Effects of a Low-Intensity Laser on Dental Implant Osseointegration: Removal Torque and Resonance Frequency Analysis in Rabbits

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The objective of this study was to investigate how a low-intensity laser affects the stability and reverse torque resistance of dental implants installed in the tibia of rabbits. Thirty rabbits received 60 dental implants with the same design and surface treatment, one in each proximal metaphysis of the tibia. Three groups were prepared (n = 10 animals each): conventional osseointegration without treatment (control group), surgical sites irradiated with a laser beam emitted in the visible range of 680 nm (Lg1 group), surgical sites irradiated with a laser beam with a wavelength in the infrared range of 830 nm (Lg2 group). Ten irradiation sessions were performed 48 hours apart; the first session was during the immediate postoperative period. Irradiation energy density was 4 J/cm^2 per point in 2 points on each side of the tibias. The resonance frequency and removal torque values were measured at 2 time points after the implantations (3 and 6 weeks). Both laser groups (Lg1 and Lg2) presented a significant difference between resonance frequency analysis values at the baseline and the values obtained after 3 and 6 weeks ($P > .05$). Although the removal torque values of all groups increased after 6 weeks ($P < .05$), both laser groups presented greater mean values than those of the control group ($P < .01$). Photobiomodulation using laser irradiation with wavelengths of 680 and 830 nm had a better degree of bone integration than the control group after 6 weeks of observation time.

Key Words: dental implants, osseointegration, low-intensity laser therapy, resonance frequency analysis, removal torque, photobiomodulation

INTRODUCTION

Several factors have been shown to have a potential influence on the incidence of dental implant success.[1,2] These factors can be divided into local and systemic risk factors that may be influential in the early or late phase of implant therapy. The local factors that affect the process of osseointegration include implant design, implant surface treatment, bone quality, surgical technique, and postoperative care.[3,4] Thus, changes and new implant models (macro-, micro-, and nano-design modifications), as well as new techniques, have recently been developed that aim to accelerate the osseointegration of dental implants. However, functional activities of the different cells and the roles of nonosteogenic cells in the healing process have not been clearly defined. A key question is how early molecular and cellular events are influenced by material surface properties in vivo and how these early events influence the organization of the surrounding tissue and its interlocking or bonding with the material surface.[5,6]

The therapeutic effects (photobiomodulation) include acceleration of healing and bone regeneration processes, restoration of neural functions, and pain reduction among others. Light-emitting diodes and, less often, broad light sources are also becoming more popular as a result of the better quality and control offered by the newer devices. Although the precise molecular mechanisms for photobiomodulation remain to be fully elucidated, their clinical effects in terms of alleviating pain, reducing inflammation, and stimulating wound healing are well established.[7,8]

Therefore, the aim of this study was a biomechanical investigation of how laser radiation affects the osseointegration process of implants placed in rabbit tibia, comparing the effects of 2 wavelengths (680 nm and 830 nm) in relation to the nonirradiated control group (conventional implantation technique).

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Figure 1. Illustration of the received signal and the implant stability quotient conversion.

Materials and Methods

Implant characteristics and group formation

This study used 60 dental implants from the same manufacturer (Frialit-2 Synchro–Friadent, Munhein, Germany). The implants had the same type of sand-blasted and acid-etched surface so that these factors presented the same level of stimulation in all samples. Implants were 3.8 mm in diameter and had a height of 8.0 mm.

Animals and surgical procedure

Thirty male New Zealand white mature rabbits weighing approximately 4 kg were used in this study. The study was approved by the ethics committee of the University of São Paulo, São Paulo, Brazil. The animals were anesthetized by an intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutica, Brasilia, Brazil). Then, a muscle relaxant (Rompum 5 mg/kg, Bayer, São Paulo, Brazil) and a tranquilizer (Acepran 0.75 mg/kg, Univet, São Paulo, Brazil) were injected intramuscularly. After the skin incision and exposure of the bone, the osteotomies were performed with burs under copious saline irrigation. One implant was inserted in the proximal metaphysis of the tibia. The insertion torque of the implants was controlled using a manual torquemeter and did not exceed 20 ± 3 N. The periosteum and fascia were sutured. Postoperatively, a single dose of 600 000 IU Benzetacil (Novartis, Bogotá, Colômbia) was used. All animals were killed with an intravenous overdose of ketamine (2 mL) and xylazine (1 mL); 5 animals per group were killed at each time point, 3 and 6 weeks after the implantations.

