The Effect of Low-Intensity Laser Therapy on Bone Healing Around Titanium Implants: A Histometric Study in Rabbits

Cecília Luiz Pereira, DDS, MS[1]/Enilson Antônio Sallum, DDS, MS, PhD[2]/
Francisco Humberto Nociti Jr, DDS, MS, PhD[2]/Roger William Fernandes Moreira, DDS, MS, PhD[3]

Purpose: *This study aimed to histometrically evaluate the influence of low-intensity laser treatment on bone healing around titanium implants placed in rabbit tibiae.* ***Materials and Methods:*** *Each tibia of 12 adult rabbits received a 3.3 × 6-mm titanium implant. The implants placed in the right tibiae were irradiated with a gallium-aluminum-arsenide diode low-intensity laser every 48 hours for 14 days postoperatively, and the left tibiae were not irradiated. After 3 or 6 weeks, the animals were sacrificed (six animals per period), and nondecalcified sections were obtained and analyzed for bone-to-implant contact (BIC) and bone area within the implant threads. Data were subjected to statistical analysis using analysis of variance (ANOVA) and the Tukey test.* ***Results:*** *BIC was significantly increased in the laser-treated group at both 3 weeks and 6 weeks. BIC did not increase significantly with time (3 weeks versus 6 weeks). Conversely, bone area within the threads was significantly increased with time (3 weeks versus 6 weeks), regardless of whether the laser was used. Considering bone area within the threads, no significant difference was found for treatment, eg, with or without laser.* ***Conclusion:*** *Low-intensity laser therapy did not affect the area of bone formed within the threads, but it may improve BIC in rabbit tibiae.* INT J ORAL MAXILLOFAC IMPLANTS 2009;24:47–51

Key words: *bone healing, dental implants, low-intensity laser therapy*

Several authors have reported high rates of long-term success using osseointegrated implants.[1,2] However, failures still occur, especially in areas with poor bone quality or reduced volume, such as the posterior regions of both arches. Mordenfeld et al[3] published a retrospective study evaluating patients rehabilitated with implants in posterior regions and found a 78.3% survival rate in the maxilla. Thus, techniques that benefit bone repair after implant placement have been sought, aiming at better prognoses in areas with less than ideal bone characteristics. The acceleration of osseointegration would also allow the implant to be loaded after a shorter period, reducing treatment time.

Low-intensity laser therapy (LILT) has been advocated as a modulator of tissue repair, and different stimulatory effects have been described. However, the precise mechanism and molecular basis of such effects remain unclear. Several studies have investigated the properties of LILT since the development of lasers in the 1960s. Among the clinical indications for the technique are the stimulation of soft tissue repair in lesions such as herpes and oral mucositis,[4] enhancement of tissue healing after oral surgery,[5] and promotion of pulpal repair.[6] This therapeutic resource has also been recommended for enhancement of bone repair and osseointegration, indicating its use after endosseous implant placement.[7]

[1]Doctoral Student, Division of Oral and Maxillofacial Surgery, Piracicaba Dental School, Campinas State University, Piracicaba, São Paulo, Brazil.
[2]Professor, Division of Periodontics, Piracicaba Dental School, Campinas State University, Piracicaba, São Paulo, Brazil.
[3]Associate Professor, Division of Oral and Maxillofacial Surgery, Piracicaba Dental School, Campinas State University, Piracicaba, São Paulo, Brazil.

Correspondence to: Prof Roger Moreira, Piracicaba Dental School, Division of Oral and Maxillofacial Surgery, 901 Limeira Av, Piracicaba, SP, Brazil. Email: roger@fop.unicamp.br

Diode lasers have a solid active medium, a semiconductor that uses a combination of aluminum, gallium, and arsenide (GaAlAs) to change electric energy into light energy. The available wavelengths for dental use range from about 800 to 980 nm, are very well absorbed by pigmented tissue, and have been proven to penetrate bone tissue.[8]

The effect of LILT on bone regeneration has become a focus of recent research, and it is based on biostimulation of tissues with monochromatic light. LILT has been shown to modulate inflammation, accelerate cell proliferation, and enhance bone healing.[9,10] Mesenchymal cell differentiation and osteoblast proliferation have been proven to be stimulated by phototherapy.[11–16] The modulation of wound healing, the reduction of postoperative pain and swelling, and some degree of bactericidal potential are attributed to LILT performed after implant placement.[17,18] In vitro and animal studies have also shown that this therapy may enhance the functional attachment of titanium implants to bone and promote bone mineralization.[7,19–23]

