The Influence of Low-Level Laser on Osseointegration Around Machined and Sandblasted Acid-Etched Implants: A Removal Torque and Histomorphometric Analyses

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Evaluation of the influence of laser application on osseointegration around implants with different surface characteristics is limited. This study aims to evaluate the influence of low-level lasers on the early stages of osseointegration. Ninety-six external hex implants (3.75 mm x 5.0 mm) were placed in 24 rabbits—one machined and one sandblasted acid-etched per tibia. The rabbits were later divided into the laser group, which received a total dose of 24 J/cm² of gallium-aluminum-arsenide laser over 15 days, and a control group. At 16 and 30 days after surgery, removal torque and histomorphometric analyses were performed. No statistical differences in removal torque or histomorphometric analyses were verified between laser and control groups regardless of implant surface (P > .05). Time was the only variable presenting significant differences between measurements (P < .05). Low-level laser had no significant short-term effect on bone-to-implant contact and removal torque values regardless of implant surface characteristics.

Key Words: implant surface, low-level laser, osseointegration, removal torque, histological analysis

INTRODUCTION

Lasers have been widely used in medicine and dentistry for almost five decades. A wide variety of low-level lasers—including gallium-aluminum-arsenide (GaAlAs), helium-neon, and argon—have been applied at different doses and frequencies, aiming to positively influence the process of tissue repair. This possible influence has been attributed to the laser’s alleged ability to modulate the production of local cytokines and other proteins responsible for cell-signaling in the process of tissue healing.

The idea that low-level lasers might be therapeutic or at least influence tissue repair and promote pain relief has been the subject of debate among researchers. Some studies have suggested that low-level lasers (ie, lasers presenting average potency of 1 to 120 nW) might induce positive tissue healing effects when applied to skin, nerve, bone, and cartilage tissues in animals, as well as in human wounds and ulcers. Conversely, other studies have reported that low-level lasers might be ineffective in positively influencing the process of tissue repair, raising doubts about their therapeutic effectiveness.

The number of studies on the possible effects of laser application on osseointegration of dental implants has reasonably increased in recent years, aimed mainly to generate postoperative comfort to patients by reducing pain and edema. In addition, indication for laser application in postoperative nerve paresthesia and treatment of peri-implantitis has been identified as a promising treatment alternative. Other reports also supported the indication of low-level laser therapy (LLLT) on implant bone healing based on results of animal studies, suggesting that the laser application might significantly improve the osseointegration response if applied during its early stages.

Surface treatment of titanium implants seems to positively affect their osseointegration by generating an increased amount of bone contacting the implant surface in short-term evaluations. When in contact with rougher implant surfaces, osteoblastic cells have a tendency to express their phenotype differently in terms of speed and amount of bone protein synthesis, compared to when in contact to smoother surfaces. A systematic review of implant-related studies analyzing mostly animal models reported a positive relationship between surface roughness and osseointegration response in short-term observations.

Despite the available literature, indications of laser therapy as an effective tool to improve or accelerate osseointegration...
around implants—as well as issues concerning the ideal type of laser, potency, and ultimate dose to elicit the best bone tissue response—still remain unclear. Consequently, the present study was designed to evaluate the possible influence of low-level laser application on the early stages of osseointegration around titanium implants presenting distinct surfaces through removal torque and histomorphometric analyses.

MATERIALS AND METHODS

Ninety-six screw-shaped, external hex, commercially pure titanium (ASTM grade 4) implants, 5.0 mm in length and 3.75 mm in diameter, were used in this study. Forty-eight implants had a machined surface (Master Screw, Conexão Sistemas de Prótese, São Paulo, Brazil), and the other 48 had a sandblasted acid-etched surface (Master Porous, Conexão Sistemas de Prótese, São Paulo, Brazil). The previously established surface roughness was 0.32 ± 0.03 μm for the machined implants and 0.73 ± 0.04 μm for the sandblasted acid-etched implants.

Twenty-four male adult New Zealand rabbits (Oryctolagus cuniculus) weighing 3.0 to 4.0 kg were used in the study, which was approved by the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS). Before surgery, anesthesia was induced by Zolazepam and Tiletamine, 15 mg/kg of body weight IM (Zoletil 50 mg, Virbac Laboratory, France).

