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Infrared Laser Light Further Improves Bone Healing When Associated with Bone Morphogenic Proteins: An *in Vivo* Study in a Rodent Model

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ABSTRACT

Objective: **This study assessed histologically the effect of laser photobiomodulation (LPBM) on the repair of surgical defects created in the femurs of Wistar rats treated or not treated with bone morphogenic proteins (BMPs) and organic bovine bone graft.** ***Background Data:*** **This paper is part of an ongoing series of works in which biomaterials are used in association with LPBM. Several previous reports by our group have shown that the use of laser photobiomodulation improves the treatment of bone defects.** ***Materials and Methods:*** **Forty-eight adult male Wistar rats were divided into four randomized groups: group I (control, n = 12); group II (LPBM, n = 12); group III (BMPs + organic bovine bone graft, n = 12); and group IV (BMPs + organic bovine bone graft + LPBM, n = 12). The irradiated groups received seven irradiations every 48 h, beginning immediately after the surgical procedure. The laser therapy (λ = 830 nm, 40 mW CW, ϕ = ~0.6 mm) consisted of 16 J/cm^2 per session divided equally over four points (4 J/cm^2 each) around the defect. The subjects were sacrificed after 15, 21, and 30 d, and the specimens were routinely embedded in wax, stained with hematoxylin and eosin and sirius red, and analyzed under light microscopy.** ***Results:*** **The results showed histological evidence of increased deposition of collagen fibers (at 15 and 21 d), as well as an increased amount of well-organized bone trabeculae at the end of the experimental period (30 d) in the irradiated animals versus the non-irradiated controls.** ***Conclusion:*** **The use of LPBM with BMPs and organic bovine bone grafts increases the positive biomodulating effects of laser light.**

INTRODUCTION

MODERN DENTISTRY is challenged daily by the need to recover bone loss due to several etiologic factors. Several autologous and xenographs have been used to provide a framework or stimulate new bone formation, and many times these grafts respond positively to the use of certain wavelengths of laser energy.[1] The use of bone morphogenic proteins (BMPs) is not new,[2–4] and they have been widely used in the reconstruction of the alveolar ridge,[5] for the recovery of bone loss, and on several types of bone defects.[6–13] Despite the growing successful application of laser photobiomodulation (LPBM) in bone repair, there are few studies assessing the association of laser light with biomaterials.[1,8–10,12] Thus there is a need for further studies to determine the most effective ways to apply LPBM combined with different biomaterials for this new type of treatment.

MATERIALS AND METHODS

This study was approved by the Animal Ethics Committee of the School of Dentistry of the Federal University of Bahia.

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Forty-eight healthy male and female young adult Wistar rats weighing 270–320 g were kept at the Laboratório de Experimentação Animal of the School of Dentistry of the Federal University of Bahia during the experimental period. The animals were fed a standard laboratory diet and had water available *ad libitum*. The animals were kept in plastic cages bedded with sterilized wood chips and were maintained in a day/night light cycle and controlled temperature during the experimental period.

Under intraperitoneal general anesthesia (10% chloral hydrate, 0.4 mL/100 g), the animals had their left legs shaved and the femurs exposed. A standardized 2 mm^2 cavity was created with a drill on the superior third of the lateral side of the bone of each animal. The animals were then randomly distributed into four groups: group I (control, bone cavity drilling only), group II (LPBM only), group III (biomaterial only), and group IV (biomaterial + LPBM). Each group was then divided into three subgroups (Table 1).

In groups I and II, the periosteum was repositioned and suturing was performed with catgut and the skin closed with nylon. In groups II and IV, the wound margins were tattooed with nankin ink at four points, and these were used to guide the application of the laser treatment. Laser light was delivered on the medial side of each mark, avoiding any interference of the dye with the absorption of the light. In groups III and IV, the cavity was completely filled with a biomaterial (organic lyophilized decalcified bovine bone [GenOx®] + collagen gel [Gencol®] + BMP pool [Genpro®]; Baumer SA, Mogi Mirim, SP, Brazil) following the technique suggested by the manufacturer, and then sutured. Tattooing was also performed on group IV.

Laser therapy (830 nm, 40 mW CW, ϕ = ~0.60 mm) (Thora Laser; DMC Equipamentos, São Carlos, SP, Brazil) was used on groups II and IV and begun immediately after suturing. It consisted of a transcutaneous application at four points around the surgical site, and was repeated every other day for 15 d. The dose per point was 4 J/cm^2, and the total dose per session was 16 J/cm^2. The total treatment dose was 112 J/cm^2.