Postoperative treatment and group formation

The rabbits were divided into 3 groups (n = 10 animals each) as follows: the control group, in which the rabbits underwent conventional osseointegration without treatment; the first laser group (Lg1 group), in which surgical sites were irradiated with a laser emitting a wavelength of 680 nm in the visible range, spot

size of 4 mm (Minilaser 2075, Helbo, Gallspach, Austria), and the second laser group (Lg2 group), in which surgical sites were irradiated with a laser emitting 830 nm in the infrared range, with spot size of 400 μm (Thera Lase, DMC Equipamentos, São Carlos, Brazil).

Both lasers had optical fibers as the beam delivery system. Exposition time was computed for each laser, taking into consideration the output area of the beam delivery system and a distance of 0.5 to 1 mm in a continuous way; this resulted in irradiations of 40 seconds for each implant site with an energy density of 4 J/cm^2 per point reaching an effect of 0.5 cm deeper in the rabbit tissues. For animals belonging to the laser groups, the first irradiation session took place during the immediate postsurgery period, and the sessions were repeated at 48-hour intervals for a total of 10 sessions.

Resonance frequency analysis

All rabbits were used for resonance frequency analysis (RFA) to measure implant stability using the Ostell (Integration Diagnostics AB, Göteborg, Sweden). For RFA, the implants were measured at installation and during euthanasia, 3 and 6 weeks after implant installation. The Implant stability quotient values were measured in 2 directions, proximal to distal and lateral to medial, and an average of each sample was determined (Figure 1).

Removal torque test

The samples were maintained in liquid solution (10% buffered formalin) and were immediately evaluated (1 hour after removal) so that they did not suffer dehydration. The 60 implants were retrieved using a manual digital torquemeter (Tohnichi STC 20, Tokyo, Japan). A special part was manufactured to adapt the external implant hexagon with parallel paw-fastening bars that worked as stabilizers to prevent paw movement or fracture during torque application (Figure 2).

Statistical analyses

For analysis of the results, nonparametric tests were used to take into consideration the nature of the studied variables. The following tests were applied[9]: the Friedman test to compare the resonance frequency measured at implant installation and after 3 and 6 weeks within each group, the Kruskal-Wallis test to compare the studied groups (Lg1, Lg2, and control groups) at every moment of the study for resonance frequency and removal torque, and the Wilcoxon test to compare the values of removal torque observed after 3 and 6 weeks separately for each group. The rejection level of the null assumption was set at 0.05. To verify the correlation between the 2 methods, all values of RFA and removal torque were converted to percentages. Then, the data were processed with Unscrambler, version 6.11 software (CAMO A/S, Trondheim, Norway).

Results

The surgical procedures were uneventful. All animals presented appropriate healing during the first week after the surgical

Figure 2. Image of the torque removal measurement.

procedure. Postsurgical inspections for 2 weeks indicated the absence of infection or inflammation. After the proposed times, all implants were osseointegrated.

The mean resistance to removal torque values and standard deviations are summarized in Table 1 and shown in the line graph of Figure 3. The mean resonance frequency values for the 3 investigated implant designs are summarized in Table 2 and shown in Figure 4. Analysis to determine correlation showed that a positive correlation exists among the mean values between the 2 methods within the proposed times: 3 weeks = 0.951, 6 weeks = 0.930.

DISCUSSION

Extrinsic stimuli of the osseointegration, such as reported with the use of a low-intensity laser, could stimulate better osseointegration of the implants and have a favorable effect on the healing and attachment of titanium implants.[10] Based on the rabbit tibia model, the present study focused on the biomechanical reaction and involved measuring the removal torque values and conducting RFA to evaluate the influence of low-intensity laser therapy (LILT) or photobiomodulation on osseointegration.