The tibial diaphysis was chosen as the experimental site. The surgical area was shaved and cleaned with an antiseptic iodine solution before skin incision and tissue dissection down to the periosteum. Periosteal flaps were then raised and 2 implant sites per tibia were prepared following a standardized drilling procedure, as recommended by the implant manufacturer. First, implant sites were marked 10 mm apart with a guide drill. They were then sequentially enlarged by a 2.0-mm twist drill, pilot drill, and 3.0-mm and 3.25-mm twist drills. Osteotomy sites were pretapped at 25 rpm and the implants inserted at 15 rpm/35 Ncm torque until they stabilized at the upper cortical bone (Figure 1a–d).

Four implants were placed in each animal, with 2 machined implants in the right tibia and 2 sandblasted acid-etched in the left. Titanium cover screws were placed manually on the implants, and the skin flap was closed using single nylon sutures (Tech Synth 3.0, Bangalore, India). After suture, 3 sites surrounding the implants were marked using 1% gentian violet.
solution (Indafarma Ltda., São Paulo, Brazil) to guide laser application throughout the study.

The animals were then randomly selected to constitute both laser and control groups. A dose of 1 J/cm² per site of GaAlAs diode laser, λ830 nm, 100 mW output, 46 mW/mm² power density (Foto Laser III, DMC Equipamentos Ltda, São Paulo, Brazil) was applied to all marked sites in the laser group, resulting in a 3 J/cm² laser dose per application at 48-hour intervals for 15 days. The total laser irradiation dose was 24 J/cm² per animal. The penetration depth in living tissues of this laser wavelength was estimated to be around 6 mm.¹¹ Control animals were subjected to the same laser application procedures but with the equipment off, going through the same daily routine of the laser group.

Postoperatively, each animal received penicillin and streptomycin (Pentabiotic, Laboratório Forte Dodge Ltda, São Paulo, Brazil) 20 000 UI/Kg IM, and ibuprofen (Alivium, Mantecorp Ltda, São Paulo, Brazil) 50 mg/ml for 5 days. Four animals were lost due to postoperative complications within 2 days after surgery, resulting in a final study group of 20 animals.

Removal torque analysis

Seven animals of each group (laser and control) were randomly selected for the removal torque analysis and sacrificed following a previously described method²⁰ at 16 and 30 days after surgery. The implant sites were surgically exposed, the cover screws were removed, and an implant mount was secured on each implant. Implant osseointegration was then evaluated for mobility, bone loss and/or signs of infection. A reverse torque was applied individually to each osseointegrated implant using a torque gauge (TSD 150 Torqueleader, MHH Engineering, Bramley, Guilford, Surrey, UK) until implant rotation was detected. Calibration of the torque device was in compliance with the requirements of ISO 6789:1992 and BSEN 26789/1994. Peak values of resistance to reverse torque were recorded in N/cm.

Histomorphometric analysis

Thirty days after surgery, 3 animals of each group (laser and control) were randomly selected for the histomorphometric analysis. The implants were removed in block with the surrounding hard and soft tissues. The implant-bone blocks were dehydrated in a graded series of alcohols and embedded in resin for microscopy (EMbed 812 Kit, Electron Microscopy Sciences, Fort Washington, Pa) to be later sectioned longitudinally to the implant axis using thin diamond blades at low speed mounted in an electric saw (Labcut 1010, Extec Corp., Enfield, UK). From each block, 3 to 4 slices approximately 120 μm thick were firstly obtained and then ground to a thickness of 10–20 μm with 600-, 800-, and 1200-grit silicon carbide paper (3M, São Paulo, Brazil) under water refrigeration, and then stained with 1% toluidine blue. The histomorphometric analysis was performed using an optic microscope (BX 50, Olympus Optical Co, Tokyo, Japan) connected to a digital camera (Sony DSC-P92, Sony Inc, Tokyo, Japan). Digital images were analyzed at ×10 magnification using the software Image Pro Plus 4.0 (Media Cybernetics, Silver Spring, Md) as described¹⁶ to measure the percentage of direct bone-to-implant contact (BIC) in relation to the overall implant surface at their upper, middle and lower portions (Figure 2a–c).

