Histologic and Resonance Frequency Analysis of Peri-Implant Bone Healing After Low-Level Laser Therapy: An In Vivo Study

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Purpose: *To evaluate the effects of low-level laser therapy (LLLT) on peri-implant bone regeneration by means of resonance frequency analysis and histologic analysis of bone-to-implant contact (BIC).* ***Materials and Methods:*** *Thirty-two male New Zealand rabbits were randomly divided into four groups of eight animals each, one control group (nonirradiated animals) and three experimental groups that received LLLT (group E5 = 5 J per session; group E10 = 10 J per session; group E20 = 20 J per session). The mandibular left incisor was surgically extracted in all animals, and a nanoparticle–treated-surface osseointegrated implant was placed immediately afterward. The experimental groups were irradiated with aluminum-gallium-arsenide laser diode every 48 hours over a 13-day period for a total of seven sessions. Implant stability quotients (ISQs) were measured at the time of implant placement and 30 days after the last LLLT session. The animals were then euthanized and dissected, and histologic slides of the implant region were obtained for BIC evaluation.* ***Results:*** *Significant differences in ISQ were detected between groups before and after LLLT, with group E20 showing significantly higher values than controls. The percentage of BIC was also significantly higher in group E20 than in control animals.* ***Conclusion:*** *Laser therapy at a dose of 20 J per treatment session, based on the irradiation protocol used in this study, was able to significantly increase ISQ values and BIC after implant placement, indicating that laser irradiation effected an improvement in peri-implant bone healing.* Int J Oral Maxillofac Implants 2015;30:1028–1035. doi: 10.11607/jomi.3882

Key words: *dental implants, laser therapy, osseointegration*

The clinical use of low-level laser therapy (LLLT) is based on its ability to promote biomodulatory effects on the biochemical processes that occur during tissue repair, leading to increased epithelial and fibroblast proliferation and increased collagen synthesis, which can accelerate the healing process.[1,2] Its effects also include normalization of hormone function, restoration of nerve function after injury, regulation of the immune system, modulation and attenuation of pain, reduction of inflammation and edema, postoperative pain management, and an increased potential for bone repair and remodeling.[3–9]

In dentistry, LLLT has proven to be effective in accelerating the healing process, even with the use of different wavelengths.[3,10,11] Given the successful application of nonablative lasers to accelerate new bone formation, some authors began to use this treatment modality to accelerate peri-implant bone healing[12–16] in efforts to reduce healing time before definitive prosthesis placement. The positive results obtained have provided a means to accelerate osseointegration, and LLLT has therefore become an adjuvant therapy in cases of rehabilitation involving implant-supported prostheses.[12–15,17–21]

In the preclinical setting, findings indicate a positive effect of LLLT on bone repair[1,7,22] and osseointegration.[12–14,17,18,23] In vivo studies of peri-implant bone

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healing have shown greater bone maturation[12,13,18,23] and higher bone-to-implant contact (BIC) percentages[24] in irradiated than in nonirradiated bone. However, despite the reported beneficial effects of LLLT on the repair process in animal models and tissue cultures,[12–14,16–19,21,22,24,25] a definitive protocol for its application in different clinical situations, particularly with regard to total energy to be used per session, remains controversial.[2,12–14,16–19,21,22,25,26]

According to the literature, the success of osseointegrated implants may be associated with their short-, medium-, and long-term stability.[27] Objective measurement of implant stability has, therefore, a direct impact on implant rehabilitation, since these data may be used for comparison at different stages of implant treatment. In this sense, resonance frequency analysis (RFA) has become a valuable tool for assessment of implant stability because it is a noninvasive technique that provides reproducible objective measurements of micromobility, which can be used at different stages of implant treatment without affecting the integrity of osseointegration.

