**In Vitro Evaluation of Implant Surface Decontamination Methods Based on Removal and Regrowth of Microorganisms**

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**Purpose:** The efficiency of bacterial removal using mechanical and chemical methods and recurrence rates was evaluated based on infected implant surface removal modalities. **Materials and Methods:** The study comprises two main parts: in vitro bacterial removal (n = 49) and regrowth (n = 42) suppression tests on implant surfaces. Microorganisms were attached to each implant, and various methods were applied to clean the infected implants. The groups were allocated as follows: (1) no treatment, (2) cotton + saline, (3) brush, (4) scaler, (5) brush + scaler, (6) cotton + 3% H2O2, and (7) brush + 3% H2O2. All groups were further divided into two subgroups of mechanical treatment (3, 4, 5) vs mechanical + chemical treatment (2, 6, 7). After treatment for each group, immunofluorescence analysis and measurement using an ultraviolet-visible light spectrophotometer were performed. **Results:** In the mechanical treatment group, the brush, scaler, and brush + scaler groups, all of which had strong polishing abilities, exhibited superior removal efficiency compared with the other groups (P < .001). In the regrowth experiment, 3% H2O2 effectively restrained biofilm formation. In particular, the brush + 3% H2O2 group exhibited significant differences from the mechanical treatment group (vs brush: P < .001, vs scaler: P = .023, vs brush + scaler: P = .005). **Conclusion:** Mechanical methods, including brushes and scalers, effectively removed bacteria. Biofilm formation was effectively restrained by H2O2. In particular, the brush + H2O2 group exhibited a superior ability to suppress bacterial regrowth compared with the other groups. Int J Oral Maxillofac Implants 2021;36:1088–1094. doi: 10.11607/jomi.8878

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Implants are a recognized treatment approach for restoring oral function. However, the origin and treatment of peri-implantitis have been reported and are currently the subject of debate.1 A recent study reported that the prevalence of peri-implantitis was 22%, ranging from 1% to 47%. Implant-related infections are a critical reason for failure.2 Biofilm formation is an associated pathogenic event involved in infection of an implant after bacterial adhesion. In addition, peri-implantitis is a mixed infection that involves several species rather than a single species.3 Several microorganisms are involved in peri-implantitis, including Actinomyces viscosus, Streptococcus sanguis, Prevotella intermedia, Porphyromonas gingivalis, and Fusobacterium nucleatum.4–7 The long-term clinical success of implants therefore depends on removing these bacteria from implant surfaces, particularly in cases of peri-implantitis.

Decontamination and alteration of implant surfaces are important because these procedures remove bacteria, prevent recurrence of peri-implantitis, and induce re-osseointegration.8 Mechanical and chemical methods have been developed to decontaminate implant surfaces. A titanium brush is one of the most powerful mechanical methods. Carral et al suggested that the removal efficacy with a titanium brush is better than that of other devices.9 Favorable results of clinical trials with titanium brushes have also been reported.10 Another mechanical treatment device is ultrasonic scalers. Scalers are less efficient at altering implant surfaces compared with titanium brushes, but they allow for assessment between the threads of an implant. Park et al demonstrated that all groups that used scalers showed noticeable decreases in bacterial attachment...
to implant surfaces. A previous study demonstrated that the decontamination efficacy of both a titanium brush and a metallic ultrasonic tip is superior to that of an air abrasive. Nonmechanical chemical options, such as hydrogen peroxide (H₂O₂), are widely used as dental disinfectants. The chemical approach can reduce bacteria and be applied to both periodontitis and peri-implantitis. H₂O₂ affects a wide range of organisms due to oxidizing action. The antibacterial effect of H₂O₂ composed of hydroxyl radicals can result in bacterial death by reacting with macromolecules like DNA and membrane lipids. H₂O₂ treatment was shown to suppress *Actinomyces actinomycetemcomitans* when it was used for subgingival irrigation in a previous study.

