Effect of Surface Roughness and Low-Level Laser Therapy on Removal Torque of Implants Placed in Rat Femurs

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The present study measured removal torque and bone-implant interface resistance of machined implants, acid-etched implants, or machined implants irradiated around the implant area with infrared low-level laser therapy (LLLT; 830 nm) immediately after surgery. There were statistically significant differences between Groups A (control) and B (rough surface) ($P = .03$). Implants with a rough surface seem to add resistance to the bone-implant interface compared with smooth titanium implants or implants treated with LLLT.

Key Words: osseointegration, dental implants, laser

INTRODUCTION

The success of implants relies on the degree of osseointegration, which is defined as the direct structural and functional connection between organized living bone and the surface of a load-carrying implant. Several studies have focused on changes in the characteristics of implant surfaces, because they may have a significant effect on bone-implant interface repair and may produce a better bone anchorage for osseointegration. Implant surface roughness has been shown to positively influence the extent of the bone-implant interface.

Implant surfaces can be roughened using acid etching, laser therapy, sputtering with different materials, or any combination of these methods. Rough surfaces improve the osseointegration of titanium implants, improve biological responses, and shorten the time necessary for osseointegration. Among the different treatments used to produce chemical and topographic changes on implant surfaces, acid etching has been shown by some researchers to provide excellent characteristics for bone-implant integration.

Previous studies using microscopic analyses and torque removal tests revealed a superior performance of implants with an increased surface roughness, which strongly supports the use of this type of surface in clinical practice. Bone-implant contact and resistance to removal tend to be greater in implants in which surface roughness is enhanced.

The use of low-level laser therapy (LLLT) in dentistry has positive effects, such as pain relief, management of premalignant lesions of the oral cavity, reduction of postoperative trismus and swelling, and faster wound healing and nerve regeneration. In implantology, LLLT seems to be a promising treatment to accelerate osseointegration, as demonstrated by its effects on bone repair.

Current studies in the field of dental implants have been directly related to the development of methods to accelerate tissue repair and increase the amount of bone-implant contact. Because the results of implant rehabilitation in patients with
poor bone quality are still unsatisfactory, achieving faster and better osseointegration remains an important challenge and a major interest in clinical research.

The objective of the present study was to measure removal torque values in order to compare the resistance of the bone-implant interface in machined implants, acid-etched implants, or machined implants irradiated around the implant area with LLLT immediately after surgery.

**MATERIALS AND METHODS**

**Design and surface treatment of titanium implants**

Twenty-four implants were placed in the femurs of 12 Wistar rats (*Rattus norvegicus albinus*) and divided into 3 groups: Group A (control) = smooth titanium; Group B = acid etching; and Group C = smooth titanium + LLLT immediately after surgery. Groups A and B included 2 animals each. Each animal received 2 implants in the upper lateral region of each femur (total of 8 implants in each group). Group C included 8 animals, and only 1 implant was placed in each animal to avoid biases potentially caused by the systemic effects of LLLT. Animals were observed for 45 days (Table 1).

Implants were screw shaped and made of pure titanium. Sixteen implants were machined (smooth surfaces, Groups A and C), and 8 had a rough surface as a result of acid-etching treatment with HF/HNO₃ for 15 minutes (Group B). All implants had a standard size of 1.4 mm in diameter and 3.3 mm in length; they were manufactured by Promm Indústria de Materiais Cirúrgicos Ltda (Porto Alegre, Brazil) especially for this study. In Group C, the animals received LLLT using an infrared laser diode (InGaAlP), \( \lambda = 830 \text{ nm} \), 40 mW, 0.60-mm spot diameter. A previously calibrated unit (DMC Equipamentos Ltda, São Carlos, Brazil) was used, and the application protocol was 4.8 J/cm² in 4 different sites around the implant area immediately after surgery. The pointer was placed perpendicularly; the unit automatically controlled the exposure time of 121 seconds.

**Implant surface analysis**

Surface machining regularity was assessed using scanning electronic microscopy (SEM; Philips XL20, Eindhoven, The Netherlands); the values of mean roughness (Ra) and maximum roughness (Rt), defined as the sum of the highest peak with the lowest valley within the measurement area, were measured with a profilometer. To gauge the roughness of implants, 5 areas were assessed in 2 implants randomly selected from each group.

**Surgical technique**

This study was approved by the Ethics Committee of the Universidade Luterana do Brasil (protocol no. 2005-006 A). Animals were anesthetized with 20 mg/kg intramuscular (IM) tiletamine-zolazepam (Zoletil 50, Virbac, France) and an additional injection of 0.2 mL/kg if necessary. Local anesthesia consisted of 0.4 ml 2% lidocaine and 1:200 000 epinephrine injected into the region of the incision.