The Osstell ISQ (Osstell AB) is a tool that uses RFA to measure implant stability. The resonance frequency of a small transducer (SmartPeg, Osstell AB) attached to the implant is measured and converted into an implant stability quotient (ISQ), which represents a linear mapping of resonance frequencies measured in kilohertz. Values are displayed on a scale of 1 to 100 ISQ, and higher ISQs suggest greater stability of the inserted implant.[28] This technology is able to provide repeated implant stability measurements during the stages of surgical placement, osseointegration, and/or loading of implants, making it possible to detect any increase or decrease in implant stability.[29]

Currently, histology is the gold-standard research tool for evaluating the efficacy of LLLT, thereby providing a benchmark against which the performance of RFA measurements on a particular bone implant can be assessed. The present study was therefore designed to evaluate the local effects of three different doses of LLLT on peri-implant bone regeneration by means of RFA measurements and histologic analysis of BIC around implants.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul (protocol no. 11/00235). Animal handling and experimentation followed the Brazilian Ethical Principles of Animal Experimentation and international standards and guidelines for the care and use of laboratory animals. All efforts were made to minimize pain and discomfort, as well as to use only the minimum number of animals required to produce reliable scientific data.

Animals

Thirty-two 3-month-old male New Zealand rabbits (*Oryctolagus cuniculus*) weighing 3 to 4 kg each were used in the study. The rabbits were numbered sequentially and randomly divided by drawing lots into four groups of eight animals each: *(1)* control: sham treatment; *(2)* E5: LLLT at a dose of 5 J per treatment session; *(3)* E10: LLLT at a dose of 10 J per session; and *(4)* E20: LLLT at a dose of 20 J per session. The animals were housed under standard conditions of temperature, humidity, and light intensity and allowed free access to solid chow (Purina, Nestlé Purina Petcare) and water.

Surgical Procedure

All rabbits underwent surgical extraction of the mandibular left incisor, followed by immediate placement of an osseointegrated implant, and this served as the baseline condition for each animal.

The rabbits were anesthetized with intramuscular ketamine (Dopalen, Vetbrands Saúde Animal; 40 mg/kg body weight) and xylazine (Anasedan, Vetbrands Saúde Animal; 3 mg/kg body weight). Before surgery, the area around the mandibular left incisor was cleaned with chlorhexidine gluconate 2% (FGM Produtos Odontológicos), and 0.5 mL of lidocaine 2% with epinephrine (1:100,000) was infiltrated to effect local vasoconstriction. The incisor was then extracted with a no. 5 pediatric extraction forceps (Edlo S/A). An implant socket was prepared using sequentially sized drills under continuous saline irrigation according to the manufacturer's instructions, and a nanoparticle-treated-surface implant (3.25 × 11.5 mm, NanoTite, BIOMET 3i) was placed. The implant insertion torque meter was set at 20 N and insertion was performed using a 20:1 contra-angle handpiece (KaVo Co) to standardize primary stability in all implants (Figs 1a and 1b). A transducer was then attached to the implant with a torque of approximately 15 N, and ISQs were obtained to assess primary stability in all implants after insertion. The transducer was removed, a cover screw was placed, and the surgical site was closed with a 4-0 monofilament nylon suture (Ethicon, Johnson & Johnson). The long axis of the implant was marked on the skin to guide later laser irradiation (Fig 1c). Tramadol (União Química) was administered intramuscularly (5 mg/kg body weight) immediately and 24 hours after surgery for analgesia. Enrofloxacin (Agener União) was administered intramuscularly (5 mg/kg body weight) once daily for 3 days for antibiotic prophylaxis.

Figs 1a to 1e *Experimental procedures.*

Fig 1a After extraction of the mandibular left incisor, an osseointegrated implant (3.25 × 11.5 mm) was inserted in the socket with contra-angle (20:1) reduction, at 15 rpm and torque of 20 N.

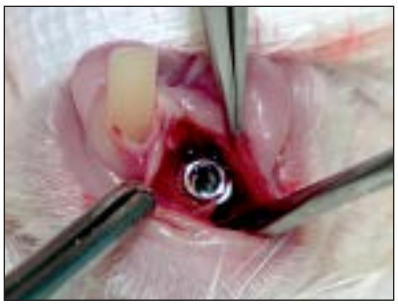


Fig 1b Occlusal view after implant placement and its correct positioning against other alveolar bone walls.