Recurrence of peri-implant mucositis and peri-implantitis after decontamination is dependent on microbial adhesion and accumulation on implant surfaces. A previous systematic review found that peri-implantitis recurred in up to 100% of treated cases over periods of longer than 1 year. An in vitro study demonstrated that bacterial recolonization on titanium specimens was evident 2 days after antimicrobial therapy. In addition, biofilm cells that survive antimicrobial treatment can grow to pretreatment levels in a short period of time. Several factors, including treatment efficacy, nutrient availability, and species richness of the biofilms, affect the regrowth rate of biofilm. The material characteristics of an implant surface are known to influence microbial adhesion. After decontamination of both rough and smooth infected implant surfaces using mechanical and chemical methods, bacterial removal and recurrence appear to be clinically important. The aim of this study was to evaluate the efficiency of bacterial removal using various mechanical and chemical methods as well as the recurrence rate using infected implant surface removal modalities.

**MATERIALS AND METHODS**

**Study Design**

This in vitro study comprised two main parts: evaluating the efficiency of biofilm removal and studying regrowth on infected implant surfaces. This study was conducted in accordance with “Qualitative Research: Standards, Challenges, and Guidelines.”

**Biofilm Removal**

A schematic explaining the approach to bacterial removal is shown in Fig. 1. Sandblasted, large-grit, acid-etched (SLA) implants (TSIII SA 4.0 × 13 mm, Osstem Implant) were prepared for this study (n = 49). One implant was placed in each well of a 24-well plate and cultured in 1 mL of culture medium (Brain heart infusion, BD) supplemented with 10 μg/mL of hemin (Sigma-Aldrich) and 0.2 μg/mL of vitamin K (Sigma-Aldrich). *Staphylococcus aureus* (KCTC 1927), *Actinomyces viscosus* (KCTC9146), and *S anguis* (KCTC 3284) were inoculated at 2 × 10⁸/mL and incubated aerobically in an atmosphere of 5% CO₂ at 37°C for 3 days. The groups were arranged as follows:

1. No treatment group (n = 7)
2. Cotton + saline group (n = 7)
3. Brush group (n = 7)
4. Scaler group (n = 7)
5. Brush + scaler group (n = 7)
6. Cotton + 3% H₂O₂ group (n = 7)
7. Brush + 3% H₂O₂ group (n = 7)
For the cotton group, biofilm on implant surfaces was removed with sterile cotton pellets with saline for 30 seconds per thread.

For the brush group, customized brushes (Smart Brush, Osstem Implant) composed of stainless steel were used to remove contaminants from the surface of implants. The brush was connected to the surgical engine (KAVO, INTRAsurg300) to polish the implant. The implant was polished from the top to the third thread by brushing at 1,500 rpm with a saline solution. Polishing was performed for approximately 30 seconds per thread.

For the scaler group, a customized scaler (Smart Scaler, Osstem Implant) made of stainless steel was applied to the implant thread for polishing for 30 seconds per thread.

For the 3% H$_2$O$_2$ group, 3% H$_2$O$_2$ was scrubbed with sterile cotton pellets to implant surfaces to decontaminate them.

**Immunofluorescence Analysis**

To evaluate bacterial removal efficiency after instrumentation, bacteria were labeled with fluorescein isothiocyanate (FITC, Sigma Chemical Company) through incubation at 37°C for 30 minutes in medium with FITC. Labeled bacteria were washed three times in Dulbecco’s phosphate-buffered saline. Fluorescence was imaged using a luminescent image analyzer (GE Life Sciences) with an F1.8 24-mm wide-angle lens and a 575DF20 Cy3 filter (GE Life Sciences). The quantity of labeled bacteria in a brushed area was measured using ImageJ software (NIH).

**Biofilm Regrowth**

Figure 2 depicts the biofilm regrowth experiment. Bacteria-cultured SLA implants were prepared using the methods described for biofilm removal (n = 42). These implants were classified into the same groups as in the removal experiment (no treatment, cotton + saline, brush, scaler, brush + scaler, cotton + 3% H$_2$O$_2$, and brush + 3% H$_2$O$_2$ groups) and applied to whole implant surfaces with each method. The implants were washed gently in 3 mL of saline solution. Each implant was placed in a well of a 24-well plate and cultured in the same manner as in the removal model. After regrowth, 500 mL of culture media was extracted to measure optical density (OD) using a 600-nm ultraviolet-visible light spectrophotometer (DU 720, Beckman).