The animals were humanely sacrificed 15, 21, and 30 d after surgery with an intraperitoneal overdose of 10% chloral hydrate. Specimens were routinely taken and kept in 4% formalin for 5 d. They were then cut, embedded in wax, and stained with hematoxylin and eosin and sirius red at the Oral Pathology Department of the School of Dentistry of the Federal University of Bahia. All slides were analyzed by light microscopy by an experienced pathologist who had previously calibrated the procedure.[6–13]

The descriptive and semi-quantitative histological analysis were performed based on these parameters: reabsorption of the cortical plate and of the biomaterial inserted into the bone defect; the presence of medullary tissue and/or granulation tissue; intensity of the inflammatory reaction; the presence of giant cells; the number of collagen fibers; number of haversian systems; and the amount and quality of the newly formed bone.

RESULTS

Controls

In control animals, at day 15 the defect was filled by medullary tissue and osteoblastic activity was seen in most specimens. Some deposition of bone matrix within the medullary tissue was also seen at this time. No cortical repair was seen in most specimens. Osteoclastic activity was seen as lacunae in the cortical area. Neither necrosis nor collagen deposition was seen within the defect (Fig. 1A). At day 21, most specimens showed early signs of cortical repair, and the defect was mostly filled by medullary tissue (Fig. 2A). At the end of the experimental period, the defect remained primarily filled by medullary tissue and most specimens showed complete cortical repair (Fig. 3A).

Laser photobiomodulation only

In the specimens from the animals receiving LPBM only, at day 15 the defect was filled by medullary tissue, and large amounts of newly formed bone were seen in most specimens. Delicate trabeculae and few collagen fibers were seen within the defect. In most specimens, cortical repair was complete at this time. Osteoclastic activity was present, as several lacunae and giant cells were seen within the defect. Collagen deposition was extensive (Fig. 1B). At day 21, some osteoblastic activity was observed. Small lacunae were present and most of the defect was filled by medullary tissue, with no apparent deposition of spongy bone or collagen fibers. Cortical repair was complete at this time (Fig. 2B). At the end of the experimental period, the picture remained similar to that seen on day 21, and cortical repair was complete in all specimens. No necrosis was seen (Fig. 3B).

Biomaterials only

In the specimens from the animals receiving the biomaterials only, at day 15 the biomaterials were seen filling the defect. Osteoclastic activity was seen, as portions of the implant were reabsorbed. Giant cells were seen close to the particles of biomaterials, and intense induction of bone formation was seen close to the grafted area. A moderate amount of collagen ma-

Table 1. Distribution of Animals in the Control and Experimental Groups

Group	*Subgroup*	N	*Treatment*
I	C15, C21, C30,	12	Control
II	CL15, CL21, CL30	12	Laser photobiomodulation only
III	P15, P21, P30	12	Biomaterials only
IV	PL15, PL21, PL30	12	Biomaterials + laser photobiomodulation

