pure titanium or titanium alloys. The micro-textured surface of most dental implants makes them prone to biofilm adherence and makes the exposed implant surface inaccessible to adequate...
cleaning.12 This has led to the investigation of a number of techniques and treatment approaches for cleaning a contaminated implant surface using mechanical and chemical means. Devices including curettes and brushes, and even burs for smoothening of the surface, are used. The use of lasers, and for this purpose especially the Er:YAG laser, has met growing popularity due to its ability to remove both soft and hard deposits, as well as its bactericidal and photobiomodulation effects.13 Another novel product for biofilm removal is a brush with resorbable chitosan filaments (BioClean, Labrida AS, Oslo, Norway). This brush appears a promising alternative for implant surfaces. Nevertheless, these treatment approaches all have shortcomings due to their limited access to the contaminated implant surface and the complexity of the attached biofilm. In addition, the treatment should not change the implant surface in a way that could interfere with its biocompatibility or implant structural integrity.15

Thus, the aims of this study were 3-fold: to evaluate the antimicrobial effects of an Er:YAG laser device, a chitosan brush, and titanium curettes on implant surfaces contaminated with an avirulent (AVIR) or a virulent (VIR) strain of P. gingivalis in an in vitro model; to compare the treatment outcomes for implant surfaces contaminated with the 2 different strains; and to determine alterations of the implant surface micro-texture resulting from the treatment.

**MATERIALS AND METHODS**

**Implants**

Forty-five Straumann SLA dental implants (D, 4.1 mm; L, 12 mm; Standard Plus, SLA, Straumann AG, Basel, Switzerland) were included. All implants were received sterile in the manufacturer’s original packaging. The implants were randomly allocated to 3 treatment groups of 10 implants, a positive control (10 implants), and a negative control (5 implants). Each treatment group and the positive control were subdivided into 2 groups of 5 implants to be contaminated with an AVIR or a VIR P. gingivalis strain (Figure 1).

**Contamination of the implants**

Two P. gingivalis strains, ATCC33277 (AVIR) and A7A1-28 (VIR), grown on blood agar under anaerobic conditions were used. The 2 strains come from the P. gingivalis library stored at the Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway. They are characterized by several methods, both multilocus sequence typing and fimA genotyping.

To prepare contamination of the implants, 5 mL bouillon (BBL enriched thioglycollate with vitamin K1 and hemin) was added to 5.5 mL human blood. The bouillon of blood (10%) was transferred to 25-mL growth flasks, and either strain was added. Ten microliters of the bacterial cultures were dissolved in 1 mL medium, and 100 μL was transferred to each flask; 4 flasks with the AVIR strain, 4 flasks with the VIR strain, and 1 flask with bouillon and blood without bacteria. Five implants were transferred to each flask of the AVIR and VIR strain, respectively, whereas 5 implants were transferred to the flask without bacteria (negative control). The flasks were incubated anaerobically (90% N2, 5% H2, 5% CO2) at 37°C (Anoxomat WS9000, Mart, Lichtenvoorde, The Netherlands) and agitated (50 rpm) for 8 days. The negative control was incubated identically under sterile conditions. Eight days was chosen based on our experience with the culture medium and broth used in this study and several other studies.

P. gingivalis adherence to the implant surface was confirmed using scanning electron microscopy (SEM) (XL30 EEM, Philips, Eidenhoven, The Netherlands). The implants were dehydrated in ethanol and critical-point dried, attached to metal studs, and sputter-coated with a 30-nm-thick layer of platinum in a Polaron E5100 sputter coater. The SEM was operated at 15 kV.

**Decontamination of the implants**

The implants were instrumented following a standardized protocol. Using a custom implant holder, the implants were randomly selected and fixed in a vertical position. Each implant was thoroughly rinsed using approximately 30 mL sterile saline. Instrumentation was performed circularly in an apical-cervical direction. Based on a prestudy test, a 3-minute instrumentation was considered sufficient for each device to accomplish implant surface decontamination. Following instrumentation, the implants were again rinsed using approximately 30 mL sterile saline. To prevent contamination, the implant holder was rinsed in ethanol and sterile saline before and between each implant instrumentation.