**Sample Size Calculation**

The sample size calculation in this study was referenced in previous similar research. The power of 0.8 and an alpha level of .05 were used to calculate the difference between groups.

**Statistical Analysis**

Statistical analysis was conducted using standard software (SPSS version 23, IBM). The Shapiro-Wilk test was used to test the normality of the distribution. As no parameters were normally distributed, a Friedman test was used to evaluate differences. The threshold for statistical significance was $P < .05$. A post hoc Tukey test was used for multiple comparisons between groups.

**RESULTS**

**Biofilm Removal**

Figure 3 illustrates the results of the removal experiment. Each value (%) describes the removal rate of all groups for the no-treatment group. There was a
significant difference in all groups \((P < .001; \text{Table } 1)\). All groups except the cotton + 3% \(\text{H}_2\text{O}_2\) group showed significant interactions with the no-treatment group \((P < .001 \text{ for each group except the brush group, for which } P = .002)\). The scaler, brush + scaler, and cotton + 3% \(\text{H}_2\text{O}_2\) groups also exhibited significant differences compared with the brush group \((P = .011, P = .007, \text{and } P = .005, \text{respectively})\). No difference was found between the brush and brush + 3% \(\text{H}_2\text{O}_2\) groups \((P = .552)\). The cotton + 3% \(\text{H}_2\text{O}_2\) group was significantly different from the brush + scaler and brush + 3% \(\text{H}_2\text{O}_2\) groups \((P < .001)\). For analysis from a different perspective, all groups were divided into two subgroups (mechanical treatment group [brush, scaler, and brush + scaler] vs mechanical + chemical treatment groups [cotton + saline, cotton + 3% \(\text{H}_2\text{O}_2\), and brush + 3% \(\text{H}_2\text{O}_2\)]) in the mechanical + chemical group. When comparing the cotton + saline and cotton + 3% \(\text{H}_2\text{O}_2\) groups, the two groups exhibited no efficiency in bacterial removal, and there was no difference in the effect between saline and 3% \(\text{H}_2\text{O}_2\) \((P = 1.000)\). However, groups using a brush (brush and brush + 3% \(\text{H}_2\text{O}_2\)) both achieved higher removal rates, and no significant difference was observed between them \((P = .552)\).

**Biofilm Regrowth**

Significant differences were found among all groups compared with the no-treatment group \((P < .001; \text{Table } 2)\). The brush, scaler, brush + scaler, cotton + 3% \(\text{H}_2\text{O}_2\), and brush + 3% \(\text{H}_2\text{O}_2\) groups showed an OD of 1.000 or less in the microorganism regrowth experiment \((\text{brush: } 0.951; \text{scaler: } 0.871; \text{brush + scaler: } 0.752; \text{cotton + 3% }\text{H}_2\text{O}_2: 0.755; \text{and brush + 3% }\text{H}_2\text{O}_2: 0.252)\). All these groups except the cotton + saline group were significantly different from the no-treatment group \((cotton + \text{saline vs no treatment: } P = .995)\). The brush, scaler, brush + scaler, cotton + 3% \(\text{H}_2\text{O}_2\), and brush + 3% \(\text{H}_2\text{O}_2\) groups also differed significantly from the cotton + saline group \((P < .001 \text{ for each, and } P = .049)\). Two groups (mechanical treatment group [brush, scaler, brush + scaler] vs mechanical + chemical treatment group [cotton + saline, cotton + 3% \(\text{H}_2\text{O}_2\), brush + 3% \(\text{H}_2\text{O}_2\)]) were observed in the regrowth study. All mechanical treatment groups exhibited significant differences in microbial restraint compared with the no-treatment group. In the mechanical + chemical treatment group, cotton + 3% \(\text{H}_2\text{O}_2\) and brush + 3% \(\text{H}_2\text{O}_2\) showed significantly less bacterial growth compared with the no-treatment group \((P < .001)\). The value (cotton + saline vs cotton + 3% \(\text{H}_2\text{O}_2\)) was significantly different \((P < .001)\). The brush + 3% \(\text{H}_2\text{O}_2\) group was significantly different from the mechanical treatment group \((\text{vs brush: } P < .001; \text{vs scaler: } P = .023; \text{vs brush + scaler: } P = .005)\).

