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Research Paper
Dental Implants

Low-level laser therapy improves peri-implant bone formation: resonance frequency, electron microscopy, and stereology findings in a rabbit model

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Abstract. Previous studies have reported positive effects of low-level laser therapy (LLLT) on bone healing. This study evaluated the effects of LLLT on peri-implant healing in vivo. Thirty-two rabbits had their mandibular left incisors removed, followed by immediate insertion of a dental implant into the fresh socket. Animals were assigned randomly to four groups: control (non-irradiated) or LLLT at three different doses per session: 5 J/cm^2, 10 J/cm^2, and 20 J/cm^2. A GaAlAs laser (830 nm, 50 mW) was applied every 48 h for 13 days, starting immediately after surgery. The implant stability quotient (ISQ) was measured using resonance frequency analysis upon implant insertion and immediately after death, 30 days after the last application. Tissues were prepared for scanning electron microscopy (SEM) and stereology. Variables measured were bone–implant contact (BIC) and bone neoformation within implant threads at three different sites. The results showed better ISQ for the 20 J/cm^2 group ($P = 0.003$). BIC values were significantly higher ($P < 0.05$) in the 20 J/cm^2 group, on both SEM and stereology. Bone area values were better in the 10 J/cm^2 ($P = 0.036$) and 20 J/cm^2 ($P = 0.016$) groups compared to the control group. Under these conditions, LLLT enhanced peri-implant bone repair, improving stability, BIC, and bone neoformation. The findings support and suggest parameters for the design of clinical trials using LLLT after implant placement.

Keywords: laser therapy; low-level; dental implants; osseointegration; scanning electron microscopy; histology.

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The rationale for the use of low-level laser therapy (LLLT) relies on its ability to exert, at the cellular level, biomodulatory effects on the molecular and biochemical processes that take place during intrinsic tissue repair.[1–10] Several in vivo and in vitro studies have suggested positive effects of LLLT on the tissue repair process, both in animal models and in culture media.[11–20] These therapeutic effects include the following: increased epithelial and fibroblast proliferation and enhanced collagen synthesis, thus speeding the process of repair; increased potential for bone remodelling and repair; restoration of nerve function after injury; normalization of hormonal function; immune regulation; reduced inflammation and oedema; modulation and relief of pain; and improved postoperative analgesia.[1–9] Even though dose is one of the most important parameters of laser therapy,[21] the data available are not sufficient to support the design of clinical studies.[11–20,22,23]

Preclinical studies have suggested that LLLT has beneficial effects on bone repair.[6,10,11] Regarding peri-implant bone healing after titanium implant placement,[12–15,24] previously published studies have shown more evident bone maturation[12,13,24] and increased bone–implant contact (BIC)[16] in LLLT-irradiated bone than in control groups. The main findings reported in the literature are summarized in Table 1.

The objective of this study was to assess the local effects of LLLT on the peri-implant healing process after implant placement in the rabbit mandible, immediately after mandibular incisor extraction, based on resonance frequency analysis (RFA), BIC, and bone neoformation area (BA) within implant threads, measured using scanning electron microscopy (SEM) and stereological analysis.

Materials and methods

Animals

The study sample comprised 32 male New Zealand rabbits (*Oryctolagus cuniculus*), weighing 3–4 kg and aged 3 months. The animals were allocated randomly to one of four different groups, with eight in each: three experimental groups treated with LLLT at different energy densities (5 J/cm^2, 10 J/cm^2, and 20 J/cm^2) and one non-irradiated control group. All animals received a solid diet and water ad libitum throughout the experiment and were housed under normal lighting, humidity, and temperature conditions in a climate-controlled environment. All animals underwent extraction of the mandibular left incisor followed by immediate placement of a dental titanium implant in the fresh socket.

Surgical protocol

Animals were anesthetized by intramuscular injection of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (3 mg/kg). The area around the mandibular left incisor was prepared with 2% chlorhexidine digluconate and local infiltration of 0.5 ml lidocaine hydrochloride 2% with epinephrine 1:100,000. The mandibular left incisor was extracted with the aid of #5 paediatric extraction forceps. The fresh extraction socket was then drilled gradually, and a dental implant (3.25 mm diameter × 11.5 mm, Nano-Tite; BIOMET 3i, Florida, USA) placed in accordance with the manufacturer's instructions. Implant stability was measured using RFA, followed by placement of a cover screw. The socket was sutured with 4–0 nylon monofilament. While the animal was still under anaesthesia, the site of laser irradiation was shaved and the long axis of the implant marked on the skin with a surgical marker. At the end of the procedure, animals received analgesia and antimicrobial prophylaxis (Fig. 1). Perioperative procedures were performed by a veterinary physician. The authors performed the surgeries and LLLT procedures.