Although progress has been made on the use of laser therapy for bone remodeling around titanium implants, there is a need for additional in vivo studies. Therefore, the aim of the present study was to histometrically evaluate the influence of low-intensity laser irradiation on direct bone-to-implant contact (BIC) and on the area of threads filled by bone (BA) around titanium implants placed in rabbit tibiae. The tested hypothesis was that laser irradiation would improve bone healing around titanium implants in this experimental model.

MATERIALS AND METHODS

Prior to the experimental phase, the study was approved by the Ethics in Animal Experimentation Committee, Campinas State University. Twelve adult female New Zealand rabbits weighing 3.5 to 4.5 kg were selected for the study. Animals were maintained in individual cages, receiving water and food ad libitum.

Preoperatively, the animals were administered intramuscular injections of 80,000 IU/kg benzatin penicillin (Benzetacil; Eurofarma Laboratórios, São Paulo, SP, Brazil) and 3 mg/kg ketoprofen (Ketofen 10%; Merial Saúde Animal, Paulínia, SP, Brazil). A subcutaneous injection of 0.08 mg/kg atropine (Wyeth Laboratórios, Itapevi, SP, Brazil) was also given. General anesthesia was obtained by an intramuscular injection of 30 mg/kg of ketamine (Dopalen, Vetbrands Saúde Animal, Jacareí, SP, Brazil) and 6 mg/kg of xylazine (Rompun, Bayer, São Paulo, SP, Brazil). Local anesthetic solution (2% lidocaine with adrenaline) was used for local hemostasis (Alphacaine, DFL, São Paulo, SP, Brazil).

Antisepsis with povidone-iodine solution and sterile draping of both hind limbs were performed. An incision was made on the medial surface of the leg through skin, the subcutaneous layer, and the muscle to the periosteum, which was reflected. Preparation for implant placement was made using sequenced surgical drills (Conexão Sistemas de Prótese, São Paulo, SP, Brazil) in a 16:1 reduction handpiece (NSK E16R, Nakanishi Dental Manufacturing, Kanuma, Japan) using an electric motor (BLM 500, VK Driller Equipamentos Elétricos, São Paulo SP, Brazil) at 1,500 rpm under copious saline irrigation. After preparation, a 3.3 × 6.0-mm cylindric self-tapping titanium implant was inserted, an implant cover screw was placed, and tissue layers were sutured. The implants were then divided into four groups (n = 6).

- Group 1: laser irradiation (right tibia) with sacrifice after 3 weeks
- Group 2: control group (left tibia) with sacrifice after 3 weeks
- Group 3: laser irradiation (right tibia) with sacrifice after 6 weeks
- Group 4: control group (left tibia) with sacrifice after 6 weeks

Immediately after the surgical procedure, the right tibiae were irradiated. A GaAlAs semiconductor active medium laser (Twin Laser, MM Optics, São Carlos, SP, Brazil), with a wavelength of 780 nm and a fluency of 7.5 J/cm^2, was employed for 10 seconds at each irradiated point. The bone around each implant was irradiated at four aspects—medial, lateral, superior, and inferior—and the implant position was determined via palpation. Irradiations were repeated every 48 hours for 14 days. All irradiations were performed by the same operator, with manual restraint of the animals. The left tibiae did not receive laser irradiation, serving as the control group.

Animals were randomly divided into two groups for sacrifice: sacrifice at 3 weeks after implant placement surgery (implant groups 1 and 2) or sacrifice at 6 weeks (implant groups 3 and 4) after surgery.