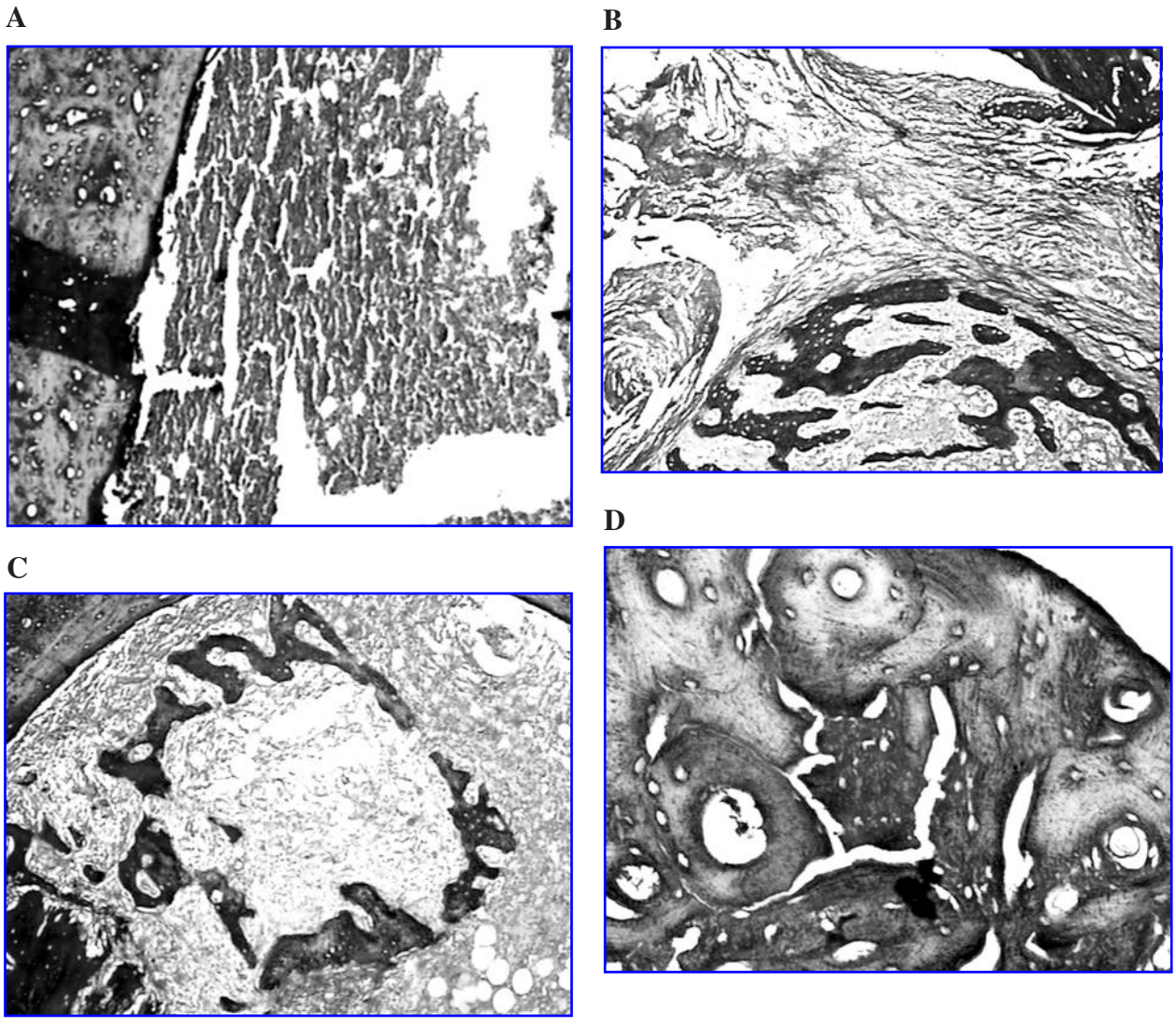

FIG. 1. Images made at day 15. (**A**) Photomicrograph of a control specimen, showing a large amount of medullary tissue filling the interior of the defect (sirius red, original magnification approximately 40×). (**B**) Photomicrograph of a specimen subjected to laser photobiomodulation only, showing bony trabeculae and a large amount of collagen matrix within the defect (sirius red, original magnification approximately 100×). (**C**) Photomicrograph of a specimen subjected to bone grafting only, showing the close relationship between the graft and the collagen matrix, along with some newly formed bone (sirius red, original magnification approximately 100×). (**D**) Photomicrograph of a specimen subjected to bone grafting and laser photobiomodulation, showing haversian system formation within the defect (sirius red, original magnification approximately 100×).

trix deposition was evident at this point. A moderate amount of diffuse lymphocytic inflammatory infiltrate was seen around the particles of biomaterials. Cortical repair was incomplete in many specimens (Fig. 1C). At day 21, cortical repair was seen in half of the specimens. Reabsorption of the particles of biomaterials was also seen at this point. An increased amount of bone formation was seen close to the implanted material, and the newly formed bone showed a normal haversian system. Collagen deposition was also seen around the biomaterial (Fig. 2C). At the end of the experimental period, bone deposition was more pronounced within the cavity, and cortical repair was largely incomplete. More condensed collagen matrix was also seen at this point (Fig. 3C).