LLLT irradiation

Spot laser irradiation was performed using a gallium–aluminium–arsenide (GaAlAs) active medium infrared diode laser (wavelength 830 nm, power 50 mW), in continuous emission mode (Thera Lase; DMC Equipamentos, São Carlos, SP, Brazil), applied every 48 h over a 13-day intervention period for a total of seven applications. The first session was started immediately after surgery.

Energy density varied among the groups. The laser was applied holding the handpiece perpendicular to the basal bone of the mandible. Animals in the 5 J/cm^2 experimental group received two spot doses of 2.5 J/cm^2 per session, one point medial and one lateral to the long axis of the implant, as marked previously on the overlying skin, for a total dose of 5 J/cm^2 per session (index dose). Animals in the 10 J/cm^2 group received twice the index dose (5 J/cm^2 per point, for a total 10 J/cm^2 per session), and those in the 20 J/cm^2 group received four times the index dose (10 J/cm^2 per point, for a total 20 J/cm^2 per session).

Non-irradiated animals (control group) underwent sham irradiation, i.e., all the procedures performed in the experimental groups were also performed in the control group, but with the laser device unpowered (Table 2).

Death

On day 45 of the experiment (30 days after the last LLLT session), the animals were sedated (same protocol used for the surgical procedure) and killed with an overdose of 1% propofol (1 ml/kg) and 10%

Table 1. LLLT protocols described in previous studies evaluating peri-implant effects.

Author	Year	Type of light	Animal model	*n*	Wavelength (nm)	Power (mW)	Total dose (J/cm^2)	No. of sessions
Dörtbudak et al.[17]	2002	Red	Monkey	5	690	100	30	5
Pinheiro et al.[10]	2003	Infrared	Rabbit	14	830	10	602	7
Khadra et al.[24]	2004	Infrared	Rabbit	12	830	150	270	10
Lopes et al.[13]	2005	Infrared	Rabbit	14	830	10	602	7
Jakse et al.[12]	2007	Red	Rabbit	12	680	75	12	3
Kim et al.[22]	2007	Infrared	Mouse	20	830	96	40.32	7
Lopes et al.[14]	2007	Infrared	Rabbit	14	830	10	602	7
Pereira et al.[26]	2009	Infrared	Rabbit	12	780	70	367.5	7
Campanha et al.[11]	2010	Infrared	Rabbit	30	830	10	602	7
Maluf et al.[15]	2010	Infrared	Mouse	24	795	120	48	6

LLLT, low-level laser therapy.