Many studies have examined the use of photobiomodulation for recovering soft oral tissues. These studies show that increased vascularization is responsible for anti-inflammatory and antiedematous effects. Furthermore, authors agree that the metabolic changes caused by LILT that promote tissue regeneration as well as proliferation and viability of reparatory cells depend on the applied dosage, that is, the appropriate energy density and power.[11–14] Some research studies have examined how low-intensity lasers affect the mechanism of bone regeneration, but there is much controversy, which indicates the need for more research to obtain certainty about how LILT afffects bone tissue.[15–20] However, in vitro and in vivo studies have showed a significant stimulation of the irradiation on the bone/implant complex, which is related to the ability of laser irradiation to accelerate cellular activity, such as improved alkaline phosphatase (ALP) activity synthesis,[21] early osteoblastic differentiation,[22] and release of growth factors.[23] The ALP is regarded as a marker of osteoblast differentiation.[24] Early progenitor cells do not express ALP activity but differentiate through a defined number of cell divisions, ultimately expressing a mature osteoblast phenotype: a postmitotic osteogenic cell with ALP activity.[25] Therefore, the effect of laser therapy on ALP activity may reflect the effect of laser irradiation on bone formation.

The 2 laser sources used in this study were semiconductor diodes emitting wavelengths of 680 nm and 830 nm, respectively. These two wavelengths have a large number of applications in dentistry because they cause photobiological effects that promote biostimulation.[7,8]

Statistical analysis of resonance frequency results has shown that for the 2 laser groups, there was a significant difference between the frequency values at the time of

Table 1			
Statistical analyses of the removal torque (Ncm) values for the groups (laser 830 nm, laser 680 nm, and control) at 3 and 6 weeks*			
	Laser 830 nm	Laser 680 nm	Control
3 weeks (mean)	35.00	37.60	24.83
6 weeks (mean)	64.61	55.03	42.45
Wilcoxon test (3 × 6 weeks)	Tcalc = 0 (P < .001)	Tcalc = 0 (P < .01)	Tcalc = 0 (P < .05)
	Tcrit = 10	Tcrit = 8.0	Tcrit = 5.0
	3 < 6	3 < 6	3 < 6
	3 weeks	6 weeks	
Kruskal-Wallis test (laser 830 nm × laser 680 nm × control)	Hcalc = 5.73	Hcalc = 6.17 (P < .05)	
	NS	Control < 830 and 680 nm	

*Tcalc indicates time calculated; Tcrit, time critic; Hcalc, H calculated; NS, not significant.
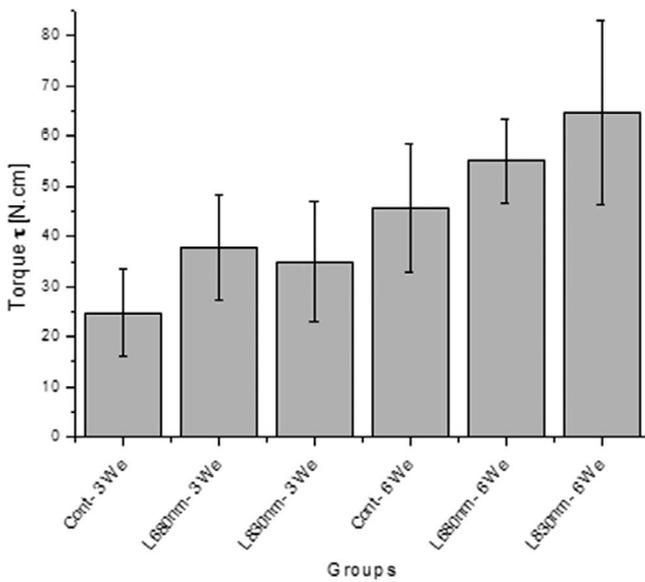
FIGURE 3. Average removal torque values (Ncm) by evaluation moment for all groups. Cont indicates control group; L, laser group; 3 We, 3 weeks; 6 We, 6 weeks; nm, nanometer.

FIGURE 4. Bar graph of the implant stability quotient values and SDs for all groups.

implant installation and the values obtained after 3 and 6 weeks (Table 2). However, statistical analysis of the resonance frequency values for these same laser groups after 3 weeks was not significantly different from the values obtained after 6 weeks.