Statistical analysis

Results of peak removal torque were analyzed by 3-way analysis of variance (ANOVA) after the application of a Kolmogorov-Smirnov test to check the normality of the data. The percentage of BIC was analyzed by Student t-test. The level of significance was set at 5% for all analyses.

Results

Tables 1 and 2 display mean values of peak removal torque for each group and results of a 3-way ANOVA, respectively. Six of the 56 implants considered for removal torque analysis did not present evidence of osseointegration according to the proposed criteria and were removed from the study. Preliminary tests indicated no significant difference due to the number of implants per group; therefore, only osseointegrated implants were considered for analysis. At 16 days, the laser group presented removal torque values of 19.15 ± 4.65 Ncm and 20.48 ± 6.72 Ncm for the machined and sandblasted acid-etched implants, respectively. At 30 days, the laser group presented removal torque values of 30.08 ± 4.14 Ncm and 23.45 ± 10.40 Ncm for the machined and sandblasted acid-etched implants, respectively. In the control group, removal torque values were 20.42 ± 3.06 Ncm and 22.67 ± 11.07 Ncm at 16 days for the machined and sandblasted acid-etched implants, and 27.42 ± 19.65 Ncm and 30.46 ± 16.06 Ncm at 30 days for machined and sandblasted acid-etched implants, respectively. Comparison between removal torque mean values presented no statistical differences between groups, indicating that laser application had no significant effect on removal torque regardless of implant surface (P > .05). Statistical analysis indicated that time was the only variable presenting significant differences between both removal torque observations (P < .05).

Tables 3 and 4 shows BIC mean values at 30 days, expressed in percentage for each analyzed group. Considering only laser irradiation, the laser group presented a mean BIC of 77.04 ± 10.17%, while the control group showed 69.51 ± 19.28% (P > .05). When comparing implant surfaces, the laser group presented results of 81.53 ± 10.15% for sandblasted acid-etched and 72.5 ± 8.73% for the machined implants, while the control group presented mean BIC values of 72.19 ± 16.77% for sandblasted acid-etched and 66.84 ± 22.79% for machined implants. Statistical analysis indicated no significant differences when considering laser application and/or implant surface (P > .05).

Discussion

To the authors’ knowledge, the present study might constitute the first reported attempt to investigate by comparison the possible effects of LLLT on early stages of osseointegration of implants with distinct surfaces. Surface treatment has been said to generate different patterns of early bone formation around
implants, so it might be hypothesized that a distinctive osseointegration pattern in terms of speed, volume, or degree of bone maturation could be expected between laser-irradiated and nonirradiated groups presenting different implant surface properties. However, contradicting this hypothesis, results from the short-term removal torque and histomorphometric analyses indicated that application of low-level laser did not significantly affect nonloaded machined and sandblasted acid-etched implants in their early stages of osseointegration, and confirmed time as a key element for the maturation of osseointegration, as observed previously.

Observation periods of 16 and 30 days for removal torque analysis were established in this investigation based on the need to evaluate the possible effects of laser irradiation during early stages of osseointegration, especially in an in vivo animal model. As the osseointegration process develops through time, differences in removal torque and BIC values tend to be negligible among implants with different surfaces. This observation emphasizes the importance of investigating the possible effects of any external influence on osseointegration during its initial biologic events. However, it might be hypothesized that such short observation period might have also negatively influenced removal torque analysis, since the

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<td><strong>Removal torque values (N/cm) for laser and control groups (P &gt; .05)</strong></td>
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<table>
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<tr>
<th>Group</th>
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<th>Period</th>
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<tr>
<td>Control</td>
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<td>19.65</td>
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<tr>
<td></td>
<td>Acid-etched</td>
<td>16 days</td>
<td>3</td>
<td>22.67</td>
<td>11.07</td>
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<tr>
<td></td>
<td></td>
<td>30 days</td>
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<td>30.46</td>
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<tr>
<td>Laser</td>
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implant surrounding bone at 16 and 30 days might still present an immature calcification and thus limit the detection capacity by mechanical tests of potential differences in bone healing between groups.