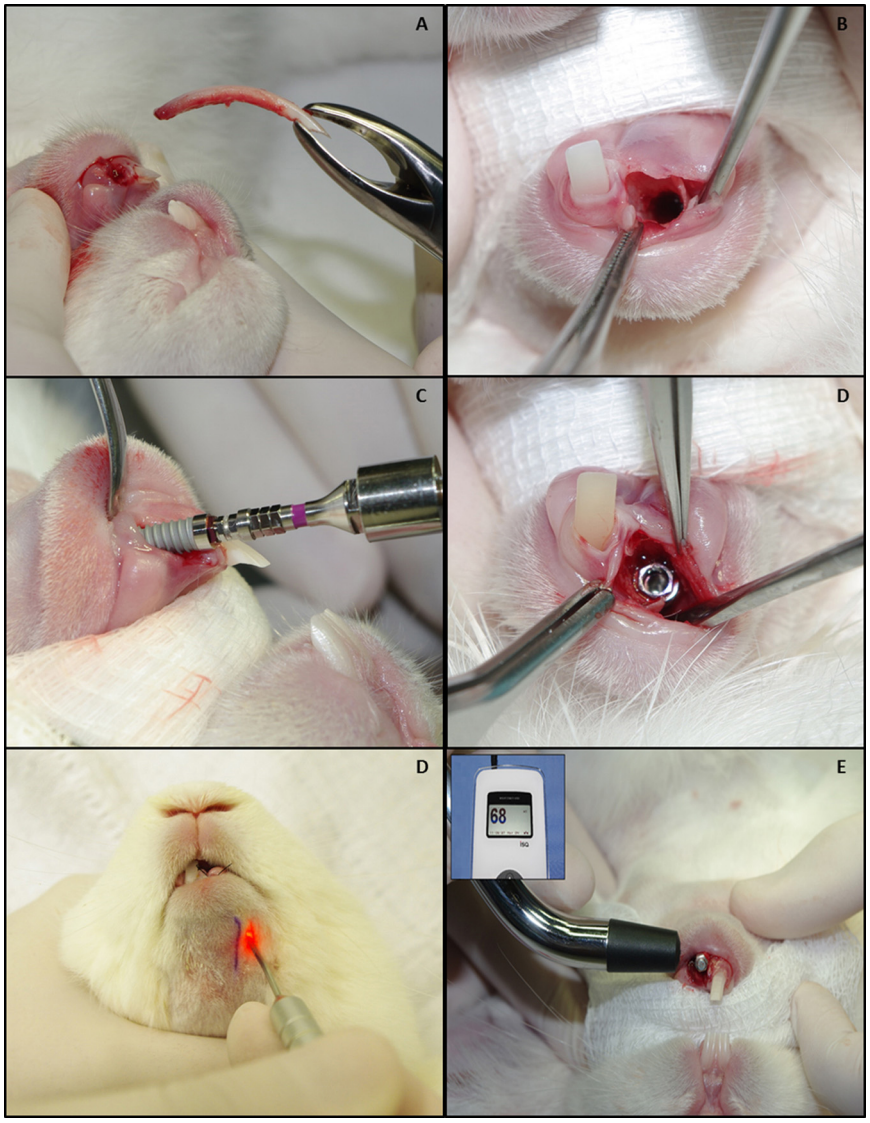

Fig. 1. Experimental surgery, low-level laser therapy (LLLT), and resonance frequency analysis (RFA) procedures. (A) Extraction of the mandibular left incisor. (B) Surgical aspect of the intact socket walls. (C) Implant insertion. (D) Operative view after complete implant insertion. (E) LLLT hand-piece tip held medial and lateral to the long axis of the implant during application. (F) RFA (inset) just after implant placement.

potassium chloride (1 ml/kg) injection. Final implant stability measurements were obtained by RFA. The mandibular halves containing the implants were removed by dissection and fixed in 10% neutral buffered formalin.

Table 2. LLLT parameters.

Parameter/group	Control	5 J/cm^2	10 J/cm^2	20 J/cm^2
Average power (mW)	–	50	50	50
Wavelength (nm)	–	830	830	830
Pulse parameters	–	CW	CW	CW
Energy per point (J/cm^2)	0	2.5	5	10
Energy density (J/cm^2)	0	5	10	20
Irradiation time per point (s)	0	51	101	201
Total dose (J/cm^2)	0	35	70	140

CW, continuous wave; LLLT, low-level laser therapy.

Stability measurement

Implant stability was measured using RFA and an Osstell device (Osstell AB, Göteborg, Sweden). The operator held the tip of the hand-held probe perpendicular to a SmartPeg attached to the implant. The device was recalibrated after each measurement. Implant stability was assessed at the time of implant placement (time point 1) and 30 days after the last LLLT session (time point 2), thus providing pre- and post-LLLT implant stability quotient (ISQ) values. ISQ is determined on an ordinal scale of 1–100 units based on the resonance frequency read by the device. The mean of four ISQ measurements (mesial, distal, buccal, and lingual aspects) was used in the analysis.

Scanning electron microscopy (SEM)

Two random samples from each group were dehydrated in a graded ethanol series

(50%, 60%, 70%, 80%, and 90%), embedded in heat- and chemically-activated resin, and sliced along the sagittal plane (long axis of the implant) using an annular saw. The resulting specimens were then sanded and buffed to enhance the surface for examination. Images were obtained using a Philips electron microscope (XL-30 FEG EDX; Philips, Eindhoven, the Netherlands) at 250× magnification from three distinct areas of the implant (apical, middle, and cervical thirds).

Stereology

The six remaining samples from each group were dehydrated in a graded alcohol series (70%, 80%, 90%, and 100%), followed by progressive infiltration with heat-cure resin for thin sectioning. Sections were obtained along the long axis of the implant (sagittal plane of the bone specimen) with a precision microtome set to a thickness of 30 μm. The sections were buffed and stained using toluidine blue. Images of three distinct areas of the implant (apical, middle, and cervical thirds) were captured by a light microscope coupled to a digital camera.