Fig 1c The long axis of the implant was marked on the skin to guide LLLT.

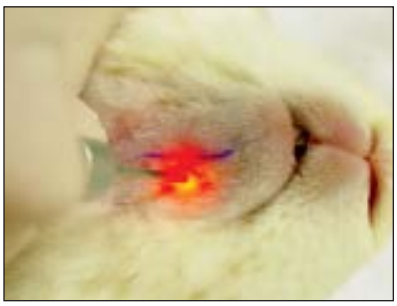


Fig 1d LLLT was performed every 48 hours after surgery (seven sessions). Irradiation was applied in two spots, one medial and one lateral to the long axis of the implant.

Fig 1e Measurements were performed with the Osstell ISQ device at four different surfaces (mesial, distal, buccal, and lingual) of the SmartPeg before and after LLLT. *(left)* Image at the moment the ISQ value is generated and shown on the display.

All surgical procedures, RFA measurements, and LLLT applications were performed by a single experienced surgeon previously trained in all aspects of assessment and procedures during a pilot study.

Laser Irradiation

Laser irradiation was performed with an aluminum-gallium-arsenide diode laser at a wavelength of 830 nm (infrared), 50 mW output power, spot area of 0.0028 cm^2, and in continuous wave mode for spot irradiation (Thera Lase, DMC Equipamentos). Animals in all three experimental groups received LLLT every 48 hours for 13 days, for a total of seven treatment sessions. Control animals underwent sham irradiation following the same protocol used for irrad iated animals, but with the laser device left unpowered. The laser parameters used in this study are described in Table 1. The LLLT therapy started immediately after implant placement and closure of the surgical site.

The total energy per session was divided into two spots, one medial and one lateral to the long axis of the implant, as marked on the overlying skin (Fig 1d). The laser probe was held perpendicular to the long axis of the implant (without overlying the implants, to prevent reflection of radiation on the implant surface) and irradiation was performed in contact with the soft tissue without pressure. Animals in group E5 received two spot doses of 2.5 J, totaling 5 J per session, over an irradiation time of 51 seconds. Animals in group E10 received double the dose administered to group E5 (5 J per spot), for a total dose of 10 J per session, over an irradiation time of 1 minute 41 seconds. Group E20 received quadruple the initial dose: 10 J per spot, for a total dose of 20 J per session, over an irradiation time of 3 minutes 21 seconds. Irradiation time was automatically adjusted by the laser unit after all other parameters were set. The same irradiation protocol was applied to all three experimental groups, except for total energy parameters (Table 1).

Thirty days after the last LLLT session, the rabbits were anesthetized (following the same protocol used for the first surgery), and an additional procedure was performed to access the implants. A linear incision was made on the skin overlying the border of the implant site, the mucoperiosteal flap was detached, the cover screw was exposed and removed, and a new transducer was attached to the implant with a torque of approximately 15 N for a second round of RFA measurements.

Table 1 Parameters Used for LLLT

	Control group	Experimental groups		
	(n = 8)	E5 (n = 8)	E10 (n = 8)	E20 (n = 8)
Light source type	None	Laser	Laser	Laser
Average power (mW)	None	50	50	50
Wavelength (nm)	–	830	830	830
Pulse parameters	–	CW	CW	CW
No. of irradiation spots	0	2	2	2
No. of laser shots per spot	0	1	1	1
Energy per spot area (J)	0	2.5	5	10
Total energy per session (J)	0	5	10	20
Irradiation time per spot (s)	0	51	101	201

CW = continuous wave.

Fig 2 Histologic appearance of BIC in the central thread of the implant. Note the direct contact between bone and the implant surface.

