Effects of titanium brush on machined and sand-blasted/acid-etched titanium disc using confocal microscopy and contact profilometry

Key words: confocal microscopy, dental implants, scanning electron microscopy, surface properties, titanium, toothbrushing

Abstract

Objective: Mechanical techniques, including scaling with metal, plastic, or ultrasonic instruments, rubber cup polishing, air-powder abrasive system and brushing with a conventional or a rotating brush, have been used for the debridement of dental implants. Recently, rotating brushes with titanium bristles (titanium brush) have been introduced for the debridement of implant surface when peri-implant osseous defects occur. The purpose of this study was to evaluate the effects of a titanium brush on machined (MA) and sand-blasted and acid-etched (SA) titanium surfaces using scanning electron microscopy, confocal microscopy and profilometry. Moreover, correlations between the two quantitative evaluation methods (confocal microscopy and contact profilometry) were assessed.

Materials and Methods: Both MA and SA discs were treated with rotating titanium brush at 300 rpm under irrigation for a total of 40 s. Roughness measurements were taken with confocal microscopy and surface profilometry. Then, the MA and SA surfaces were evaluated using scanning electron microscopy to determine the changes of the surface properties.

Results: Untreated MA surface demonstrated uniform roughness with circumferential machining marks, and scratch lines over the original surfaces were observed after treatment with the titanium brush. Similarly, the titanium brush produced noticeable changes on the SA titanium surfaces. However, this treatment with titanium brush did not significantly change the roughness parameters, including the arithmetic mean height of the surface (Sa) and the maximum height of the surface (Sz), in both MA and SA surfaces. Correlations between two evaluation methods showed a Pearson correlation coefficient of 0.98 with linear regression $R^2$ of 0.96.

Conclusion: This study showed that the treatment with the titanium brush did not significantly change the roughness parameters, including Sa and Sz, in both MA and SA surfaces. Correlations between confocal microscopy and surface profilometry showed high correlation with a Pearson correlation coefficient of 0.98.

Peri-implantitis is defined as an inflammatory process affecting the tissues around an osseointegrated implant, resulting in the loss of the supporting bone (Amoroso et al. 2006). Recent reports showed that the prevalence of peri-implantitis is assumed to be between 10% and 20% (Duddeck et al. 2012). An increasing number of dentists are including dental implant therapy in their clinics, and this may lead to an increasing number of cases with peri-implantitis (Wohlfahrt & Lyngstad aas 2012). Mechanical techniques, including scaling with metal, plastic, or ultrasonic instruments, rubber cup polishing, air-powder abrasive system and brushing with a conventional or a rotating brush, have been used for the debridement of dental implants (Alhag et al. 2008; Park et al. 2012a,b, Park et al. 2013). Recently, rotating brushes with titanium bristles (titanium brush) have been introduced for the debridement of peri-implant osseous defects (Wohlfahrt & Lyngstad aas 2012).

Scanning electron microscopy, confocal microscopy and profilometry may be used to evaluate the characteristics of the surfaces (Park et al. 2012a,b). Scanning electron microscopy is one of the most widely used methods to evaluate the surface topography, and a magnified view may show the configuration and the characteristics of the surface (Brookshire et al. 1997, Ahn et al. 2011).
Quantitative evaluation of the change of the surfaces may be evaluated with confocal microscopy and contact profilometry (Park et al. 2012a,b). Confocal microscopy utilizes the reconstruction of surface topography from optical sections either by reflection or by fluorescence imaging (Evans et al. 2001; Howell et al. 2002; Park et al. 2012a,b). Contact profilometry utilizes a stylus, which is moved vertically in contact with a sample and then moved laterally across the sample (Kim et al. 2008; Zhang et al. 2010).

This study was performed to evaluate the effects of the titanium brush on machined (MA) and sandblasted and acid-etched (SA) titanium surfaces using scanning electron microscopy, confocal microscopy and profilometry. Additionally, correlations between the two qualitative evaluation methods (confocal microscopy and contact profilometry) were assessed.

Material and methods

Specimen preparation for profilometry
Six MA titanium discs and six SA discs were used in this study. The discs measured 10 mm in diameter and 2 mm in thickness. The MA and SA titanium discs were instrumented with a titanium brush (Tigran Peri-brush, Tigran Technologies AB, Malmö, Sweden) at 300 rpm under irrigation for 40 s by a single operator (JP) (Fig. 1). Calibrations were performed before the experiments, and the average force applied in this study was approximately 15 g.