BIC and BA analysis

UTHSCA Image Tool 3.0 software (University of Texas Health Science Center at San Antonio, Texas, USA) was used to assess (1) BIC, expressed as the ratio of bone–implant contact to total linear

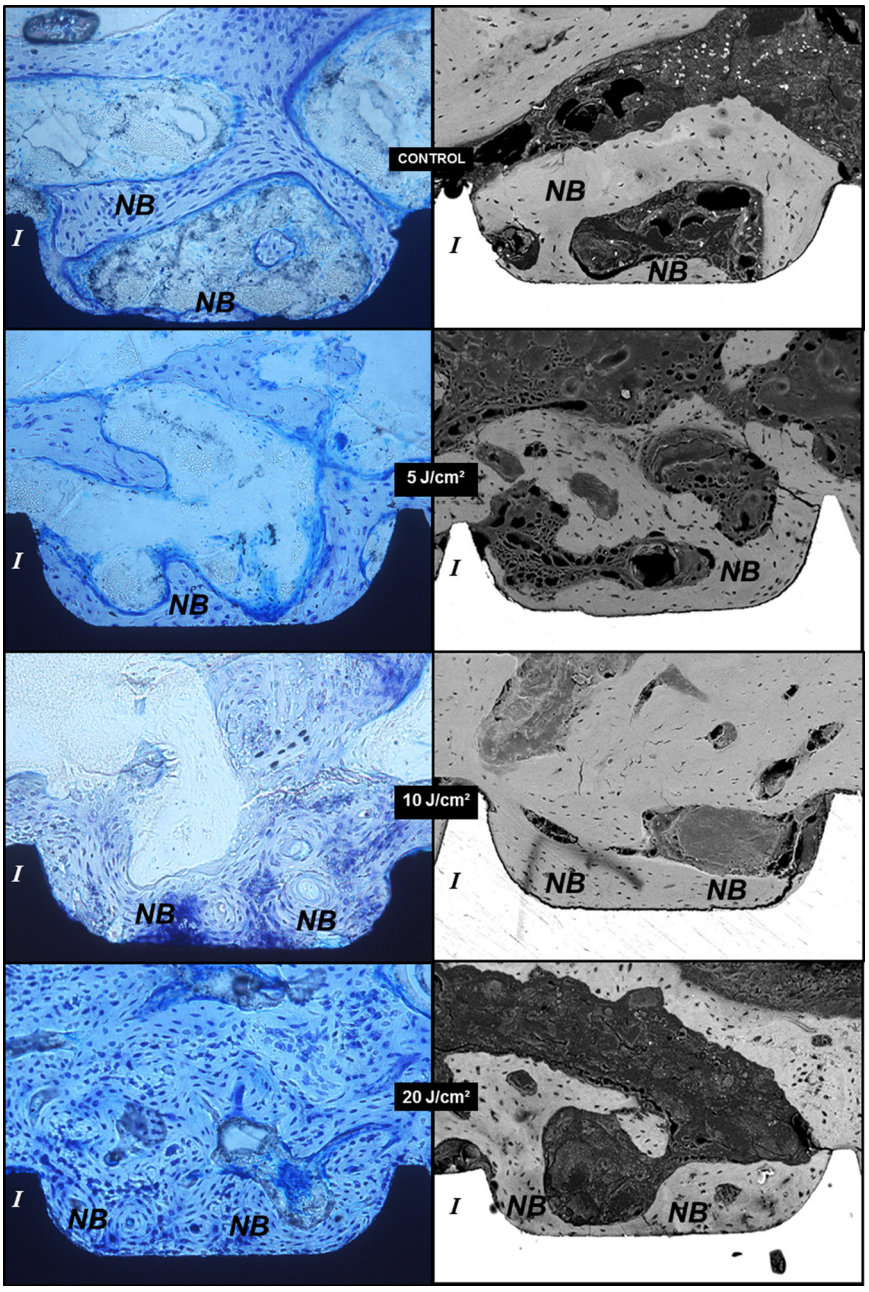

Fig. 2. Representative specimens of rabbit mandibles from the four study groups. *C* = 0 J/cm^2; 5 J/cm^2; 10 J/cm^2; 20 J/cm^2. Left: light microscopy images (250× magnification, 10% toluidine blue stain) showing the bone–implant interface. Right: SEM images (250× magnification) showing the bone–implant interface. *I* indicates the titanium implant surface between threads. *NB* indicates bone neoformation, most evident in the 20 J/cm^2 LLLT group.

Table 3. Mean (SD) values for BIC and BA after LLLT.

Analysis	Energy density	BIC (μm)	BA (μm^2)
SEM	Control	807.8^{b,c} (174.9)	91,599.7^{a} (45,770.3)
	5 J/cm^2	761.5^{c} (56.1)	129,465.5^{a,b} (33,028.8)
	10 J/cm^2	977.9^{a,b} (67.4)	96,763.7^{a,b} (33,754.3)
	20 J/cm^2	1021.1^{a} (110.6)	122,573.7^{b} (57,385.9)
Stereology	Control	757.9^{c} (148.3)	63,740.3^{b} (36,828.3)
	5 J/cm^2	884.7^{b,c} (169.6)	87,428.3^{a,b} (44,328.5)
	10 J/cm^2	902.4^{b} (131.4)	100,068.4^{a} (35,443.3)
	20 J/cm^2	1045.3^{a} (162.7)	103,934.5^{a} (40,229.7)

BA, bone neoformation area; BIC, bone–implant contact; LLLT, low-level laser therapy; SD, standard deviation; SEM, scanning electron microscopy.
^{a,b,c}Different letters indicate significant differences by ANOVA, followed by Tukey's multiple comparison test, at the 5% significance level.

surface area with potential for contact and (2) BA, expressed as the ratio of the area of newly formed bone within the implant threads to total area of possible bone formation. Both variables were measured at each third of the implant (threads 1, 5, and 9), and the mean for each of the three regions was calculated.