**DISCUSSION**

Numerous methods have been introduced to treat and slow the progression of peri-implantitis by decontaminating implant surfaces. However, no method has been shown to be superior.\(^{23}\) Treatment techniques include several mechanical methods, such as titanium curettes, brushes, scalers, air-abrasion, and implantoplasty. In addition, chemical methods, such as citric acid and \(\text{H}_2\text{O}_2\), have been proposed. Both mechanical and chemical methods are associated with controversial therapeutic effects.

The present study evaluated the efficiency of bacterial removal and regrowth by comparing various mechanical and chemical methods of decontaminating infected implant surfaces and changing rough to smooth surfaces. The results indicate that methods that strongly polish the surface (brush, scaler, brush + scaler, and brush + 3% \(\text{H}_2\text{O}_2\)) produced higher removal rates compared with no treatment, cotton + saline, and cotton + 3% \(\text{H}_2\text{O}_2\). A chemical method with cotton (cotton + saline and cotton + 3% \(\text{H}_2\text{O}_2\)) had no effect on microbial removal from infected implant surfaces regardless of whether the microorganisms were alive or dead. The results of regrowth indicated that 3% \(\text{H}_2\text{O}_2\) effectively restrained biofilm formation regardless of whether mechanical instruments were involved (cotton + 3% \(\text{H}_2\text{O}_2\) and brush + 3% \(\text{H}_2\text{O}_2\) groups). In particular, the use of a brush + 3% \(\text{H}_2\text{O}_2\)
De Tapia et al reported that the use of a titanium brush in vitro and in vivo. A previous study reported reduced inflammatory infiltration around the implant surface with the mechanical treatment group (vs brush, scaler, and brush + scaler). The study results indicate that mechanical methods, including brushes and scalers, affect the effective removal of biofilm, and 3% H2O2 efficiently suppressed microbial regrowth.

In this study, brushes were used as a mechanical method. Various brushes have been tested both in vitro and in vivo. A previous study reported reduced inflammatory infiltration around the implant surface after using a titanium brush compared with gauze. De Tapia et al reported that the use of a titanium brush for regenerative treatment of peri-implantitis resulted in reducing probing pocket depth (PPD) after 1 year.25 Meto et al reported that a brush removed 76% of biofilm from titanium disks.26 The present authors made similar observations using a brush and a brush + 3% H2O2. This suggests that a brush, which can strongly polish a surface, was effective at removal work, and 3% H2O2 was not effective. In connection with this finding, cotton + saline, which can only weakly polish a surface, did not affect biofilm removal rates on implant surfaces.

Another mechanical treatment device is the ultrasonic scaler. Park et al reported a statistically significant

### Table 1 Biofilm Removal on Infected Implant Surfaces Based on Treatment (%)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotton + saline</td>
<td>7</td>
<td>-18.93 (49.39)</td>
<td>-32.44</td>
<td>-61.88</td>
<td>17.63</td>
<td>-67.54</td>
<td>56.02</td>
<td></td>
</tr>
<tr>
<td>Brush</td>
<td>7</td>
<td>49.27 (16.77)</td>
<td>50.23</td>
<td>38.39</td>
<td>52.38</td>
<td>31.17</td>
<td>81.93</td>
<td>†</td>
</tr>
<tr>
<td>Scaler</td>
<td>7</td>
<td>56.48 (14.70)</td>
<td>54.73</td>
<td>49.88</td>
<td>61.43</td>
<td>35.71</td>
<td>82.30</td>
<td>†, #</td>
</tr>
<tr>
<td>Brush + Scaler</td>
<td>7</td>
<td>56.71 (13.95)</td>
<td>53.99</td>
<td>48.71</td>
<td>61.63</td>
<td>39.61</td>
<td>82.66</td>
<td>§, **</td>
</tr>
<tr>
<td>Cotton + 3% H2O2</td>
<td>7</td>
<td>-11.37 (37.90)</td>
<td>-20.94</td>
<td>-21.66</td>
<td>11.86</td>
<td>-77.93</td>
<td>38.87</td>
<td>††, †††,</td>
</tr>
<tr>
<td>Brush + 3% H2O2</td>
<td>7</td>
<td>53.44 (15.29)</td>
<td>50.26</td>
<td>46.21</td>
<td>57.89</td>
<td>34.42</td>
<td>81.20</td>
<td>†*</td>
</tr>
</tbody>
</table>