Determination of surface properties with confocal microscopy
Roughness parameters [arithmetic mean value of the profile [Ra], maximum height of the profile [Rz], skewness of the assessed profile [Rsk], arithmetic mean height of the surface [Sa], maximum height of the surface [Sz], skewness [Ssk], developed interfacial area ratio [Sdr], and kurtosis [Sku]] were measured before and after instrumentation using a confocal laser microscope (LSM5 Pascal, Zeiss, Jena, Germany). All the values were determined at a cutoff length of 0.04 mm in 50 sections, and the stack size of z-sections was 0.80 μm. Each scanning covered an area of 460.7 × 460.7 μm, and a Gaussian filter was used to determine the surface values. Roughness measurements were calculated by using the proprietary software [Topography package; Zeiss] [Stubinger et al. 2010]. Three discs were used for each group, and measurements were taken at five random areas from each disc.

Measurement of surface roughness with contact profilometry
The surface roughness of MA and SA titanium discs was measured using a contact profilometer [SURFPAK-SV, Mitutoyo, Hiroshima, Japan]. The average roughness [Ra] was used to characterize the roughness.

Examination of the titanium discs treated with titanium brush using scanning electron microscopy
Following the treating of the MA and SA titanium discs with titanium brush, the surfaces were washed twice with phosphate-buffered saline [PBS] to remove debris. The surfaces were evaluated using scanning electron microscopy to determine the changes of the surface properties after the instrumentation. The samples were mounted on stubs. The discs were then air-dried on a clean bench via evaporation of hexamethyldisilazane sputter-coated with gold palladium and observed using a scanning electron microscope (S-4700, Hitachi, Tokyo, Japan) at 15 kV and 2000× magnification. Images were randomly captured from each disc and were saved as TIFF.

Statistical methods
Data are represented as mean ± standard deviation. Non-parametric Mann–Whitney U-test was used to test for statistical differences between the test and the control groups with commercially available statistical software (SPSS 12 for Windowsl SPSS Inc., Chicago, IL, USA). Statistical significance was set at \( P < 0.05 \). Correlations between the two evaluation methods were assessed with Pearson’s correlation analysis.

Fig. 1. Titanium brush used in this study.

Fig. 2. The morphology of the surface of the titanium discs after treatment. (a) Untreated MA surface; (b) MA titanium brush; (c) SA control (no treatment); (d) SA titanium brush. MA, Machined; SA, sand-blasted and acid-etched.
Results

The gross morphology of the surface of the MA and SA titanium discs after treatment with titanium is shown in Fig. 2. Differences in colour and surface texture between MA and SA and changes after the instrumentation with titanium brush could be discriminated by visual inspection alone. The change in the surface morphology after treatment was obvious when compared with the untreated MA and SA surfaces. The surface properties measured with the confocal microscope are shown in Fig. 3. The untreated MA surface demonstrated uniform roughness with circumferential machining marks (Fig. 3a). The untreated MA surface showed a relatively flat topographic configuration with isotropic grooves. Scratch lines over the original surfaces were observed after treatment with titanium brush (Fig. 3b). The untreated SA surface demonstrated uniform roughness (Fig. 3c), and the titanium brush produced noticeable changes on the SA titanium surfaces (Fig. 3d). The surface characteristics of examined areas are listed in Fig. 4, and the profiles of each group are listed in Fig. 5. Fig. 6 shows the images obtained with contact profilometry.

The qualitative analyses of roughness parameters using two different methods are seen in Tables 1 and 2. There were no significant changes of Ra value after treatment in MA and SA discs when evaluations were performed with confocal microscopy and contact profilometry ($P > 0.05$). Similarly, no significant changes of Sa and Sz values were noticed in MA and SA surfaces after treatment ($P > 0.05$). The changes of Ssk, Sdr and Sku after treatments are shown in Table 1. Ssk values were increased after the treatment, and Sdr and Sku values were decreased after the treatment. Correlations in Ra measurements between two evaluation methods showed a Pearson correlation coefficient of 0.98 with linear regression $R^2$ of 0.96.

Fig. 7 shows the images obtained with scanning electron microscopy from untreated MA, treated MA, untreated SA and treated SA implant discs. SEM images of untreated MA showed smooth configuration with machining marks (Fig. 7a). Scratch lines over the original surfaces were observed after treatment with titanium brush (Fig. 7b). Untreated SA surface demonstrated rough surface with sharp spikes and deep pits (Fig. 7c). The titanium brush produced noticeable changes on the SA titanium surfaces (Fig. 7d).

Discussion

This study showed that treatment with titanium brush did not produce significant changes on the roughness parameters, including Sa and Sz, in both MA and SA surfaces. Secondly, high correlations between two evaluation methods using Spearman’s correlation analysis with coefficient of 0.98 suggested that both methods (confocal microscopy and contact profilometry) can provide reliable and consistent results.
contact profilometry) might be useful for the evaluation of the surface characteristics.