The implants in the laser group were treated using an Er:YAG laser at a wavelength of 2940 nm (LightWalker, Fotona D.D., Ljubljana, Slovenia) equipped with a H14-N hand piece and a cylindrical ø1.3–×-8-mm sapphire fiber tip. The settings were medium-short pulse, 80 mJ/10 Hz with water and air both set to level 5 according to the manufacturer’s recommendations for decontamination of implant surfaces. The fiber tip was used perpendicularly to the implant surface at a distance and a speed of approximately 1 mm and 2 mm/s, respectively. Care was taken to overlap with even strokes covering the complete surface of the implants in an apical-cervical direction.

The implants in the chitosan brush group were treated using a rotating brush (BioClean, Labrida AS). Before use, the sterile packaged brush was soaked in sterile saline for 3 minutes and then mounted in a handpiece with a gearing of 1:1 connected to a micromotor set to 1500 rpm. The brush was used parallel to the long axis of the implant in a gentle manner with care taken not to push the metal wire inside the brush.

![Flow chart of the study.](image-url)
against the implant surface. A new brush was used for each implant.

The implants in the titanium curette group were treated with titanium curettes specifically made for debridement of titanium implants (Implant Mini Universal curette, LM-instruments Oy, Parainen, Finland). The curettes were used with vertical strokes with the working edge against the implant surface, as well as with horizontal strokes with the point of the curettes in the valleys and against the flanks of the implant threads.

Microbiological sampling, DNA extraction, and checkerboard analysis

Following instrumentation, DNA was extracted from the implants and quantified by checkerboard DNA–DNA hybridization. The DNA in the positive and negative controls, with and without P. gingivalis, was used as reference values. In each group, the remaining bacterial implant surface DNA was applied as the inverse determinant of decontamination.

Before DNA extraction, the treated implants were transferred to tubes containing 200 μL TE solution (10 mM Tris-HCl and 1 mM EDTA) and 200 μL 0.5 M NaOH (lysis buffer). From the solution, 200 μL was used for the checkerboard analysis. A total of 200 μL of the lysis sample from each implant was boiled for 10 minutes and then 800 μL 5 M NH₄ acetate was added before loading the nylon membrane. The membrane was loaded with samples of standard preparations of 10⁵, 10⁶, and 10⁷ bacterial cells for quantification references in 3 lanes at the top of the membrane. Then, DNA samples from each implant were loaded one by one in specific lanes of the membrane corresponding to the respective test groups.

The DNA probe for P. gingivalis labeled with digoxigenin was applied perpendicular to the standard preparations. The hybridization between the P. gingivalis probe, and each sample DNA was detected by an antidigoxigenin antibody conjugated with alkaline phosphatase and a chemiluminescence substrate. DNA was detected by an antidigoxigenin antibody conjugated with alkaline phosphatase and a chemiluminescence substrate.

Quantitative surface characterization

A light interferometer (MicroXAM, Phaseshift, Tucson, Ariz) was used for quantitative surface roughness characterization of implants with threads at the micrometer level. The surface evaluation was performed at ×50 magnification and 0.62 zoom measuring an area of 264 × 200 μm.

To characterize the implant surface, 3 measurements were taken in different areas, from the tops, the valleys, and the flanks of the threads, totaling 9 recordings from each unit. In addition, 3 sampling units were evaluated for each implant group. The undulations from the machining process and the shape were considered separately to adequately describe the roughness obtained following instrumentation. A standard filtering process, with a 50 × 50-μm Gaussian filter was applied to make the separation and to evaluate the micrometric roughness. For this purpose, a surface scan software (Sonomicro Instrument, Lyon, France) was used, which also provides visual images and numerical descriptions. The following parameters were selected for the numerical description of the implant surface texture: Sₐ, arithmetic mean of the roughness area from the mean plane; Sₜ, density, or the number of peaks per unit of area; Sₐr, hybrid parameter representing the ratio between the developed surface area and a flat reference area.

Qualitative surface characterization

Alterations in implant surface texture were identified using SEM (Supra 55VP, Zeiss, Oberkochen, Germany) following the manufacturer’s recommendations. Implants were attached to sample studs and fully inspected at 20 kV at a magnification of ×50. Representative surface areas were recorded at a ×3000 magnification.