Histomorphometric Analysis

After sacrifice, bone blocks containing the implants were removed and fixed in 4% neutral formalin. The blocks were dehydrated using an ascending series of ethanols (60% to 100%) and embedded in glycomethacrylate (Technovit 7200, Heraeus Kulzer, Wehrheim, Germany). Subsequently, 20- to 30-mm sections were obtained and stained using 1%

Table 1 Bone-Implant Contact (BIC) and Bone Area (BA) (Means ± SDs) by Treatment and Time

	BIC		BA	
Time	Control	Laser	Control	Laser
3 weeks	32.5 ± 6.97[a]	35.9 ± 11.58[b]	59.2 ± 10.32[a]	63.4 ± 11.23[a]
6 weeks	29.3 ± 6.78[a]	37.1 ± 6.67[b]	69.2 ± 10.05[b]	74.0 ± 6.44[b]

Means followed by different letters are statistically different (ANOVA, $P < .05$).

Fig 1 Group 1: Control tibia, sacrifice after 3 weeks (toluidine blue, 2.5% magnification).

Fig 2 Group 2: Laser-irradiated tibia, sacrifice after 3 weeks (toluidine blue, 2.5% magnification).

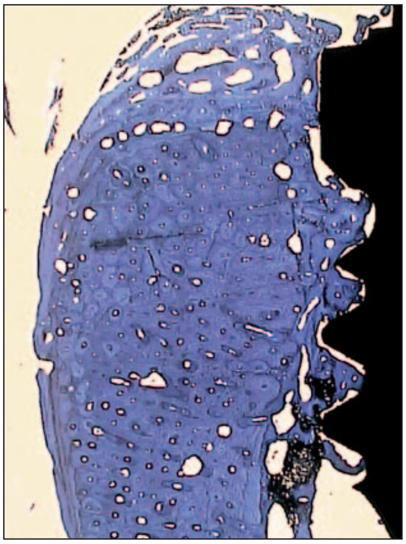

Fig 3 Group 3: Control tibia, sacrifice after 6 weeks (toluidine blue, 2.5% magnification).

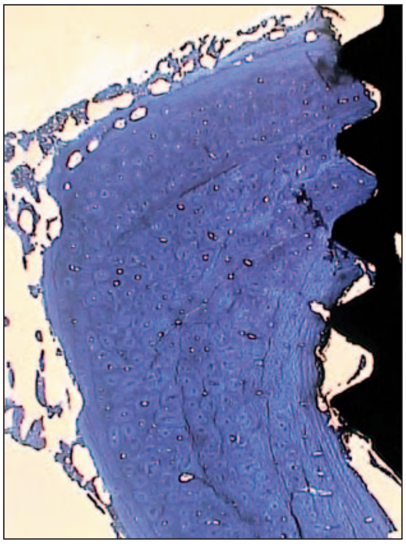

Fig 4 Group 4: Laser-irradiated tibia, sacrifice after 6 weeks (toluidine blue, 2.5% magnification).

toluidine blue. The percentage of direct BIC and BA within the most crestal three or four threads of the implants, which corresponded to the threads inserted in cortical bone, was calculated. These data were obtained by a masked examiner using image analysis software (Image Pro, Media Cybernetics, Silver Spring, MD). The obtained data were subjected to statistical analysis with analysis of variance and the Tukey test, with statistical significance considered at the 5% level ($P < .05$).

RESULTS

The mean contact percentages and standard deviations found in all groups are shown in Table 1. Figures 1 to 4 show characteristic histologic sections for all the groups.

There was a significant increase ($P = .003$) in BIC in groups 1 and 3, when compared to groups 2 and 4, at both sacrifice periods. Regarding time (3 weeks versus 6 weeks), there was no statistically significant increase in BIC ($P = .09$).

There was no significant interaction between laser and time ($P = .41$) for BA. Means were statistically similar between the groups, both with and without laser treatment ($P = .07$), at both time points. BA showed a significant improvement over time, regardless of the use of laser ($P < .0001$).

DISCUSSION

This study aimed to evaluate the influence of LILT on bone repair around titanium implants placed in rabbit tibiae. Histomorphometric evaluation is considered today as a standard analysis in implant research, and the analysis used in this study suggested more BIC in the irradiated groups as compared to the control groups at both time points. Several studies in the literature have obtained similar results that suggest LILT may stimulate bone repair, affecting cellular proliferation, differentiation, and adhesion.[24]

When the two sacrifice periods were compared, BIC did not show significant differences with time, regardless of the use of laser. This may be explained by bone remodeling around implants, which might reduce BIC in an early period after implant insertion. In the control group, although it was not statistically significant, BIC values were slightly reduced at the 6-week period. It was observed that, although bone formation continued, as evidenced by the increase in BA, the final BIC had been achieved after 3 weeks.