Biomaterials plus laser photobiomodulation

In the specimens receiving both bone grafting and LPBM, at day 15 the cortical repair was complete in most specimens. The defect was filled by the biomaterial and its particles were replaced by newly formed bone that showed early signs of formation of the haversian system. Giant cells were seen close to the implanted material reabsorbing portions of the particles. Within the implanted material, delicate collagen fibers and moderate amounts of chronic inflammatory infiltrate and newly formed blood vessels were seen. No reabsorption of the cortical bone was seen at this point (Fig. 1D). At day 21, complete cortical repair was seen in half of the specimens. Reabsorption

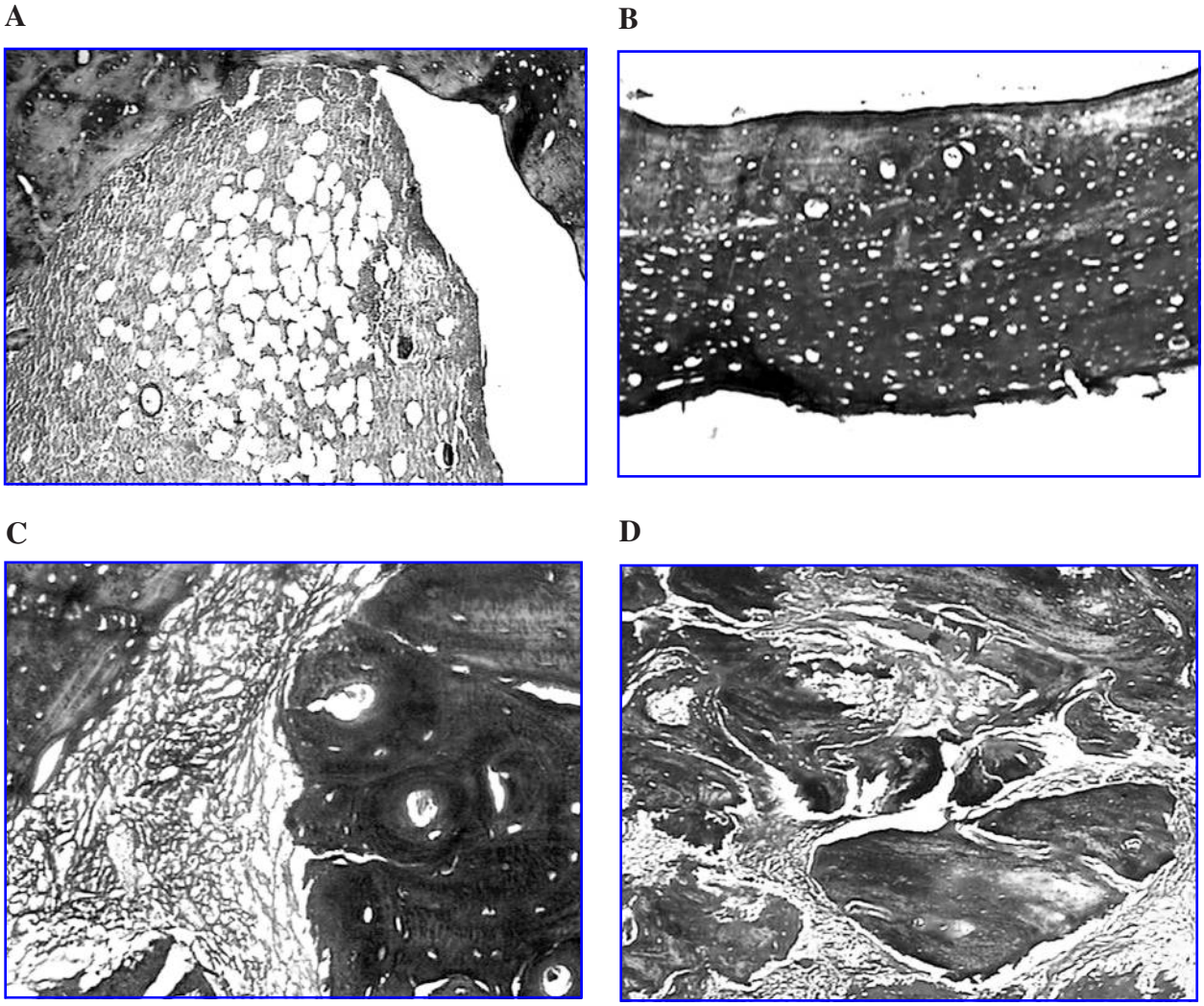

FIG. 2. Images made at day 21. (**A**) Photomicrograph of a control specimen, showing a large amount of medullary tissue filling the bone defect, along with many adipocytes (sirius red, original magnification approximately 40×). (**B**) Photomicrograph of a specimen subjected to laser photobiomodulation only, showing complete cortical repair (sirius red, original magnification approximately 40×). (**C**) Photomicrograph of a specimen subjected to bone grafting only, showing the close relationship between the haversian system and the collagen matrix (sirius red, original magnification approximately 100×). (**D**) Photomicrograph of a specimen subjected to bone grafting and laser photobiomodulation, showing remnants of the graft encircled by many collagen fibers, along with the deposition of osteoid matrix and newly formed bone (sirius red, original magnification approximately 100×).

of the biomaterial and its replacement with newly formed bone was seen. Thicker collagen fibers were also seen at this stage, along with slight inflammation (Fig. 2D). At the end of the experimental period, the characteristics seen were nearly the same as those seen on day 21 (Fig. 3D).