After the animals were anesthetized, the upper regions of the right and left tibia were shaved and cleaned with a 2% iodophor-in-alcohol solution, after which the surgical field was draped. The animal was placed in position, and a longitudinal incision of about 20 mm was made into the skin and subcutaneous tissues to access the tibia in the upper medial region, followed by dissection and separation of tissues. After that, the implants were placed according to usual clinical guidelines. Absorbable 4.0 catgut suture (Shalon, São Luís de Montes Belos, Brazil) was used for layered closure.

At the end of the surgery, 40 000 IU/kg penicillin G benzathine was administered in a single dose as antibiotic prophylaxis. The animals received oral analgesics (acetaminophen, liquid Tylenol, Janssen-Cilag, S. J. dos Campos, Brazil) for 7 days after the surgery.

**Removal torque tests**

The animals were killed 45 days after surgery by administration of an IM tiletamine-zolazepam (Zoletil 50 mg) overdose. Implant sites were surgically exposed via sharp dissection, the leg was stabilized,
and the implant was removed under reverse torque rotation using a manual torque gauge (BTG Tohnichi, Tokyo, Japan). The equipment was used to measure the peak value of resistance to reverse torque rotation in Newton centimeters (Ncm).

**Statistical analysis**

The torque to remove each implant was measured and compared using analysis of variance (ANOVA). Multiple comparison tests were used to determine possible differences between groups. The level of significance was set at 0.05% ($P < .05$). Results are presented as means with corresponding standard deviations.

**RESULTS**

All rats recovered well from anesthesia and surgery. The wounds healed without any signs of infection, and all animals gained weight at a similar pace. No side effects or signs of pain were observed. No implants were excluded from the study because of clinical instability at the time of the removal torque test, carried out immediately after the IM tiletamine-zolazepam overdose was injected.

The Ra and Rt parameters and SEM findings revealed that surfaces submitted to acid etching for 15 minutes (Group B) had a substantially homogeneous, typically rough surface as a result of the action of the acid (Figure 1). Microscopic findings of machined surfaces (Groups A and C) confirmed their smooth appearance.

According to ANOVA and the Tukey multiple comparison test, Ra and Rt parameters were significantly different between the 2 experimental groups with smooth surfaces (A and C) versus Group B, which had rough surfaces ($P = .01$) (Table 2).

The results of ANOVA and the Tukey multiple comparison test revealed significant differences in removal torque values between the groups. Group B (rough surface) presented higher mean removal torque values compared with Group A (control, smooth surface). Group C (smooth surface + LLLT) showed no significant differences from the other 2 groups ($P = .05$), as shown in Table 3 and Figure 2.

**DISCUSSION**

The metabolic process of bone repair; the characteristics of the materials used; and the shape, roughness, and topography of an implant all play an important role in the early and late phases of biological response to implants during osseointegration, a process that is accompanied by protein adsorption and cell adhesion. Torque removal forces have been used to study biomechanical anchorage, or endosseous integration, and the greater forces required to remove implants may be interpreted as an increase in the strength of bone integration. Torque removal values in this study were in agreement with other results in the literature, which suggest a significant increase in bone integration when implants with a rough surface are used. In this study, acid-etched implants (Group B) presented higher removal torque values than the machined implants (Group A). This result can be explained by cell phenomena, especially proliferation and replication, which depend on implant surface morphology (the production of the extracellular matrix is sensitive to surface roughness). Cell proliferation varies with surface roughness, as cells can identify surface roughness. In the present work, removal torque indirectly quantified the osseointegration mechanism.

In the complex process of bone formation on the bone-implant interface, an increased surface roughness achieved by specific techniques is an important parameter, and it affects biological responses: rough surfaces improve cell responses and play an important role in osteoblastic differentiation. As a result, osseointegration increases with greater implant surface roughness. Subtractive methods (eg, blasting, acid etching) have been used to increase surface area and to alter the microtopography, or texture, of implant surfaces. Similar to what was observed in our study, the significantly higher removal torque values obtained in acid-etched implants (Group B) in relation to control implants (Group A), in addition to an improved bone-implant interface and increased resistance to failure, have also been reported in studies designed to test acid-etched implant surfaces in combination with other surface treatments. Moreover, there is less correlation between removal torque and the difference in HF volume percent used.

Laser therapy has emerged as an efficient noninvasive treatment for stimulating osteogenesis and accelerating bone healing because it promotes cell proliferation and the differentiation of osteo-
blasts, provoking an increase in the number of differentiated osteoblasts as well as bone formation. LLLT also improves blood and oxygen flow; stimulates capillary regeneration and formation by releasing growth factors; and enhances the activity of neutrophils, macrophages, and fibroblasts as well as the metabolism of damaged cells.