BA was not affected by laser irradiation. In a recent study, Jakse et al[25] observed similar results when studying the influence of LILT on bone repair after sinus grafting and implant insertion in sheep. The authors observed no beneficial impact of laser treatment on bone formation within the sinus grafts. Interestingly, the authors also observed a tendency toward a higher percentage of bone formation (BA) ($P < .053$) and a significant increase in BIC, as was observed in the present study.

Laser irradiation has a wide range of effects on tissues, and the clinical and experimental results can be affected by the therapeutic protocol. The GaAlAs laser with a 780-nm wavelength was selected on the basis of successful results obtained both in vitro and in vivo by different authors. The studies published by Khadra et al,[9,10] which also used a GaAlAs laser, showed significantly higher percentages of cell attachment of both gingival fibroblasts and osteoblastlike cells cultured on titanium. Fujihara et al[14] irradiated rat calvaria osteoblastlike cell cultures with a 780-nm GaAlAs laser, observing better cell proliferation in the irradiated cultures, regardless of the use of corticoids. The improvement in BIC observed in this study was previously reported by Khadra et al,[20] who investigated the effect of this wavelength on bone healing around implants placed in rabbit tibiae using removal torque, histometric analysis, and mineralization on the implant surface. Guzzardella et al,[7] using histometric analysis to evaluate implants placed in rabbit tibiae, found similar results, with improved bone repair around implants that had been irradiated with a GaAlAs laser. These results corroborate the findings of this study, with increased BIC at both time periods.

The GaAlAs laser is also known to have a high depth of penetration in comparison to other types of lasers and thus offers the clinician a tool of great efficiency. It has been reported that a high tissue penetration could be observed at 820 to 840 nm because of the low water absorption at that wavelength.[26] Bossy et al[27] concluded, in an in vitro study of low-energy beam penetration in compact bone, that a laser with near infrared wavelength could give a penetration of about 18 mm in the bone axis direction and approximately 6 mm in the corticomedullary direction. Considering clinical application in implant dentistry, these penetration rates would allow irradiation of the bone-implant interface, even in cortical bone in the mandible.

Satisfactory results obtained in experimental studies suggest that LILT may be a noninvasive option for enhancement of bone repair after the placement of implants. In the present study, irradiation was performed every 2 days for 2 weeks, and this protocol might be difficult for patients because they would have to appear at the professional's office to receive laser therapy at these specific times. For this reason, the authors do not consider LILT a routine protocol for all patients receiving implants, but an important complementary therapeutic resource in patients with unfavorable systemic or local factors that could interfere with osseointegration, such as smoking habits or poor bone quality.

In vivo experiments using lasers have been criticized for the lack of control groups and a wide variation of treatment settings. Although an effort was made to control as many variables as possible, the results of the present study are limited to the specific irradiation settings that were used (GaAlAs diode laser, 780 nm). However, the less expensive semiconductor diode lasers, such as that tested, have become increasingly popular among clinicians and are presently more accessible for use in daily practice. In addition to its increasing popularity, the promising results obtained in recent studies suggest that LILT may be an easy, noninvasive therapeutic means for wound healing and bone repair modulation after implant placement.

Based on previous studies and the present results, it can be concluded that LILT may have beneficial effects on bone repair, as proved by both in vitro and in vivo studies. Further investigations should focus on establishing the ideal laser wavelengths, energy density, and irradiation protocols to be used in implant dentistry.

CONCLUSION

The use of low-intensity laser did not affect the bone area within the threads, but the irradiation was effective in improving bone-to-implant contact of titanium implants inserted in rabbit tibiae.