The laser irradiation protocol applied here was in accordance with other previous reports, where aspects such as laser type, wavelength, application time, irradiation area, and total laser dose were monitored to produce an effective bone response. The laser dose of 3 J/cm² per application was described as adequate to produce a significant bone formation response in animal models, recommended to range from 1 to 10 J/cm² per application for bone LLLT. Although supported by previous studies, differences involving laser settings used in the present investigation compared to other reported studies might have influenced osseointegration in both groups and consequently the observed results. Additionally, the use of distinct animals for laser and control groups, instead of an intra-individual test-control model, was observed to eliminate a possible bias caused by laser potential systemic effects. Also, although hardly quantifiable, differences regarding postoperative tissue swelling and overall animal comfort could be clearly observed in the laser group, confirming the suggested analgesic and anti-inflammatory effects associated with postoperative local laser application.

Although not fully supported by the literature, a possible explanation for the influence of lasers on living tissues has been said to be related to the activation of specific tissue photoreceptors, such as rhodopsin, and nonspecific photoreceptors such as flavoproteins, which may absorb photons and modulate the production of proteins and other tissue biochemical mediators to positively interfere on cell metabolism. However, some studies have shown even osteoblastic growth inhibition linked to LLLT, suggesting that any positive effect, if applicable, might be directly linked to the applied laser settings in terms of wavelength, dose, and application timing, as well as characteristics of the utilized cell lines and type of matrix compounds. Although some reports have stated that LLLT might have a positive effect on osseointegration around dental implants, major experimental differences in terms of studied subjects, type of laser, applied settings, and total laser dose make impossible any comparison attempt between distinct studies.

The implants used in the present investigation were selected based on their design and surface treatment. Aspects of surface texture have been said to play an important role in the osseointegration process. Machined implants usually present a resultant anisotropic surface produced during their manufacturing process, with asperities following a specific directional pattern, while sandblasted acid-etched implants present an isotropic surface with randomly distributed asperities. Although the predictability for an acceptable osseointegration outcome has been shown for both surfaces, isotropic textures have been said to produce a faster osseointegration. Under mechanical and histological evaluations, both implant surfaces have already shown significant differences in BIC at specific observation periods. Although significant differences in torque removal were verified between 16 and 30 days of observation, confirming the positive maturation of the osseointegration in both groups, no differences were present between both surfaces in histomorphometric and mechanical analysis at 30 days, suggesting that similar bone responses might be expected even in short-term observations. Also, the implant length and diameter applied here were chosen to fit the average size of the tibial diaphysis of an adult rabbit, minimizing the risk of possible postoperative bone fractures.

Regarding histomorphometric analysis, results were in accordance with previous reports, which presented a mean 10% BIC difference between laser irradiated and nonirradiated groups, though not statistically significant (Figure 3a and b). Microscopy of the implant surrounding bone indicate a distinct trabecular organization and a superior volume of both blood vessels and Haversian canals for the laser group, suggesting some influence of laser irradiation on bone formation (Figure 4a and b). Although not quantified in the present investigation and therefore merely speculative, this possible influence on the vascular tissue configuration was also mentioned in previously reported studies, which have also emphasized the crucial role played by an adequate blood supply on osteoblastic phenotype expression and protein biosynthesis.

Based on the present observations, further studies seem to be needed to better understand the possible benefits of the association between local laser irradiation and osseointegration of dental implants. Both short- and long-term events present in
osseointegration have been said to be influenced by LLLT at some extent, even though this influence might induce only minor consequences at molecular or cell levels. The remaining challenge consists of determining to what extent this influence might really induce treatment benefits to osseointegration and to establish a precise and more reliable methodology to induce and quantify them.

CONCLUSION

Low-level laser irradiation did not significantly influence the percentage of bone-to-implant contact and the removal torque for both machined and sandblasted acid-etched implants at short-term observation. Statistical analysis confirmed time as the only variable presenting significant differences between removal torque observations at 16 and 30 days \( (P < .05) \).

ABBREVIATIONS

ANOVA: analysis of variance
BIC: bone-to-implant contact
GaAlAs: gallium-aluminum-arsenide
LLLT: low-level laser therapy

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