Statistical analysis

Bacterial counts ranging from <10⁵ to >10⁷ from the checkerboard analysis were given scores from 1 to 7. Descriptive statistics, median bacterial score, and range were calculated for each treatment group and for each strain. Because the outcomes of the bacterial counts were organized in categories and not normally distributed, the Kruskal-Wallis rank test was applied to examine the overall differences in bacterial reduction among the treatment groups. The same test was further used to examine differences in bacterial counts between the groups, the overall difference between the strains, and differences between each strain within each treatment group.

The parameters Sₐ, Sₜ, and Sₐr were measured from 3 different sites of every implant. Box plots showing the raw data for the 3 parameters are presented. Because each implant was measured at 3 sites, linear mixed effects models were used. The linear model (assuming a normal distribution and an identity link function) included a fixed effect part applying a dummy coding for the 3 sites and the 4 treatment categories. Implant was entered as a random effect adjusting for the correlation between different measures from the same implant. From these analyses, estimated marginal mean values were presented based on the estimated fixed effects. Post hoc analyses for multiple comparisons of differences between treatment groups and sites were adjusted using Scheffe’s method. Results were considered statistically significant for P < .05. Stata version 13 (Stata Corp, College Station, Tex) was used for all analyses.

Results

Microbiological analysis

SEM revealed adherence of both P. gingivalis strains to the implant surface following inoculation and incubation. The bacteria were often seen in clusters and mostly on the microtextured surface of the body of the implant (Figure 2).

Compared with the positive control, implants from all 3 treatment groups showed a highly significant reduction in attached bacteria following instrumentation regardless of approach (P < .001). The median bacterial score was reduced from 7 to 4 (range, 2–5) for the Er:YAG laser, from 7 to 5 (range, 2–7) for the chitosan brush, and from 7 to 4.5 (range, 2–6) for
the curette group. Overall, there were no significant differences between treatment groups ($P = .310$; not tabulated).

Comparing the overall remaining bacteria of the 2 $P$. gingivalis strains following instrumentation, a significant higher median bacterial score was found for VIR ($P = .007$). At treatment level, a significantly higher number of VIR was detected for the chitosan brush group and for the curette group ($P = .009$ for both), but not for the Er:YAG laser group ($P = .95$; Table 1). When looking at the remaining bacteria of AVIR following instrumentation, there was a significant difference between the treatment groups with the Er:YAG laser showing a lower median bacterial score than the chitosan brush and the curette groups ($P = .007$). For VIR, there was no significant between-group difference ($P = .878$; not tabulated).

**Surface characterization**

Following instrumentation, a significant difference in implant surface roughness was detected. For both the $S_a$ and $S_{ds}$ parameters for the valley and top sites, the overall difference was highly significant ($P < .001$; Table 2). For the $S_a$ parameter and the valley and top site, the titanium curette group showed a significantly lower mean value (1.67 and 1.35 μm, respectively) compared with the positive control (2.31 and 2.02 μm, respectively), whereas the Er:YAG laser (2.24 and 2.05 μm) and the chitosan brush group (2.25 and 2.25 μm, respectively) showed comparable mean values. This indicates that the curette instrumentation flattened some of the peaks in contrast to the Er:YAG laser and the chitosan brush. The same trend was seen for the $S_{ds}$ parameter and the valley and top site; the titanium curette group showed a significantly higher mean (0.12% and 0.15%, respectively) than the positive control (0.11% and 0.12%, respectively), the Er:YAG laser (0.11% and 0.11%, respectively), and the chitosan brush group (0.11% and 0.11%, respectively), indicative of causing shallow scratches on the implant surface following instrumentation. The flank area showed similar values for all parameters and treatment groups. No significant differences were observed for the $S_{dr}$ parameter (Table 2; Figure 3a through c).

SEM of the implant surfaces revealed significant alterations in surface morphology following titanium curette instrumentation. Compared with the other groups, showing only minor visible surface alterations, the surface of the curette treated implants seemed to have lost the original surface microstructure. The rough part of the implant was smoothed out and flattened, whereas the turned neck showed clearly vertical scratches (Figure 4).