Statistical analysis

The Shapiro–Wilk test confirmed the normality of data distribution. Analysis of variance (ANOVA) and Tukey's post hoc test, at a significance level of 5%, were used to evaluate differences in SEM and stereological variables. ISQ values were calculated using generalized estimating equations (GEE), at the same significance level.

Results

All rabbits survived the surgery and other experimental procedures. No implants were lost or showed physical signs of failure, infection, or inflammatory reaction during the experimental period. Figure 2 shows SEM and light microscopy images. Table 3 summarizes BIC and BA measurements.

On SEM analysis, BIC values were significantly higher in the 20 J/cm^2 group (1021.1 μm, $P = 0.018$) and in the 10 J/cm^2 group (977.9 μm, $P = 0.016$) than in the control group. There were no differences between the 5 J/cm^2 group (761.5 μm) and the control group (807.8 μm, $P = 0.072$). Regarding BA, significantly greater values were found for the 20 J/cm^2 group vs. the control group (122,573.7 vs. 91,599.7 μm^2; $P = 0.018$). There were no significant differences among the other groups.

The stereological analysis showed significantly higher BIC values for the 20 J/cm^2 group (1045.3 μm, $P = 0.000$) as compared to the control group (757.9 μm). The 10 J/cm^2 (902.4 μm, $P = 0.013$) and 5 J/cm^2 groups (884.7 μm, $P = 0.034$) also had significantly higher BIC values than the control group (757.9 μm) (Fig. 3). Regarding BA, the 20 J/cm^2 group (103,934.5 μm^2) and the 10 J/cm^2 group (100,068.4 μm^2) were statistically similar ($P = 0.991$), but presented significantly higher results than the control group (63,740.3 μm^2) ($P = 0.016$ and $P = 0.036$, respectively).

ISQ increased from baseline to death (Table 4 and Fig. 4). A significant increase was observed in the 20 J/cm^2 group (56.26 to 68.81 ISQ) compared to the control group (56.34 to 61.43 ISQ) ($P = 0.003$).

Discussion

Despite its potentially positive effects, LLLT still lacks clearly defined dosage protocols for different types of treatment. Studies describing the clinical use of LLLT in several fields and for a variety of clinical applications have grown in number, despite the low-quality evidence supporting its use. These observations encourage and justify the performance of basic and clinical research into the potential applications of LLLT using different