Each value describes the removal rate of all groups to the no-treatment group. *Significantly different among seven groups. †Significantly different from cotton + saline and brush groups. ‡Significantly different from cotton + saline and scaler groups. §Significantly different from cotton + saline and brush + scaler groups. §§Significantly different from cotton + saline and brush + 3% H2O2 groups. §§§Significantly different from brush + scaler and cotton + 3% H2O2 groups. ¶¶Significantly different from brush and scaler groups. ¶¶¶Significantly different from brush + scaler and cotton + 3% H2O2 groups. †††Significantly different from brush + scaler and cotton + 3% H2O2 groups. Statistical significance level was P < .05.

### Table 2 Biofilm Regrowth on Infected Implant Surfaces Based on Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>6</td>
<td>1.143 (0.025)</td>
<td>1.129</td>
<td>1.128</td>
<td>1.160</td>
<td>1.123</td>
<td>1.179</td>
<td></td>
</tr>
<tr>
<td>Cotton + saline</td>
<td>6</td>
<td>1.121 (0.054)</td>
<td>1.139</td>
<td>1.099</td>
<td>1.156</td>
<td>1.028</td>
<td>1.173</td>
<td></td>
</tr>
<tr>
<td>Brush</td>
<td>6</td>
<td>0.951 (0.026)</td>
<td>0.957</td>
<td>0.942</td>
<td>0.965</td>
<td>0.907</td>
<td>0.983</td>
<td>†‡</td>
</tr>
<tr>
<td>Scaler</td>
<td>6</td>
<td>0.871 (0.078)</td>
<td>0.892</td>
<td>0.806</td>
<td>0.924</td>
<td>0.771</td>
<td>0.959</td>
<td>§, ††</td>
</tr>
<tr>
<td>Brush + scaler</td>
<td>6</td>
<td>0.752 (0.406)</td>
<td>0.928</td>
<td>0.648</td>
<td>0.995</td>
<td>0.008</td>
<td>1.079</td>
<td></td>
</tr>
<tr>
<td>Cotton + 3% H2O2</td>
<td>6</td>
<td>0.755 (0.028)</td>
<td>0.753</td>
<td>0.751</td>
<td>0.754</td>
<td>0.715</td>
<td>0.803</td>
<td>¶, §§, ***</td>
</tr>
<tr>
<td>Brush + 3% H2O2</td>
<td>6</td>
<td>0.252 (0.394)</td>
<td>0.002</td>
<td>0.001</td>
<td>0.487</td>
<td>0.001</td>
<td>0.859</td>
<td>*§, †††,</td>
</tr>
</tbody>
</table>

Each value describes optical density (OD) for the bacterial regrowth of all the groups. *Significantly different among seven groups. †Significantly different from no-treatment and cotton + saline groups. ‡Significantly different from no-treatment and brush groups. §Significantly different from no-treatment and scaler groups. ¶Significantly different from cotton + saline and brush + scaler groups. ¶¶Significantly different from cotton + saline and brush + 3% H2O2 groups. §§Significantly different from cotton + saline and brush + 3% H2O2 groups. ¶¶¶Significantly different from cotton + saline and brush + 3% H2O2 groups. ¶¶¶¶Significantly different from cotton + saline and brush + 3% H2O2 groups. ¶¶¶¶Significantly different from cotton + saline and brush + 3% H2O2 groups. Statistical significance level was P < .05.
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