**DISCUSSION**

The aim of the present study was to evaluate the potential of an Er:YAG laser, a chitosan brush, and titanium curettes to decontaminate a micro-textured dental implant contaminated with 2 different strains of $P$. gingivalis and to evaluate any adverse effects on the implant surface due to instrumentation. Compared with the positive control, all treatments significantly reduced the bacterial contamination, although without significant differences between treatments, whereas the

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**FIGURE 2.** SEM images of the SLA implant surface inoculated with *Porphyromonas gingivalis*: (a) Magnification $\times 10,000$ and (b) magnification $\times 30,000$. Asterisks indicate presence of *P. gingivalis* on the implant surface.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Median Bacterial Score (Range)</th>
<th>$P$ Value for AVIR vs VIR for Each Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:YAG laser</td>
<td>AVIR</td>
<td>4 (4–5)</td>
<td>.95</td>
</tr>
<tr>
<td></td>
<td>VIR</td>
<td>2 (2–5)</td>
<td></td>
</tr>
<tr>
<td>Chitosan brush</td>
<td>AVIR</td>
<td>6 (6–7)</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>VIR</td>
<td>2 (2–4)</td>
<td></td>
</tr>
<tr>
<td>Curette</td>
<td>AVIR</td>
<td>5 (5–6)</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>VIR</td>
<td>2 (2–4)</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>AVIR</td>
<td>7 (7–7)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>VIR</td>
<td>7 (7–7)</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>0 (0–0)</td>
<td></td>
</tr>
</tbody>
</table>

*AVIR indicates avirulent *Porphyromonas gingivalis* strain ATCC33277; VIR, virulent *P. gingivalis* strain A7A1-28.
titanium curette uniquely altered the implant surface. These observations indicate that the Er:YAG laser, chitosan brush, and the titanium curette all are able to reach and dislodge the bacteria from the micro-textured implant surface.

Similarly designed in vitro studies have documented a reduction of bacteria following instrumentation using Er:YAG lasers, titanium curettes, brushes, and other available methods. Contrary to our findings, a study comparing an Er:YAG

### TABLE 2

Marginal mean values and standard deviations (in parenthesis) for each parameter and site for all treatments (overall *P*-value for treatments tested against each other), calculated from linear mixed effects models

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Site</th>
<th>A: Positive Control</th>
<th>B: Er:YAG Laser</th>
<th>C: Chitosan Brush</th>
<th>D: Titanium Curette</th>
<th>Overall P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa (μm)</td>
<td>Valley</td>
<td>2.31 (2.15–2.47)</td>
<td>2.24 (2.08–2.39)</td>
<td>2.25 (2.09–2.40)</td>
<td>1.67 (1.51–1.82)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>2.02 (1.86–2.18)</td>
<td>2.05 (1.89–2.20)</td>
<td>2.25 (2.09–2.41)</td>
<td>1.35 (1.19–1.51)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Flank</td>
<td>1.82 (1.63–2.01)</td>
<td>1.60 (1.41–1.79)</td>
<td>1.66 (1.47–1.85)</td>
<td>1.51 (1.32–1.70)</td>
<td>.132</td>
</tr>
<tr>
<td>Sds/mm²</td>
<td>Valley</td>
<td>0.11 (0.10–0.11)</td>
<td>0.11 (0.10–0.11)</td>
<td>0.11 (0.10–0.11)</td>
<td>0.12 (0.12–0.13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>0.12 (0.11–0.13)</td>
<td>0.11 (0.11–0.12)</td>
<td>0.11 (0.10–0.12)</td>
<td>0.15 (0.14–0.16)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Flank</td>
<td>0.12 (0.12–0.13)</td>
<td>0.13 (0.12–0.13)</td>
<td>0.12 (0.12–0.13)</td>
<td>0.13 (0.13–0.14)</td>
<td>.222</td>
</tr>
<tr>
<td>Sdr (%)</td>
<td>Valley</td>
<td>255.64 (133.80–377.48)</td>
<td>350.57 (228.73–472.41)</td>
<td>324.72 (202.88–446.56)</td>
<td>258.51 (136.67–380.35)</td>
<td>.622</td>
</tr>
<tr>
<td></td>
<td>Top</td>
<td>256.59 (163.00–350.18)</td>
<td>252.98 (159.39–346.57)</td>
<td>245.14 (151.55–338.73)</td>
<td>174.61 (81.02–268.20)</td>
<td>.577</td>
</tr>
<tr>
<td></td>
<td>Flank</td>
<td>297.07 (196.51–397.64)</td>
<td>357.60 (257.03–458.16)</td>
<td>332.75 (232.18–433.31)</td>
<td>367.58 (267.01–468.14)</td>
<td>.771</td>
</tr>
</tbody>
</table>

Capital letters (A, B, and C) in superscript to the mean values of the titanium curette group denote a significant difference (*P* < .05) between the titanium curette group and the other groups, based on the Scheffé method.