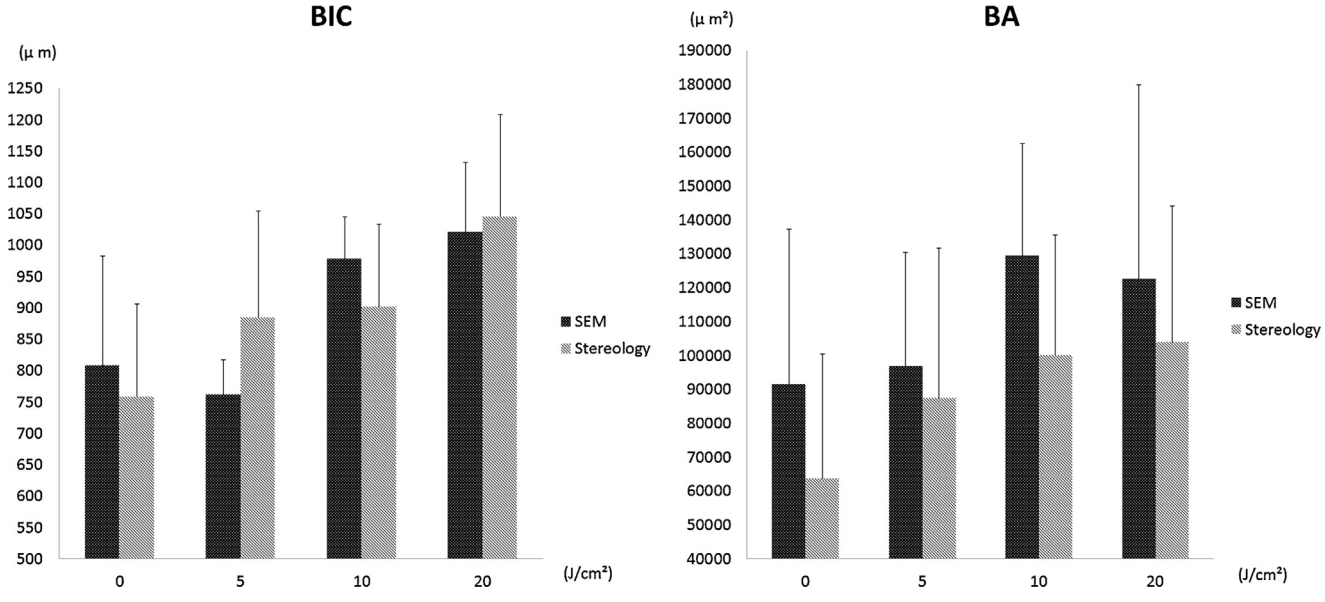

Fig. 3. Mean results obtained for BIC (μm) and BA (μm^2) on SEM and stereological analysis of rabbit mandibles from the four study groups. BIC values were greater in the 20 J/cm^2 group with both methods of evaluation. BA values were similar across groups according to SEM results; for the stereological analysis, mean BA values were higher in the experimental groups, with the best results for the 20 J/cm^2 group.

Table 4. Mean (SD) values for ISQ.

Energy density	Time	
	Baseline	30 days after LLLT
Control	56.34[a] (1.26)	61.43[b] (1.30)
5 J/cm^2	56.46[a] (1.41)	63.84[a,b] (1.63)
10 J/cm^2	57.21[a] (0.87)	64.21[a,b] (1.26)
20 J/cm^2	56.26[a] (1.40)	68.81[a] (0.39)

ISQ, implant stability quotient; LLLT, low-level laser therapy; SD, standard deviation.
[a,b]Different letters indicate significant differences by ANOVA, followed by Tukey's multiple comparison test, at the 5% significance level.

models and power settings.[13,15,25–27] One potential use of particular interest is the enhancement of peri-implant tissue repair.[11–15,19,24–27] However, the different protocols currently used for LLLT hinder interpretation of the effects of laser on the osseointegration process and comparison of results across different studies, and this indicates the need for better-defined protocols to enable more reliable comparisons.

To assess the effects of LLLT in a routine clinical situation, the mandibular left incisor was extracted and a dental implant placed in the fresh socket in all animals in the control and experimental groups, using the same surgical technique. As in other studies,[11,12,14,15,18,19,28] the rabbit model was employed due to its ease of handling and more particularly to the size of the fresh mandibular incisor socket, which is suitable for placement of conventional, off-the-shelf implants. We chose the mandible as the site of study rather than the tibia or other bones in order to better mimic a potential clinical situation.[20,29]

In agreement with recent studies, we employed the GaAlAs infrared laser (λ = 830 nm)[11–15,17–19,22] due to its greater tissue penetration capacity as compared to those with other wavelengths.[11–13,15–17,22] Infrared lasers can penetrate deeper into the subcutaneous tissues due to poor absorption by water and skin pigments.[3] The laser power was set at 50 mW, and the total dose was divided across two non-overlapping application sites, with the laser hand-piece tip held close to the implant site (thus preventing reflection of the laser energy irradiated into the tissues); this was performed every 48 h for seven LLLT sessions.[11,12,14,15] Each experimental group was exposed to a different dose: 5 J/cm^2, 10 J/cm^2, or 20 J/cm^2.

Comparison of ISQ values obtained by RFA showed a significant improvement in stability in the 20 J/cm^2 group. These results corroborated the SEM and stereological data, which showed that the group exposed to the highest LLLT dose per session (20 J/cm^2 group) achieved the highest BIC values within 45 days of implant placement. BA values were also significantly higher in the experimental groups than in the control group. Previous animal studies[11,15] have already described the positive effects of LLLT on peri-implant bone healing. Maluf et al.[15] reported better attachment between bone and implant. Campanha et al.[11] also described improved stability, even in implants without initial stability. Finally, Pereira et al.[26] found improvement of BIC on histological analysis using a similar model.

The LLLT doses used in this study were lower than those reported in previous animal model studies available in the literature. In this regard, the reader should bear in mind that bone formation in rabbits is different from humans, and that the clinical use of LLLT needs to be supported by clinical trials. Nevertheless, the definition of energy parameters to be used in clinical trials could be based on findings from animal studies. Taking into consideration that the lowest possible dose of radiation producing the desired effect should be indicated, we suggest that further preclinical and clinical studies of the effects of LLLT on peri-implant bone repair include lower doses (such as the ones used here) in their methods.