DISCUSSION

Despite several previous reports indicating that the use of BMPs can be effective in improving bone healing *in vivo*,[1,5,6–14] few studies have been published to date[1,8–10,12] on the use of these techniques in association with LPBM. To the best of our knowledge, ours is the first report on the combination of BMPs with LPBM.

The results of our study do not agree with those of a previous report,[4] as those researchers did not see haversian system formation at early stages of healing as we did. Formation of these systems has been reported between 4 and 6 weeks after the use of BMPs. We believe that implementation of a more effective protocol for LPBM resulted in a synergistic effect on the healing process, which caused accelerated healing compared to either treatment (LPBM or biomaterial) used alone. These positive results were observed throughout the experimental period in our study, as we have reported in the past.[1,6–13]

In this study, we used infrared laser light because of its ability to more deeply penetrate the tissues, especially the subcutaneous tissues.[1,6–13] Several studies have shown the effectiveness of LPBM in accelerating healing of both bone defects and fractures. However, some authors have not found the same re-

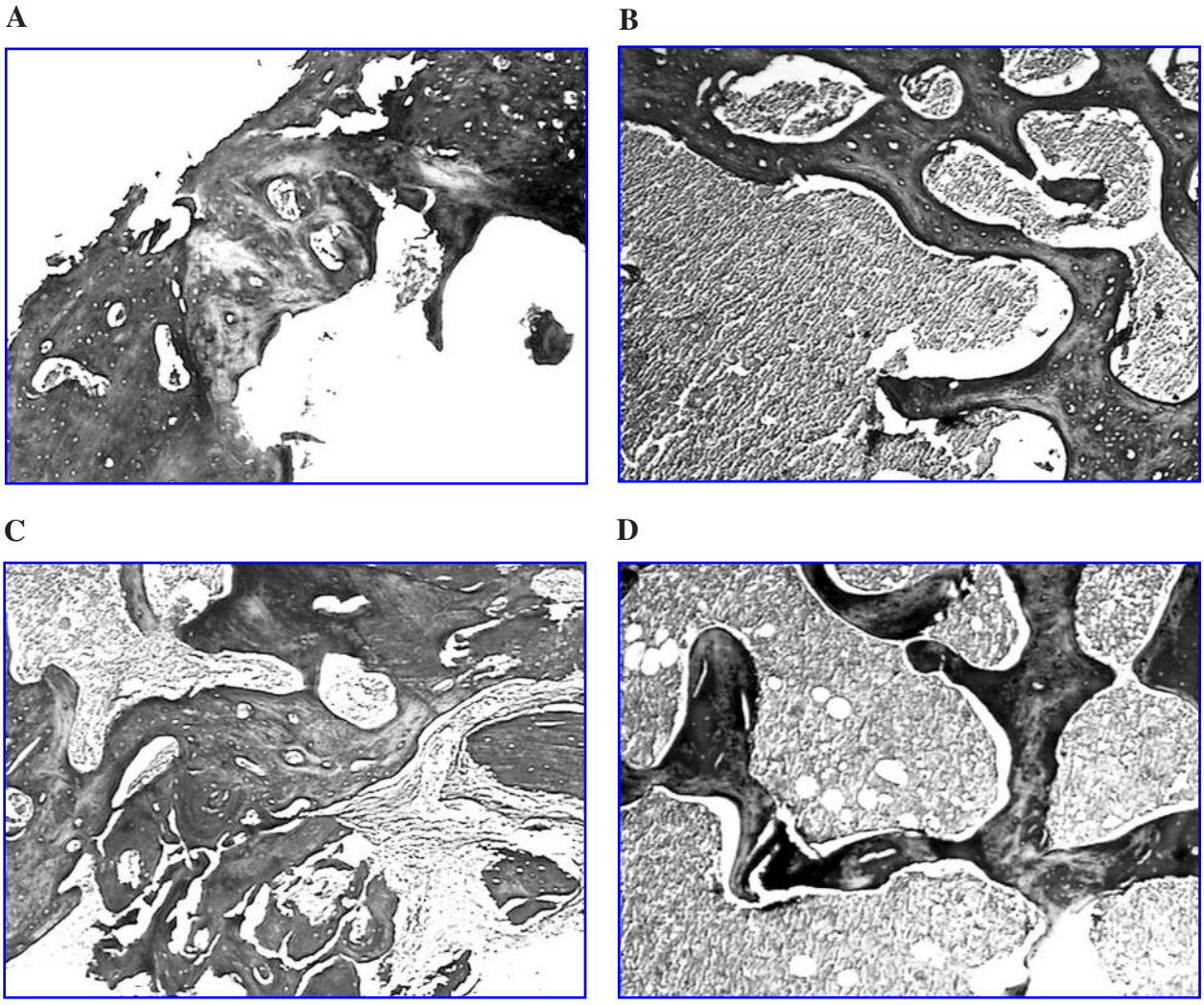

FIG. 3. Images made at day 30. (**A**) Photomicrograph of a control specimen, showing characteristics similar to those seen on day 21.. However, there is a closer relationship between the margins of the defect and its thickness, which remained thinner than usual (sirius red, original magnification approximately 40×). (**B**) Photomicrograph of a specimen subjected to laser photobiomodulation only, showing that the newly formed bone trabeculae from the cortical margin is in a more mature stage compared to controls (sirius red, original magnification approximately 100×). (**C**) Photomicrograph of a specimen submitted to bone grafting only, showing nearly complete cortical repair and some collagen matrix formation. The cortical bone is still thinner then usual (sirius red, original magnification approximately 40×). (**D**) Photomicrograph of a specimen subjected to bone grafting and laser photobiomodulation, with newly formed bone trabeculae and medullary tissue seen within the defect (sirius red, original magnification approximately 100×).

sults as ours. Previous reports from our group in which we used both organic and inorganic bone grafts and resorbable membrane found that LPBM had a positive biomodulating effect on healing bone.[1,6–13]

The most significant finding of this study was seen at day 15, when complete cortical repair was seen in most specimens, with newly formed bone similar to that in untreated areas. A great deal of new bone replaced the implanted material, and remodeling haversian systems were also seen at day 15. This accelerated healing continued throughout the experimental period, and the new bone had characteristics similar to those of mature bone. Bone trabeculae were seen at the center of the defect, as well as their spread from the cortical area toward the center of the defect. Many collagen fibers and new osteoid tissue were also seen growing around the remnants of the implanted biomaterial.