Resonance Frequency Analysis

RFA was used to assess implant stability based on measurements performed with the Osstell ISQ device, which measures the resonance frequency of a small transducer (SmartPeg) attached to the implant. An ISQ is generated and shown on the display, reflecting the implant's stability. This was done immediately after implant placement (time point 1) and 30 days after the last LLLT session (time point 2). According to the literature, acceptable stability levels range from 55 to 85 ISQ, with an average level of 70 ISQ.[29–33] At time point 1, four ISQ measurements were obtained on the mesial, distal, buccal, and lingual surfaces of the transducer that was attached to the implant, with the tip of the handheld probe held perpendicular to the transducer (Fig 1e). The device was recalibrated after each measurement. The ISQ value for each rabbit was calculated as the average of these four measurements. The same protocol was followed for measurements at time point 2, thus providing pre- and post-LLLT ISQ values for each rabbit.

Histologic Analysis

Immediately after the second round of ISQ measurements, the rabbits were sedated following the same protocol used for the surgical procedure and killed with an overdose of propofol 1% (1 mL/kg body weight, B. Braun S.A. Laboratories) and potassium chloride 10% (1 mL/kg body weight, Isofarma Pharmaceutical Industrial Ltda) injected intravenously. The left half of the mandible was resected, and the bone portion containing the osseointegrated implant was processed to obtain 100-µm sections using a microtome (Diamond Band Saw, Diamond Cutting Band, 0.2 mm, D64, Exakt). The sections were polished to a thickness of 30 µm using a grinding and polishing system (Exakt) and stained with 10% toluidine blue. The slides were examined under a light microscope at ×300 magnification (Olympus BX51, Olympus Corporation) by two previously calibrated observers who were blinded to the treatment groups. The photomicrographs were histomorphometrically evaluated and compared using an image-analysis system (ImageTool for Windows, version 3.0, University of Texas Health Science Center at San Antonio). BIC linear values were calculated for the central thread of the implant (Fig 2) for each rabbit individually and for the group as a whole.

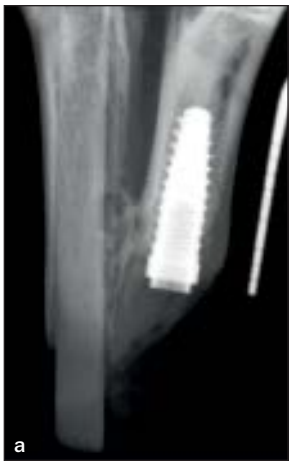
Statistical Analysis

Categorical variables were expressed as absolute frequencies and ISQs and BIC values were expressed as means (standard deviations). Pre- and post-LLLT ISQ values were compared between groups to determine whether laser irradiation had an effect on the osseointegration process. One-way analysis of variance (ANOVA) was used to calculate the difference between the final and initial ISQs for independent samples and to analyze the histomorphometric data (BIC values). Statistical analysis was performed using SPSS, version 17.0 (SPSS Inc). The level of significance was set at 5% ($P \leq .05$). A power analysis was performed using WinPepi (version 11.28, Pepi-for-Windows, Hebrew University), resulting in a power of 99% for this study design.

Table 2 ISQ Measurements and Histomorphometric Measurements of BIC

	ISQ*		ΔISQ	%BIC in the central
	Time 1	Time 2	(time 1 – time 2)	thread of the implant
Control				
01	55.25	62.5	7.25	72.62
02	61.5	53.5	–8	73.36
03	57	63	6	86.73
04	52	58.75	6.75	68.16
05	56	63.25	7.25	73.06
06	62.25	65.5	3.25	49.45
07	52.25	60	7.75	90.29
08	54.5	65	10.5	72.91
Group means†	56.12 ± 3.75	61.12 ± 4.05		73.32 ± 12.33
E5				
01	58.75	69.75	11	55.24
02	62	63.75	1.75	78.30
03	58.5	66	7.5	85.60
04	55	61.75	6.75	77.84
05	57.75	65.75	8	78.12
06	56	53	–3	87.71
07	47.25	64.5	17.25	69.36
08	56.5	66.25	9.75	94.03
Group means†	56.12 ± 4.25	63.37 ± 4.80		78.27 ± 11.95
E10				
01	61	65	4	77.90
02	59.5	63.5	4	73.07
03	57	59.75	2.75	74.07
04	59.25	68.75	9.5	60.14
05	54.75	67	12.25	74.03
06	53	68.5	15.5	73.68
07	56.25	62.75	6.5	74.49
08	57	58.5	1.5	78.15
Group means†	57.00 ± 2.67	63.75 ± 3.91		73.19 ± 5.61
E20				
01	63.25	70	6.75	82.24
02	49.5	67.75	18.25	92.41
03	53.75	70.5	16.75	96.45
04	59.75	68.75	9	98.13
05	58	68.5	10.5	96.33
06	56.5	69.5	13	87.66
07	56.25	66.75	10.5	95.67
08	53	68.75	15.75	94.33
Group means†	55.87 ± 4.29	68.25 ± 1.38‡		92.90 ± 5.38‡