In conclusion, the use of the LLLT protocol described herein improved peri-implant bone repair and implant stability, as evidenced by significant increases in ISQ, BIC, and BA values within implant threads, particularly at a dose of 20 J/cm^2 per session. Our findings support the design of clinical trials using LLLT after implant placement and may be considered as suggested parameters for its use.

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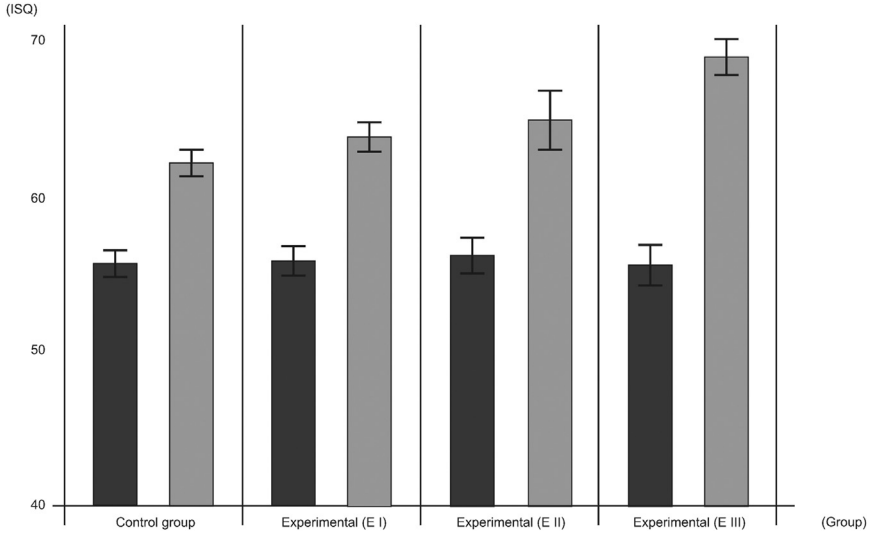

Fig. 4. Mean ISQ measurements of implants inserted in rabbit mandibles at baseline (black) and 30 days after LLLT (grey). ISQ values improved after LLLT, with significantly higher stability in the 20 J/cm^2 group.

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Competing interests

None.

Ethical approval

This study was approved by the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) Animal Experimentation Ethics Committee with protocol number 11/00235, and by the Universidade Federal do Rio Grande do Sul (GPPG/HCPA) Experimentation Ethics Committee with protocol number 12-0112. All study procedures were conducted in compliance with the Brazilian Ethical Principles for Animal Experimentation, as set forth in Law 11794 of 8 October 2008 (the Arouca Act), and the Ethical Principles of Experimental Research described by the Brazilian Society for Laboratory Animal Science (formerly the Brazilian College of Animal Testing). All efforts were made to minimize animal suffering throughout the experiments, as well as to use only the number of animals that was essential to produce reliable scientific data.