REFERENCES

1. Albrektsson T, Dahl E, Enbom L, et al. Osseointegrated oral implants: A Swedish multicenter study of 8139 consecutively inserted Nobel-Pharma implants. J Periodontol 1988; 59:287–296.
2. Adell R, Eriksson B, Lekholm U, Brånemark P-I, Jemt T. A long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. Int J Oral Maxillofac Implants 1990;5:347–359.
3. Mordenfeld MH, Johansson A, Hedin M, Billstrom C, Fyrberg KA. A retrospective clinical study of wide-diameter implants used in posterior edentulous areas. Int J Oral Maxillofac Implants 2004;19:387–392.
4. Wong SF, Wilder-Smith P. Pilot study of laser effects on oral mucositis in patients receiving chemotherapy. Cancer J 2002;8:247–254.
5. Myers TD, McDaniel JD. The pulsed Nd:YAG dental laser: Review of clinical applications. J Calif Dent Assoc 1991; 19:25–30.
6. Jayawardena JA, Kato J, Moriya K, Takagi Y. Pulpal response to exposure with Er:YAG laser. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:222–229.
7. Guzzardella GA, Torricelli P, Nicoli-Aldini N, Giardino R. Osseointegration of endosseous ceramic implants after post-operative low-power laser stimulation: An in vivo comparative study. Clin Oral Implants Res 2003;14:226–232.
8. Coluzzi DJ. An overview of laser wavelengths used in dentistry. Dent Clin North Am 2000;44:753–765.
9. Khadra M, Kasem N, Lyngstadaas SP, Haanaes HR, Mustafa K. Laser therapy accelerates initial attachment and subsequent behaviour of human oral fibroblasts cultured on titanium implant material. A scanning electron microscopic and histomorphometric analysis. Clin Oral Implants Res 2005; 16:168–175.
10. Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K. Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. Biomaterials 2005;26:3503–3509.
11. Abramovitch-Gottlib L, Gross T, Naveh D, et al. Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix. Lasers Med Sci 2005;20:138–146.
12. Dortbudak O, Haas R, Mallath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. Clin Oral Implants Res 2000;11:540–545.
13. Hamajima S, Hiratsuka K, Kiyama-Kishikawa M, et al. Effect of low-level laser irradiation on osteoglycin gene expression in osteoblasts. Lasers Med Sci 2003;18:78–82.
14. Fujihara NA, Hiraki KR, Marques MM. Irradiation at 780 nm increases proliferation rate of osteoblasts independently of dexamethasone presence. Lasers Surg Med 2006;38:332–336.
15. Stein A, Benayahu D, Maltz L, Oron U. Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. Photomed Laser Surg 2005;23:161–166.
16. Ozawa Y, Shimizu N, Kariya G, Abiko Y. Low-energy laser irradiation stimulates bone nodule formation at early stages of cell culture in rat calvarial cells. Bone 1998;22:347–354.
17. Shibli JA, Martins MC, Theodoro LH, Lotufo RF, Garcia VG, Marcantonio EJ. Lethal photosensitization in microbiological treatment of ligature-induced peri-implantitis: A preliminary study in dogs. J Oral Sci 2003;45:17–23.
18. Arnabat-Dominguez J, Espana-Tost AJ, Berini-Aytes L, Gay-Escoda C. Erbium:YAG laser application in the second phase of implant surgery: A pilot study in 20 patients. Int J Oral Maxillofac Implants 2003;18:104–112.
19. Dortbudak 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. 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;15:325–332.
21. Khadra M, Kasem N, Haanaes HR, Ellingsen JE, Lyngstadaas SP. Enhancement of bone formation in rat calvarial bone defects using low level laser therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:693–700.
22. Pinheiro AL, Limeira Junior FA, Gerbi ME, et al. Effect of 830-nm laser light on the repair of bone defects grafted with inorganic bovine bone and decalcified cortical osseous membrane. J Clin Laser Med Surg 2003;21:301–306.
23. Ueda Y, Shimizu N. Effects of pulse frequency of low-level laser therapy (LLLT) on bone nodule formation in rat calvarial cells. J Clin Laser Med Surg 2003;21:271–277.
24. Merli LA, Santos MT, Genovese WJ, Faloppa F. Effect of low-intensity laser irradiation on the process of bone repair. Photomed Laser Surg 2005;23:212–215.
25. 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.
26. Yamagishi H, Shinohara C, Saito S, Sasaki H, Kanegae H, Shibasaki Y. A basic study on the use of penetrative sensitivity on living tissue. J Jpn Soc Laser Dent 1994;5:13–22.
27. Bossy I, Chevalier JM, Sambuc P. In vitro survey of low energy laser beam penetration in compact bone. Acupunct Electrother Res 1985;10:35–39.