In this study, different methods were used to evaluate the changes of the surface properties. Scanning electron microscopy showed the change of the surface in MA and SA surfaces very clearly. This method is very useful, but additional sample preparation is needed, and quantitative analysis is difficult (Brookshire et al. 1997; Lu et al. 2012). Confocal microscopy can measure various roughness parameters and has been applied in various applications in dentistry, including teeth and titanium (Howell et al. 2002; Park et al. 2012a,b). Contact profilometry, which utilizes stylus, has several advantages because most of the surface finish standards in the world are written for this profilometry, which is less influenced by a dirty environment with contaminants (Durakbasa et al. 2011). It should be kept in mind that the stylus readings are influenced by the radius of the stylus tip, the pressure of the stylus tip on the surface and the hardness of material (Wen et al. 1996; Durakbasa et al. 2011).

In this study, surface topography was quantitatively evaluated by confocal microscopy to measure roughness parameters Ra, Rz, Rsk, Sa, Sz, Ssk, Sdr and Sku, and by contact profilometry to evaluate Ra. Ra is one of the most widely used parameters of roughness, and it remains useful as a general guideline on the surface texture (Park et al. 2012a,b). However, it has been shown to be too general to describe the surface’s functional nature in today’s ever-increasing complexity of applications because Ra averages all peaks and valleys of the roughness profile (Taylor et al. 2006). Rz reflects outlying points better because it averages only the five highest peaks and the five deepest valleys (Demirciglu & Durakbasa 2011). Moreover, Ra and Rz give information only from a profile, but Sa and Sz provide information about a surface area, resulting in three-dimensional parameters with higher reliability (Wennerberg & Albrektsson 2010). Ssk shows the skewness of surface height distribution and represents the degree of symmetry of the surface heights about the mean plane (Gruhn et al. 2012), and it is useful in monitoring for different types of wear conditions (Whitehouse 2000). If Ssk is greater than zero, it indicates the predominance of peaks; if Ssk is less than zero, it means that valley structures have more quantity (Tudose et al. 2007). Sdr presents information about the number and the height of peaks of a given surface, and it is defined as the developed interfacial area ratio and expresses the increment of the interfacial
Table 1. Surface topographic properties of the MA and SA discs (mean ± standard deviation, median) after titanium brush (Tigran PeriBrush, Tigran Technologies AB, Malmö, Sweden) for 40 s at 300 rpm under saline irrigation using confocal microscopy. Three discs were used for each group, and measurements were taken at five random areas from each disc. A total of 15 measurements were taken for each group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ra (µm)</th>
<th>Rz (µm)</th>
<th>Rsk</th>
<th>Sa (µm)</th>
<th>Sz (µm)</th>
<th>Ssk</th>
<th>Sdr (%)</th>
<th>Sku</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA untreated</td>
<td>0.72 ± 0.50</td>
<td>2.59 ± 1.48</td>
<td>0.71 ± 0.74</td>
<td>0.82 ± 0.54</td>
<td>4.33 ± 1.77</td>
<td>0.66 ± 0.68</td>
<td>62.77 ± 67.40</td>
<td>4.54 ± 2.76</td>
</tr>
<tr>
<td>MA titanium brush</td>
<td>0.39 ± 0.15</td>
<td>1.52 ± 0.56</td>
<td>0.78 ± 0.72</td>
<td>0.41 ± 0.14</td>
<td>3.57 ± 1.02</td>
<td>0.94 ± 0.31</td>
<td>8.28 ± 4.98</td>
<td>5.66 ± 1.28</td>
</tr>
<tr>
<td>P-value</td>
<td>0.32</td>
<td>1.34</td>
<td>0.66</td>
<td>0.33</td>
<td>2.98</td>
<td>0.87</td>
<td>5.80</td>
<td>5.59</td>
</tr>
<tr>
<td>SA untreated</td>
<td>0.345</td>
<td>0.233</td>
<td>0.935</td>
<td>0.161</td>
<td>0.233</td>
<td>0.486</td>
<td>0.116</td>
<td>0.106</td>
</tr>
<tr>
<td>SA titanium brush</td>
<td>1.87 ± 0.21</td>
<td>7.25 ± 0.63</td>
<td>−0.21 ± 0.57</td>
<td>1.94 ± 0.09</td>
<td>13.52 ± 0.76</td>
<td>0.10 ± 0.13</td>
<td>111.35 ± 7.68</td>
<td>3.18 ± 0.22</td>
</tr>
<tr>
<td>P-value</td>
<td>1.92</td>
<td>7.21</td>
<td>−0.21</td>
<td>1.92</td>
<td>13.54</td>
<td>0.10</td>
<td>110.74</td>
<td>3.15</td>
</tr>
<tr>
<td>SA titanium brush</td>
<td>1.73 ± 0.20</td>
<td>6.35 ± 0.66</td>
<td>0.33 ± 0.39*</td>
<td>1.89 ± 0.10</td>
<td>13.26 ± 0.66</td>
<td>0.43 ± 0.41*</td>
<td>89.13 ± 12.35*</td>
<td>4.28 ± 1.50*</td>
</tr>
<tr>
<td>P-value</td>
<td>1.71</td>
<td>6.13</td>
<td>0.39</td>
<td>1.88</td>
<td>13.06</td>
<td>0.38</td>
<td>87.31</td>
<td>3.64</td>
</tr>
</tbody>
</table>