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References

1. Takeda Y. Irradiation effect of low-energy laser on alveolar bone after tooth extraction. Experimental study in rats. *Int J Oral Maxillofac Surg* 1988;**17**:388–91.
2. Manfredi M, Merigo E, Guidotti R, Meleti M, Vescovi P. Bisphosphonate-related osteonecrosis of the jaws: a case series of 25 patients affected by osteoporosis. *Int J Oral Maxillofac Surg* 2011;**40**:277–84.
3. Agha-Hosseini F, Moslemi E, Mirzaii-Dizgah I. Comparative evaluation of low-level laser and CO_2 laser in treatment of patients with oral lichen planus. *Int J Oral Maxillofac Surg* 2012;**41**:1265–9.
4. Silva Júnior AN, Pinheiro AL, Oliveira MG, Weismann R, Ramalho LM, Nicolau RA. Computerized morphometric assessment of the effect of low-level laser therapy on bone repair: an experimental animal study. *J Clin Laser Med Surg* 2002;**20**:83–7.
5. do Nascimento PM, Pinheiro AL, Salgado MA, Ramalho LM. A preliminary report on the effect of laser therapy on the healing of cutaneous surgical wounds as a consequence of an inversely proportional relationship between wavelength and intensity: histological study in rats. *Photomed Laser Surg* 2004;**22**:513–8.
6. Weber JB, Pinheiro AL, de Oliveira MG, Oliveira FA, Ramalho LM. Laser therapy improves healing of bone defects submitted to autologous bone graft. *Photomed Laser Surg* 2006;**24**:38–44.
7. Mohammed IF, Al-Mustawfi N, Kaka LN. Promotion of regenerative processes in injured peripheral nerve induced by low-level laser therapy. *Photomed Laser Surg* 2007;**25**:107–11.
8. Freddo AL, Rodrigo SM, Massotti FP, Etges A, de Oliveira MG. Effect of low-level laser therapy after implantation of poly-L-lactic/polyglycolic acid in the femurs of rats. *Lasers Med Sci* 2009;**24**:721–8.
9. Karu T. Photobiology of low-power laser effects. *Health Phys* 1989;**56**:691–704.
10. Pinheiro AL, Lopes CB, Sathaiah S, Duarte J. Laser biomodulation in bone implants: a Raman spectral study. *Int Congr Series* 2003;**1248**:449–51.
11. Campanha BP, Gallina C, Geremia T, Loro RC, Valiati R, Hübler R, de Oliveira MG. Low-level laser therapy for implants without initial stability. *Photomed Laser Surg* 2010; **28**:365–9.
12. Jakse N, Payer M, Tangl S, Berghold A, Kirmeier R, Lorenzoni M. Influence of low-level laser treatment on bone regeneration and osseointegration of dental implants following sinus augmentation. An experimental study on sheep. *Clin Oral Implants Res* 2007;**18**:517–24.
13. Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Martins MM. Infrared laser light reduces loading time of dental implants: a Raman spectroscopic study. *Photomed Laser Surg* 2005;**23**:27–31.
14. Lopes CB, Pinheiro AL, Sathaiah S, Da Silva NS, Salgado MA. Infrared laser photobiomodulation (lambda 830 nm) on bone tissue around dental implants: a Raman spectroscopy and scanning electronic microscopy study in rabbits. *Photomed Laser Surg* 2007;**25**:96–101.
15. Maluf AP, Maluf RP, Brito CR, França FM, de Brito Jr RB. Mechanical evaluation of the influence of low-level laser therapy in secondary stability of implants in mice shinbones. *Lasers Med Sci* 2010;**25**:693–8.
16. Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scand J Plast Reconstr Surg* 1969;**3**:81–100.
17. Dörtbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implant sites. *Clin Oral Implants Res* 2002;**13**:288–92.
18. Petri AD, Teixeira LN, Crippa GE, Beloti MM, de Oliveira PT, Rosa AL. Effects of low-level laser therapy on human osteoblastic cells grown on titanium. *Braz Dent J* 2010;**21**:491–8.
19. Pinheiro AL. Advances and perspectives on tissue repair and healing. *Photomed Laser Surg* 2009;**27**:833–6.
20. de Jong WC, Korfage JA, Langenbach GE. Variations in habitual bone strains in vivo: long bone versus mandible. *J Struct Biol* 2010;**172**:311–8.
21. Otulakowska J, Nicholson JW. Scanning electron microscopy and energy dispersive X-ray study of a recovered dental implant. *J Mater Sci Mater Med* 2006;**17**:277–9.
22. Kim YD, Kim SS, Hwang DS, Kim SG, Kwon YH, Shin SH, Kim UK, Kim JR, Chung IK. Effect of low-level laser treatment after installation of dental titanium implant—immunohistochemical study of vascular endothelial growth factor: an experimental study in rats. *Laser Phys Lett* 2007;**4**:681–5.
23. Sennerby L, Meredith N. Implant stability measurements using resonance frequency analysis: biological and biomechanical aspects and clinical implications. *Periodontology 2000* 2008;**47**:51–66.
24. Khadra M, Ronold HJ, Lyngstadaas SP, Ellingsen JE, Haanaes HR. Low-level laser therapy stimulates bone–implant interaction: an experimental study in rabbits. *Clin Oral Implants Res* 2004;**15**:325–32.
25. Khadra M. The effect of low level laser irradiation on implant–tissue interaction. In vivo and in vitro studies. *Swed Dent J Suppl* 2005;**172**:1–63.
26. Pereira CL, Sallum EA, Nociti Jr FH, Moreira RW. The effect of low-intensity laser therapy on bone healing around titanium implants: a histometric study in rabbits. *Int J Oral Maxillofac Implants* 2009;**24**:47–51.
27. Carlsson L, Röstlund T, Albrektsson B, Albrektsson T, Brånemark PI. Osseointegration of titanium implants. *Acta Orthop Scand* 1986;**57**:285–9.
28. Garg AK. Osstell Mentor: measuring dental implant stability at placement, before loading, and after loading. *Dent Implantol Update* 2007;**18**:49–53.
29. Ahmad OK, Kelly JR. Assessment of the primary stability of dental implants in artificial bone using resonance frequency and percussion analyses. *Int J Oral Maxillofac Implants* 2013;**28**:89–95.

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