### Figure 3

(a) Box plot showing mean roughness area (Sa) for 4 treatment categories of the implants from the tops, the valleys, and the flanks of the threads. (b) Box plot showing density (Sds), number of peaks per unit of area, for 4 treatment categories of the implants from the tops, the valleys, and the flanks of the threads. (c) Box plot showing the hybrid parameter (Sdr), representing the ratio between the developed surface area and a flat reference area, for 4 treatment categories of the implants from the tops, the valleys, and the flanks of the threads.
laser, an ultrasonic device, and plastic curettes supplemented with a chlorhexidine rinse demonstrated significant group differences.\textsuperscript{20} Titanium SLA surfaces attached to intraoral removable splints were contaminated with a multibacterial biofilm and then treated. With this setup, the Er:YAG laser (100 mJ/10 Hz) removed the biofilm more effectively than an ultrasonic device and plastic curettes as evaluated using light microscopy. Compared with a natural developing biofilm, contamination of the implants with a \textit{P. gingivalis} monoculture may present a limitation to the present study. \textit{P. gingivalis} is regarded as a late colonizer and is mostly seen attached to early colonizers in an already established biofilm.\textsuperscript{23} Early colonizers may have specific “adhesion” molecules providing a strong surface attachment indicating that an established biofilm will be more difficult to remove than a homotypic biofilm of \textit{P. gingivalis}. This may in part also explain the diverging results from our study compared with other in vitro setups.

A recently published review article summed up the outcomes of peri-implantitis treatment following decontamination with different cleaning alternatives.\textsuperscript{24} In this complex clinical setting, patient-related factors and differences in surface decontamination efficacy might affect the final outcomes. It has been challenging to document long-term differences in clinical parameters as change in probing depth and attachment level following different methods of surface decontamination. The importance of a long-term evaluation was clearly demonstrated in a study where surgical therapy of peri-implantitis was compared with Er:YAG laser or plastic curettes for surface decontamination. The gain in clinical attachment level was significant for both groups at 12 months. However, at the 24-month evaluation, the gain was not significantly different compared with baseline.\textsuperscript{25}

When looking at the 2 different strains, the \textit{P. gingivalis} \textit{fimA} genotype II (VIR) seemed to be easier to remove than the \textit{fimA} genotype I strain (AVIR). This was consistent for all instrumentation modalities, although the difference was borderline significant for the Er:YAG laser group. The results indicate a stronger adherence of AVIR to the implant surface and further that the laser treatment might be more effective in removing strains strongly attached to the implant surface.

The fimbriae with fimbrial adhesins, expressed by \textit{P. gingivalis}, seem to mediate a bacterial adherence to a wide variety of molecules and oral substrates.\textsuperscript{26,27} Both short and long fimbriae are apparently involved in the development of periodontitis.\textsuperscript{28} It has, however, been demonstrated that the longer fimbriae corresponding to the \textit{fimA} genotype II (VIR) have a greater ability to adhere to and invade human epithelial cells than other genotypes.\textsuperscript{29} In our study, VIR was more easily removed from the implant surface than AVIR. This may indicate that the higher virulence potential seen with VIR may be due to
other factors than differences in adhesive properties. Invasion of oral tissue and activation and release of proinflammatory cytokines are probably more significant factors in a clinical setting.30

The disruption and removal of biofilm including pathogenic bacteria from the implant surface appears paramount to the treatment of peri-implantitis. However, the decontamination procedure should not alter the implant surface micro-structure and in turn compromise biocompatibility of the implant. In our study, an optical interferometer was used to quantify surface morphology changes following instrumentation. The titanium curette significantly altered the surface structure evaluated using the $S_a$ and $S_{ds}$ parameters. When using the chitosan brush and the Er:YAG laser, significant surface alterations could not be quantified. The reductions in $S_a$ following the use of titanium curettes were also visualized using SEM illustrating that the SLA peak structure was clearly flattened. In perspective, instrumentation of titanium implant surfaces using stainless steel curettes has been controversial.20,31,32 Titanium curettes have therefore been fronted as a preferred alternative. This recommendation is not fully supported by our findings, which indicate that titanium curettes also may alter the implant surface structure.