**Table 2.** Surface topographic properties of the MA and SA discs (mean ± standard deviation, median) after titanium brush (Tigran PeriBrush) using surface profilometry.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ra (µm)</th>
<th>Rsk</th>
<th>Sa (µm)</th>
<th>Sz (µm)</th>
<th>Ssk</th>
<th>Sdr (%)</th>
<th>Sku</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA untreated</td>
<td>0.37 ± 0.08</td>
<td>0.42</td>
<td>0.17 ± 0.58</td>
<td>0.37 ± 0.08</td>
<td>0.42</td>
<td>0.17 ± 0.58</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>95% confidence of interval</td>
<td>0.30 ± 0.43</td>
<td>0.436</td>
<td>1.36 ± 0.11</td>
<td>1.40</td>
<td>1.27 ± 1.44</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>SA untreated</td>
<td>6.35 ± 0.66</td>
<td>0.33 ± 0.39*</td>
<td>1.89 ± 0.10</td>
<td>13.26 ± 0.66</td>
<td>0.43 ± 0.41*</td>
<td>89.13 ± 12.35*</td>
<td>4.28 ± 1.50*</td>
</tr>
<tr>
<td>95% confidence of interval</td>
<td>3.18 ± 0.22</td>
<td>3.64</td>
<td>87.31</td>
<td>3.64</td>
<td>3.15</td>
<td>4.28 ± 1.50*</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Statistically significant differences were noted between untreated and treated groups.

**Fig. 7.** SEM images of each group. (a) MA control (no treatment). (b) MA titanium brush. (c) SA control (no treatment). (d) SA titanium brush. MA, Machined; SA, sand-blasted and acid-etched.
surface. However, there were no significant changes of Sa and Sz after treatment. A statistically significant decrease in Sdr value suggests that spatial intricacy was decreased after treatment. In SA surfaces, significant changes were noted in Ssk, Sdr and Sku. A decrease in Sdr value suggests that spatial intricacy was reduced, and an increase in SSk and Sku suggests that there was a higher tendency for the predominance of peaks.

Titanium brush was suggested to offer easier access to narrow spaces and implant threads [Tigran Technologies AB 2011]. Compared with other mechanical instrumentation, including ultrasonic scaling, titanium brush may be considered gentler to the implant surface because less force is applied on the titanium surface than in previous studies (40–300 g) [Sato et al. 2004; Ramaglia et al. 2006; Duddeck et al. 2012]. Titanium brushes may adapt closely to the architecture of the implant, and rotating titanium brush at 300–600 rpm under continuous irrigation may shorten treatment time [Duddeck et al. 2012].

It is reported that material that is less hard than titanium may leave remnants on the surface after treatment [Unursaikhan et al. 2012; Ruhling et al. 1994; Schwarz et al. 2003]. Plastic- and teflon-coated instruments may leave plastic contaminationsthat are macroscopically visible [Gosau et al. 2010], and the biocompatibility of the titanium surfaces may be impaired by the contaminants [Schwarz et al. 2006]. Stainless steel particles abraded from the metal brush may become embedded in the titanium surface, and this may initiate galvanic corrosion [Tigran Technologies AB 2009]. A significant decrease in the number of osteoblast-like cells was seen in the presence of debris of the carbon fibres [Schwarz et al. 2003]. Similarly, fibroblasts grown on stainless steel instrumented surfaces showed a somewhat rounded morphology and a relatively reduced degree of spreading [Dmytryk et al. 1990]. Negative impact coming from plastic or stainless steel tools may be avoided by using titanium brush [Tigran Technologies AB 2009]. Further research is needed to evaluate the efficiency of titanium brush on removing the contaminants and adhering bacteria and effects of treatment on the cellular attachment, proliferation and differentiation.

Conclusions

This study evaluated the effects of titanium brush on MA and SA titanium discs using confocal microscopy, contact profilometry and scanning electron microscopy. Treatment with titanium brush did not significantly change the roughness parameters, including Sa and Sz, in both MA and SA surfaces. Correlations between confocal microscopy and surface profilometry showed high correlation with a Pearson correlation coefficient of 0.98.

Acknowledgements: This research was supported by Seoul St. Mary’s Hospital Clinical Medicine Research Program year of 2013 through the Catholic University of Korea. The authors acknowledge Dentium (Seoul, Korea) for donating the titanium discs for this study.

References


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