*Values are the mean of four measurements (mesial, distal, buccal, and lingual surfaces).
†One-way analysis of variance (ANOVA). Values are expressed as means ± standard deviations.
‡Significantly different from controls ($P < .05$, ANOVA).



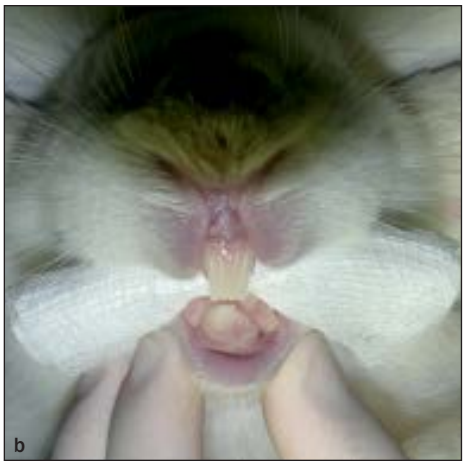


Fig 3a Occlusal radiograph immediately before death showing the implant in place along the axis of the mandibular symphysis. Note correct implant positioning and absence of radiolucent areas around the implant.

Fig 3b Clinical appearance 45 days after surgery. Features are normal, and there are no signs of inflammation in the peri-implant soft tissue.

RESULTS

The ISQs obtained at the time of implant placement and 30 days after the last LLLT session are shown in Table 2. All implants were considered osseointegrated by 30 days after LLLT, and all showed normal clinical and radiographic features and no signs of inflammation in the peri-implant soft tissue (Figs 3a and 3b). All groups showed very similar ISQs at the time of implant insertion (time point 1) and displayed an increase in ISQs after LLLT (Table 2). Group E20 implants had significantly higher ISQs than controls ($P = .004$).

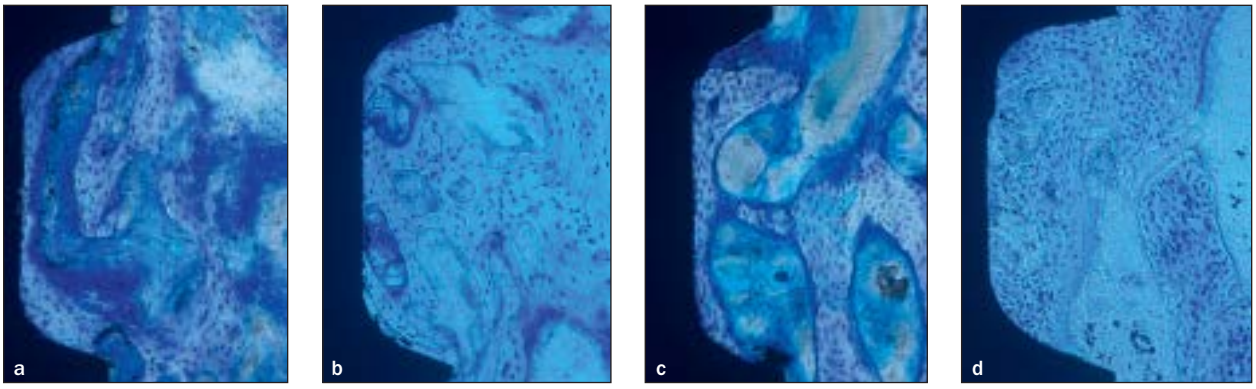


Fig 4 Light microscopic views of specimens from *(a)* control group, *(b)* experimental group E5, (*c*) experimental group E10, *(d)* experimental group E20. In all images, note the vertical position of the osseointegrated implant and newly formed bone (toluidine blue; magnification ×300).