The adherence of bacteria and the subsequent biofilm formation are dependent on the roughness of the implant surface, as a higher roughness accumulates more bacteria.33 Thus, the decrease in the roughness parameter $S_a$, following instrumentation with the titanium curette, could potentially be a positive treatment outcome.

The surface decontamination effect of the chitosan brush has not received extensive evaluation. In a recent report, a significant reduction of inflammation of implants affected by mild peri-implantitis has been demonstrated after treatment with a chitosan brush.14 The concept of using a rotating brush is not novel. The effectiveness of biofilm removal on SLA surface titanium discs in vitro using a rotating titanium filaments brush was recently evaluated. Using stainless steel curettes as control, the titanium brush instrumentation showed a mean residual biofilm area approximating 9%, which was significantly lower compared with 29% following curette instrumentation. Further, surface structure alteration was minor following titanium filaments brush decontamination verified by SEM.34

At higher irradiation, the Er:YAG laser is capable of melting the titanium surface.35 Such changes were not observed in the present study using the Er:YAG laser setting of 80 mJ/10 Hz with water cooling. In vitro, Er:YAG laser irradiation of 60 mJ/10 Hz ensures reliable removal of bacterial cytotoxic components from implant surfaces without altering the surface morphology of titanium micro-textured surfaces.36 To estimate changes to the implant surface following higher laser irradiation, SLA surfaced implants were irradiated at 100, 140, and 180 mJ/10 Hz. Evaluated using light interferometer, no significant between-group differences in surface roughness could be detected. However, a melting or fusion surface effect was demonstrated by SEM in the 180-mJ group.37

The most common mean surface roughness ($S_a$) used in the manufacture of dental implants at present resides in the 1- to 2-$\mu$m interval. This surface roughness appears to induce a stronger bone response than surfaces of lesser and higher roughness.15 If an increase in attachment or osseointegration is desired, it may be desirable to preserve the original micro-texture of the implant surface. In that regard, the Er:YAG laser and the chitosan brush appear the best options as they to a higher degree preserve the original surface structure of the implant. On the other hand, if the goal of the treatment mainly is to facilitate a surface less prone to bacterial attachment, the use of metal curettes seems to be a proper treatment modality as it may reduce the surface roughness and thereby bacterial adherence.33

We acknowledge that the use of a monoculture of the bacteria $P. gingivalis$ is a limitation of the study. This bacterium is not found exclusively on the surface of an infected dental implant, but in coexistence with other oral bacteria. One should therefore be careful to generalize the decontamination effect of the different cleaning devices to an in vivo situation. On the other hand, the use of a monoculture enabled evaluation of differences in the capacity of removal of 2 strains of $P. gingivalis$, presumably having different adherence properties. The decision of splitting each treatment group of 10 implants into 2 subgroups of 5 implants and the subsequent contamination with 2 different strains of $P. gingivalis$ can be discussed. This design adds strain specific $P. gingivalis$ information but undermines the power of the statistical analysis. Further, in this in vitro model, only 1 brand of dental implants was evaluated. Due to implant type differences, an extrapolation of the present results to a clinical setting should be performed with caution.38

In conclusion, the Er:YAG laser, chitosan brush, and titanium curettes appear equally effective in reducing the number of bacteria adhering to the micro-textured SLA implant surface, whereas the titanium curettes uniquely altered the implant surface structure. The Er:YAG laser and the chitosan brush might be an appropriate alternative in the management of peri-implantitis lesions.

**ABBREVIATIONS**

AVIR: avirulent $P. gingivalis$ strain ATCC33277

VIR: virulent $P. gingivalis$ strain A7A1-28

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**NOTE**

The authors report no conflicts of interest related to this study.
Decontamination of P. gingivalis-Infected Implant Surfaces

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