The control group did not show any statistically significant difference during RFA. However, the control group showed surprisingly high resonance frequency values at the time of the implant installation compared with the values obtained for the laser group. These higher values may have been responsible for the smaller evolution of resonance frequency values with time. This fact confirms the findings of a previous study,[26] where implants installed in high-density bone do not show a large evolution of resonance frequency over time, and it may even decrease in some situations.

In 1997, Meredith et al[27] analyzed the resonance frequency of implants installed in rabbit tibia and concluded that the values increased with time, but the authors indicated a trend
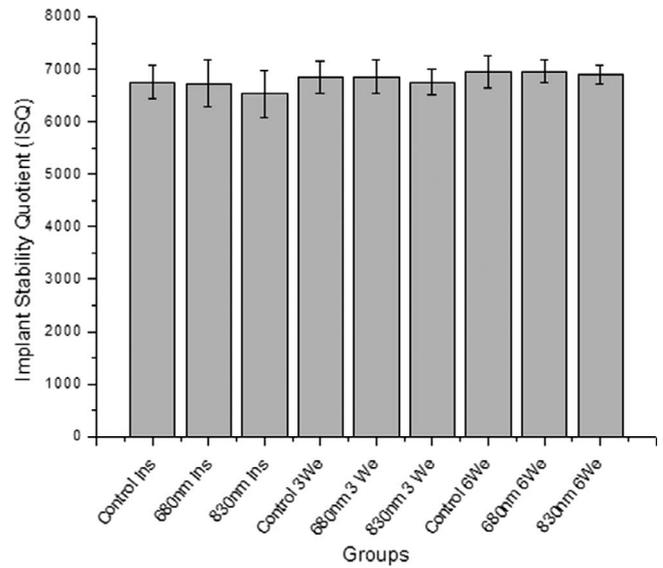
for this increase to reach equilibrium after 40 days. It should be noted that in this study, the increase achieved equilibrium after 21 days, which indicates the biostimulating effect of low-intensity laser radiation.

The high removal torque values for the laser groups show that, in this study, the low-intensity laser radiation had a biostimulating effect on the bone integration process of implants because higher values of removal torque are related to closer contact between bone and implant surfaces.[4,28] Statistical analysis also showed that the laser groups had higher average removal torque values after 3 weeks, but the critical value required for achieving significance between the groups was 5.99 ($P < .05$), and the value obtained was 5.73 (Table 1). This result indicates an acceleration of the bone integration process of implants after a period of 3 weeks. The removal torque values after 6 weeks showed that, in this study, laser radiation emitted at a low intensity was capable of promoting bone integration comparable to values achieved in previously published studies only after long periods of 3 to 12 months.[4,29–31]

TABLE 2

Statistical analyses of the resonance frequency (Hz) values for the groups (Laser 830 nm, Laser 680 nm, and Control) at 3 and 6 weeks*

	Laser 830 nm	Laser 680 nm	Control
Installation (mean)	6205.3	6337.2	6780.0
3 weeks (mean)	6895.4	6833.3	6886.8
6 weeks (mean)	6908.0	6961.7	6948.0
Freedman test (installation × 3 weeks × 6 weeks)	χ^2calc = 9.90 ($P < .02$)	χ^2calc = 10.76 ($P < .02$)	χ^2calc = 0.9
χ^2crit = 5.99	Initial < 3 and 6 weeks	Initial < 3 and 6 weeks	NS
	Initial	3 weeks	6 weeks
Kruskal-Wallis test (Laser 830 × laser 680 × control)	Hcalc = 5.26	Hcalc = 0.52	Hcalc = 0.34
Hcrit = 5.99	NS	NS	NS

*χ^2calc indicates χ^2calculed; χ^2crit, χ^2critic; NS, not significant; Hcalc, H calculated.

CONCLUSION

With the conditions of this study, the results indicate that there is an acceleration of the bone integration process of implants irradiated by a laser emitting wavelengths of 680 nm or 830 nm in comparison with the control group.

ABBREVIATIONS

ALP: alkaline phosphatase activity
LILT: low-intensity laser therapy
RFA: resonance frequency analysis

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