Histomorphometric evaluation showed significantly higher BIC values in the threaded area of interest in group E20 than in the control group ($P = .033$). No statistically significant differences in BIC values were observed between the other groups (Table 2, Fig 4).

DISCUSSION

LLLT has proven effective and beneficial as an adjuvant therapy for several dental treatments. However, because there is no well-defined protocol for different types of LLLT treatment, its clinical application has been the subject of research in several health fields. The use of LLLT to accelerate peri-implant bone healing after implant placement stands out among the various indications for LLLT reported in the literature.[2,12–21,22,25] In this study, to obtain similar initial clinical conditions for application of LLLT, all animals underwent surgical extraction of the mandibular left incisor, followed by immediate placement of an osseointegrated implant using the same operative technique; all procedures were performed by the same surgeon. Statistical analysis of the ISQs revealed great similarity between the results obtained at the time of implant placement, indicating successful standardization of the surgical procedure (Table 2). Furthermore, a minimally traumatic surgical technique was used to maintain peri-implant bone tissue integrity for implant placement.

Rabbits were chosen as the experimental animal model for this study, as was the case in previous studies,[2,12,14,17,21,22] because of ease of handling, surgical preparation, postoperative follow-up, and, particularly, the adequate size of this animal, which enables placement of conventional osseointegrated implants into fresh extraction sockets. This region was chosen for implant placement, rather than the tibia, to mimic as closely as possible the situations encountered in clinical practice. In addition, this method provides higher reliability, because the mandibular alveolar bone receives a masticatory load that is different from that to which the rabbit tibia is subjected.[34]

Likewise, in accordance with the most recent literature, LLLT was performed with an infrared laser[2,12–14,16–19,21,22,25] at a wavelength of 830 nm,[12,14,16,17,22,24,25] which was applied to two spots near (not directly over) the dental implant. A total of seven sessions of irradiation were performed,[12,14,17,22] with 48-hour intervals between sessions,[12–18,21,22,25] and different doses were used in each experimental group.

RFA using the Osstell ISQ device is currently a reliable noninvasive method for assessment of implant stability before loading of implants.[29–31,33,35,36] As a diagnostic tool, the Osstell ISQ system enables the dentist to optimize any stage of the healing process, prosthetic rehabilitation, or surgical protocol to be used, making it possible to reduce the time required before insertion of the definitive rehabilitation. This method has also been used in scientific research to prevent the compromise of samples during measurement of both primary and secondary implant stability, particularly when the purpose is to assess the amount of BIC.[37] Although a recent study using RFA in 3,786 dental implants concluded that ISQs are not reliable in predicting early implant failure,[38] a trustworthy cutoff ISQ that would differentiate between success and early implant failure remains to be determined. In contrast to these findings[38] but in agreement with much of the literature,[29–31,33,35,36] the current authors observed that, in scientific research performed on a scheduled basis in animals under ideal conditions for assessment, with measurements properly recorded by calibrated examiners and strict use of the same procedures at the same intervals, without interference from inadequate occlusal or prosthetic factors, ISQs corresponded closely to the histologic observations.

In the present study, the protocol employed for assessment of implant stability was based on the mean of four ISQ measurements (mesial, distal, buccal, and lingual surfaces) in an attempt to eliminate measurement bias by including all bone walls. When the ISQs obtained at implant placement (time 1) and 30 days after the last LLLT session (time 2) were compared, an increase was observed in ISQs at the end of the experiment (time 2) for all implants tested, regardless of group, which may be attributed to the osseointegration phenomenon. However, the irradiated rabbits always showed ISQs that were higher than those of controls at time point 2, with group E20, which received total energy of 20 J per session, showing a significant increase in ISQ compared to all other groups ($P = .004$). It is worth noting that, although all groups showed a mean increase in ISQs caused by the process of osseointegration itself, this finding was not observed in all animals, which may be attributed to specific changes related to the biologic aspects of each individual in the sample.