Depending on the wavelength used, laser therapy produces different effects on irradiated tissues. Infrared laser acts indirectly on mitochondria, producing a photophysical or photoelectric effect on these structures, changing the membrane potential and therefore stimulating cell proliferation. Bones irradiated with infrared wavelengths show increased levels of osteoblastic proliferation, collagen deposition, and bone neoformation compared with nonirradiated bone.

The osteogenic potential of mesenchymal cells depends on several genetic factors, as well as on systemic and local inducer factors. Laser therapy may act as such an inducer factor. Laser therapy improves bone matrix production because of the improved vascularization and anti-inflammatory effects. Therefore, both the release of mediators and microvascularization might increase, which might subsequently accelerate bone healing.

Laser therapy is more effective if the treatment is carried out at early stages, when intense cell proliferation takes place. Vascular responses have also been described as one of the possible mechanisms responsible for the positive clinical results observed after laser therapy. It remains unclear whether bone stimulation by laser light is a general effect or whether the isolated stimulation of osteoblasts is possible.

The use of LLLT may induce greater mechanical

**Figure 1.** Micrographs showing implants with (a) smooth surface and (b) rough surface at 500 μm and ×130 magnification, and implants with (c) smooth surface and (d) rough surface at 50 μm and ×1000 magnification.
resistance of the bone-implant interface because of an increase in metabolism, which may lead to a faster healing process. However, our study did not find any significant differences between Groups A (control) and C (smooth surface + LLLT). The fact that only 1 implant was placed in each animal in Group C (ie, absence of internal controls) is justified by the suggestion made by several reports that LLLT does not act only on the sites of irradiation but also produces systemic effects.

No consensual LLLT irradiation protocol has yet been defined. In our study, laser was used only once, immediately after implant placement. In the study conducted by Khadra et al, however, LLLT was used immediately after surgery and repeated daily for 10 consecutive days. The tensile tests, histomorphometric evaluation, and energy-dispersive X-ray microanalysis conducted by those authors showed that LLLT had a positive effect on the functional attachment of titanium implants to the bone. Further studies should be conducted to determine an LLLT application protocol that is able to produce a strong bone-implant contact.

More than one method of evaluation should be used for the topographic description of a surface because no single technique is capable of faithfully detecting all relevant topographic details. There is a great variety of 2- and 3-dimensional techniques designed to describe surfaces. Based on a previous study by Keller et al, 2 techniques were selected for our study: SEM, which provides excellent depth of focus and a qualitative analysis of topography, and profilometry, which provides a quantitative assessment of surface topography using the Ra and Rt parameters. Our surface roughness values were similar to those described in the literature, with dual acid-etched surfaces usually presenting roughness values of Ra ranging from 0.5 to 2.0 mm. These values are known to result in better in vivo performances compared with machined surfaces.

Further longitudinal studies should be conducted to determine the ideal 3-dimensional configuration for bone-implant contacts. The nanometric surface configuration of implants seems to be promising to guide the development of future studies.

Data found in this study suggest that implants with a rough surface promote significantly improved bone-implant contact compared with smooth implants, whereas no differences were observed in the comparison of rough surface implants with smooth implants irradiated with LLLT. These results suggest that increases in the surface roughness of titanium implants promote bone formation and greater bone-implant integration.

**ABBREVIATIONS**

ANOVA: analysis of variance

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**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ra Mean</th>
<th>Ra Standard Deviation</th>
<th>P</th>
<th>Rt Mean</th>
<th>Rt Standard Deviation</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Smooth surfaces (A and C)</td>
<td>0.15</td>
<td>0.02</td>
<td>.01*</td>
<td>1.17</td>
<td>0.16</td>
<td>.01*</td>
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<tr>
<td>Rough surfaces (B)</td>
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<td>0.07</td>
<td></td>
<td>4.49</td>
<td>0.78</td>
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*Significant at \( P < .05 \).

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**TABLE 3**

<table>
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<tr>
<th>Group*</th>
<th>N</th>
<th>Mean†</th>
<th>Standard Deviation</th>
<th>F</th>
<th>P</th>
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<tr>
<td>Smooth surface</td>
<td>8</td>
<td>11.8</td>
<td>1.3</td>
<td>3.43</td>
<td>.05</td>
</tr>
<tr>
<td>Smooth surface + LLLT</td>
<td>8</td>
<td>14.0</td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rough surface</td>
<td>6</td>
<td>16.8</td>
<td>4.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LLLT indicates low-level laser therapy.
†Means followed by the same letter indicate absence of statistical difference.
FIGURE 2. Comparison of mean removal torque values between study groups.

IM: intramuscular  
LLLT: low-level laser therapy  
Ra: mean roughness  
Rt: maximum roughness  
SEM: scanning electron microscopy

REFERENCES