The effect of the infrared laser energy could be seen at the earliest stages of healing, perhaps indicating that the effects of LPBM are exacerbated at the early stages of healing, when cellular proliferation is most active.[1] These results were also corroborated by a previous study of ours.[1,6–13] Another group[14] also found positive effects of LPBM on bone remodeling in rats as assessed by computerized morphometric analysis.

Although laser energy applied in the early stages of healing was effective in hastening bone healing, treatment with LPBM in later stages of healing might play an important role in bone

regeneration. For this reason, the LPBM protocol used here included irradiation in the postoperative period that was repeated every 48 h over 15 d.

Effects of LPBM on neovascularization were also suggested as one possible mechanism responsible for the positive clinical results seen in our study. Vascularization is an important factor contributing to wound healing and pain relief, and may be one of the mechanisms responsible for the clinical effectiveness seen in this study.

The true mechanism behind the positive effect of laser light on injured tissue remains obscure, and thus comparative analysis of our results to those of others is difficult. Several hypotheses have been proposed to explain this mechanism.

The magnitude of the biomodulating effect depends on the physiologic status of the cells at the time of irradiation. This may explain why the biomodulating effect is not always detectable. The stimulant effects of laser energy occur during the initial phases of proliferation and differentiation of undifferentiated cells, but it does not occur during more advanced stages of cell growth. These mechanisms may explain the differences seen between irradiated and non-irradiated tissues in our study.

The treatment protocol used in this investigation was the same as that used in our group's past experiments, as no existing parameters are universally accepted, and other authors who used similar protocols have reported conflicting results. A single parameter that produces a replicable photobiological response does not exist, but the combination of several parameters appears to have a cumulative effect.[1] It remains uncertain if bone stimulation by laser light is a systemic effect, or if stimulation of isolated osteoblasts is possible.

CONCLUSION

The use of infrared laser light was effective for accelerating healing of bone defects filled with organic bovine bone graft, as evidenced by increased collagen deposition, faster cortical repair, and earlier development of haversian systems.

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