After strict sample preparation for histomorphometric analysis of BIC in the central thread of the implant, it was seen that group E20 had statistically higher BIC scores. The other two experimental groups, which received lower doses of LLLT, showed results very similar to those of controls. The literature,[2,22–26] however, still lacks a therapeutic laser protocol that covers, using a single total energy value, all the possibilities of beneficial effects on bone tissue. Nevertheless, the 20-J-per-session LLLT protocol used in this study promoted the largest amount of newly formed bone in contact with the implant, as detected not only by RFA but also by histologic analysis, which is the gold standard for this type of study.

Although this study was carefully designed to minimize bias, it had some limitations that deserve consideration. First, the lack of a definitive protocol for LLLT had a direct impact on the objectives of the study, particularly the lack of a standard value for the total energy that must be delivered to the tissue to achieve the desired effect on peri-implant bone healing. Second, with respect to ISQs, the information provided by the manufacturer of the Osstell ISQ device and data from the literature indicate which ISQ ranges correspond to variations that show lower and higher implant stability (< 50, 50 to 70, and > 70), with the average level of 70 ISQ indicating mainly acceptable loading of an implant, whereas < 50 ISQ is related to poor stability and > 70 ISQ to excellent implant stability, as measured by RFA. In an attempt to elucidate the relationship between these values and the osseointegration phenomenon, the authors compared the results of RFA measurements and histologic analysis of BIC around implants in an attempt to establish a connection between mean ISQs and BIC percentages, especially when comparing subtle variations in ISQs. Finally, RFA is a noninvasive method that provides objective measurements that can be reproduced in vivo, and this approach allows a clinical assessment of the effects of LLLT on implant stability. However, further studies, including assessments (of ISQ and BIC) at different points in time, are warranted to investigate variations at the very early stages of implant placement, as well as to determine the course of any LLLT-related increases in ISQ and BIC in the long term.

CONCLUSIONS

The present study showed significant differences in implant stability quotients (ISQs) and percentage of bone-implant contact obtained after application of low-level laser therapy (LLLT). The current findings demonstrate that LLLT was able to significantly increase ISQs after implant placement, reflecting greater implant stability and suggesting that this laser therapy is effective in improving peri-implant bone healing when used in the parameters described here. Likewise, when ISQs and histologic results were compared, bone-implant contact values were also favored by the use of LLLT, showing an increase in the amount of newly formed bone on the implant surface.

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REFERENCES

1. Karu T. Photobiology of low-power laser effects. Health Phys 1989; 56:691–704.
2. 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.
3. Takeda Y. Irradiation effect of low-energy laser on alveolar bone after tooth extraction. Experimental study in rats. Int J Oral Maxillofac Surg 1988;17:388–391.
4. Rigau J, Trelles MA, Calderhead RG, Mayayo E. Changes in fibroblast proliferation and metabolism following in vitro helium-neon laser irradiation. Laser Ther 1991;3:25–33.

5. Silva AN Jr, 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;20:83–87.
6. 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;22:513–518.
7. 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.
8. 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;25:107–111.
9. 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;24:721–728.
10. 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;40:277–284.
11. 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;41:1265–1269.
12. Campanha BP, Gallina C, Geremia T, et al. Low-level laser therapy for implants without initial stability. Photomed Laser Surg 2010;28:365–369.
13. 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;18:517–524.
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. García-Morales JM, Tortamano-Neto P, Todescan FF, de Andrade JC Jr, Marotti J, Zezell DM. Stability of dental implants after irradiation with an 830-nm low-level laser: A double-blind randomized clinical study. Lasers Med Sci 2012;27:703–711.
16. Kim YD, Kim SS, Hwang DS, et al. 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;4:681–685.
17. Lopes CB, Pinheiro AL, Sathaiah S, Duarte J, Cristinamartins M. Infrared laser light reduces loading time of dental implants: A Raman spectroscopic study. Photomed Laser Surg 2005;23:27–31.
18. Maluf AP, Maluf RP, Brito Cda R, França FM, de Brito RB Jr. Mechanical evaluation of the influence of low-level laser therapy in secondary stability of implants in mice shinbones. Lasers Med Sci 2010;25:693–698.
19. Dörtbudak O, Haas R, Mailath-Pokorny G. Effect of low-power laser irradiation on bony implant sites. Clin Oral Implants Res 2002;13:288–292.
20. 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;21:491–498.
21. Pereira CL, Sallum EA, Nociti FH Jr, 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.
22. Pinheiro ALB, Lopes CB, Sathaiah S, Duarte J. Laser biomodulation in bone implants: A Raman spectral study. Int Congr Ser 2003;1248:449–451.
23. Shibli JA, Theodoro LH, Haypek P, Garcia VG, Marcantonio E Jr. The effect of CO_2 laser irradiation on failed implant surfaces. Implant Dent 2004;13:342–351.
24. Khadra M, Rønold 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;15:325–332.
25. Kim YD, Kim SS, Hwang DS, et al. Effect of low-level laser treatment after installation of dental titanium implant-immunohistochemical study of RANKL, RANK, OPG: An experimental study in rats. Lasers Surg Med 2007;39:441–450.
26. Pinheiro AL. Advances and perspectives on tissue repair and healing. Photomed Laser Surg 2009;27:833–836.
27. Javed F, Almas K, Crespi R, Romanos GE. Implant surface morphology and primary stability: Is there a connection? Implant Dent 2011;20:40–46.
28. Becker W, Sennerby L, Bedrossian E, Becker BE, Lucchini JP. Implant stability measurements for implants placed at the time of extraction: A cohort, prospective clinical trial. J Periodontol 2005;76:391–397.
29. Garg AK. Osstell Mentor: Measuring dental implant stability at placement, before loading, and after loading. Dent Implantol Update 2007;18:49–53.
30. Sennerby L, Meredith N. Implant stability measurements using resonance frequency analysis: Biological and biomechanical aspects and clinical implications. Periodontol 2000 2008;47:51–66.
31. 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.
32. Cricchio G, Imburgia M, Sennerby L, Lundgren S. Immediate loading of implants placed simultaneously with sinus membrane elevation in the posterior atrophic maxilla: A two-year follow-up study on 10 patients. Clin Implant Dent Relat Res 2014 Aug;16(4):609–617. Epub 2013 Jan 11.
33. Bornstein MM, Hart CN, Halbritter SA, Morton D, Buser D. Early loading of nonsubmerged titanium implants with a chemically modified sand-blasted and acid-etched surface: 6-month results of a prospective case series study in the posterior mandible focusing on peri-implant crestal bone changes and implant stability quotient (ISQ) values. Clin Implant Dent Relat Res 2009;11:338–347.
34. de Jong WC, Korfage JA, Langenbach GE. Variations in habitual bone strains in vivo: Long bone versus mandible. J Struct Biol 2010;172:311–318.
35. Winter W, Möhrle S, Holst S, Karl M. Parameters of implant stability measurements based on resonance frequency and damping capacity: A comparative finite element analysis. Int J Oral Maxillofac Implants 2010;25:532–539.
36. Karl M, Palarie V, Nacu V, Krafft T. Effect of intraoperative bone quality testing on bone healing and osseointegration of dental implants. Int J Oral Maxillofac Implants 2013;28:1254–1260.
37. Dagher M, Mokbel N, Jabbour G, Naaman N. Resonance frequency analysis, insertion torque, and bone to implant contact of 4 implant surfaces: Comparison and correlation study in sheep. Implant Dent 2014;23:672–678.
38. Monje A, Ortega-Oller I, Galindo-Moreno P, et al. Sensitivity of resonance frequency analysis for detecting early implant failure: A case-control study. Int J Oral Maxillofac Implants 2014;29:456–461.