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Assessment of Bone Repair Associated with the Use of Organic Bovine Bone and Membrane Irradiated at 830 nm

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ABSTRACT

Objective: **The aim of the present investigation was to assess histologically the effect of LLLT (GaAIAs, 830 nm, 40 mW, CW, (ϕ) ~0.6 mm, 16 J/cm² per session) on the repair of surgical defects created in the femur of the Wistar Albinus rat. The defects were filled to lyophilized bovine bone (Gen-ox®, organic matrix) associated or not to GTR (Gen-derm®).** ***Background data:*** **A major problem on modern Dentistry is the recovery of bone defects caused by trauma, surgical procedures or pathologies. Several types of biomaterials have been used in order to improve the repair of these defects. These materials are often associated to procedures of GTR. Previous studies have shown positive effects of LLLT on the repair of soft tissue wounds, but there are a few on its effects on bone healing.** ***Methods:*** **Surgical bone defects were created in 42 animals divided into five groups: Group I (control, 6 animals); Group II (Gen-ox®, 9 animals); Group III (Gen-ox® + Laser, 9 animals); Group IV (Gen-ox® + Gen-derm, 9 animals); Group V (Gen-ox® + Gen-derm® + Laser, 9 animals). The animals on the irradiated group received 16 J/cm² per session divided into four points around the defect (4 J/cm²) being the first irradiation immediately after surgery and repeated seven times at every 48 h. The animals were humanly killed after 15, 21, and 30 days.** ***Results:*** **The results of the present investigation showed histological evidence of improved amount of collagen fibers at early stages of the bone healing (15 days) and increased amount of well organized bone trabeculae at the end of the experimental period (30 days) on irradiated animals compared to non irradiated ones.** ***Conclusions:*** **It is concluded that a positive biomodulative effect on the healing process of one defect associated or not to the use of organic lyophilized bone and biological bovine lyophilized membrane on the femur of the rat.**

INTRODUCTION

THE TREATMENT OF BONE DEFECTS using biomaterials has been extensively studied in the dental field.[1–7] Since the pioneerion work by Urist[7] demonstrated heterotropic formation of bone induced by devitalized desmineralized bone matrix, a new possibility of treating bone defects has surfaced. Demineralized bone matrix has osteoinductive properties due to the presence of soluble growth factors in its composition.[7] The idea of using desmineralized bone in the treatment of bone defects is not new.[8] Senn reported the use of such material in the treatment of osteomielytes, and later its use in several other conditions was also reported.[9,10] Guided tissue regeneration is widely used for treating periodontal defects, and recently it has been used to

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treat other bone defects, where it is known as guided bone regeneration (GTR).[8,11] The use of organic bovine bone and membrane has been well described.[7,8,12]

Although the use of low-level laser therapy (LLLT) on the biomodulation of bone repair has been growing steadily and several studies have demonstrated positive results on the healing of bone tissue,[13–25] there are no reports on the association of LLLT and biomaterials. The aim of this study was to assess the effect of LLLT on the healing of bone defects treated with inorganic bovine bone associated or not with decalcified cortical osseous membrane.

MATERIALS AND METHODS

Forty-two healthy adult male and female *Wistar Albinus* rats weighing 270–320 g were kept under natural conditions of light, humidity, and temperature at the Laboratório de Experimentação Animal (LEA) of the Faculdade de Odontologia da Universidade Federal da Bahia. The animals were fed with standard laboratory pelted diet and had water *ad libidum.* The animals were divided into five groups, and each group was divided into three subgroups (Table 1).

Under intraperitoneal general anesthesia (10% chloral hydrate, 0.4 mL/100 g), the animals had the right leg shaved and the femur exposed. Standardized 3 mm^2 cavities were created on the superior third of the lateral side of the bone. On Group I, the periosteum was repositioned and suturing was performed with catgut and the skin closed with nylon. On groups II, III, IV, and V, the cavities were completely filled with organic bovine bone (Gen-ox®, Baumer S/A Mogi Mirim, SP, Brazil). Group IV and V cavities were covered with decalcified cortical osseous membrane (Gen-derm®, Baumer S/A, Mogi Mirim, SP, Brazil). All wounds were routinely sutured. The animals of Groups III and V were submitted to seven sessions of LLLT (Thera Lase, DMC Equipamentos, São Carlos, SP, Brazil; λ 830 nm, 40 mW, θ ~0.60 mm, CW) at 48-h intervals. The irradiation was performed transcutaneously, and the first session was performed immediately after surgery. A dose of 4 J/cm^2 was applied to four points around the defect, giving a total of 16 J/cm^2 per session and a total treatment dose of 112 J/cm^2. Doses used here were based upon previous studies carried out by Pinheiro,[26] who recommended doses ranging from 1.8 to 5.4 J/cm^2. Following irradiation, the animals were humanely killed at 15, 21, and 30 days after surgery by an overdose of general anesthetic. The samples were taken and kept on 4% buffered paraformaldehyde solution for 5 days. The samples were decalcified with 10% nitric acid and routinely stained with H&E and Picrosirius.[27]

RESULTS

On day 15, on control defects, a small amount of granulation tissue, cancellous bone, and a few small trabeculae were observed. Bone neoformation was observed to be initiating the repair of the cortical area of the defect. At 21 days after surgery the aspect was similar to day 15; however, cortical repair was more pronounced. On day 30, the cortical area was repaired, but the thickness of the area was smaller than normal (Fig. 1).

When the Gen-ox®–Organic was used, it was observed on day 15 that the defect was mostly filled by the graft, which was encircled by delicate collagen fibers and cancellous bone. Immature bone trabeculae were observed on the periphery of the graft and on the internal cortical surface, progressing towards the center of the cavity. Two specimens showed complete cortical repair at this stage. On day 21, a discrete to mild chronic inflammatory infiltrate could be seen, and discrete areas of reabsorption of the cortical bone and the graft by osteoclasts could also be observed. The graft was encircled by diffuse collagen fibers, and incipient bone matrix deposition amongst them could be seen. At the end of the experimental period, some particles of the graft were still present, but the process of reabsorption was more evident at this stage. Osteoid tissue was also more evident at this stage, and incomplete cortical repair was also observed (Fig. 2).

When LLLT was added in the Gen-ox®–Organic, it was noticed on day 15 after surgery that the cavity was partially filled by particles of the Gen-ox®, which was mostly encircled by granulation tissue. Intense fibroblastic proliferation was observed at this stage, and the cortical area showed the presence of mononuclear inflammatory infiltrate. An increased amount of collagen fibers could be observed within most of the cavity, and a discrete bone formation originated from both the cortical area and from the particles of the graft. On day 21, the Gen-ox® particles were encircled by collagen fibers at the center of the cavity, and some particles showed signs of reabsorption; deposition of newly formed bone could be seen on the resulting lacunae. The cavity was mostly filled by a less cellular connective tissue, which showed rich and well-distributed and organized

TABLE 1. GROUPS AND SUBGROUPS

Group	*Subgroups*	*n*	*Procedure*
I	C15/C21/C30	6	Control
II	B15/B21/B30	9	Organic bovine bone
III	BL15/BL21/BL30	9	Organic bovine bone + LLLT
IV	BM15/BM21/BM30	9	Organic bovine bone + decalcified Cortical osseus membrane
V	BML15/BML21/BML30	9	Organic bovine bone + decalcified Cortical osseus membrane + LLLT

C, control; B, bone; M, membrane; L, laser.

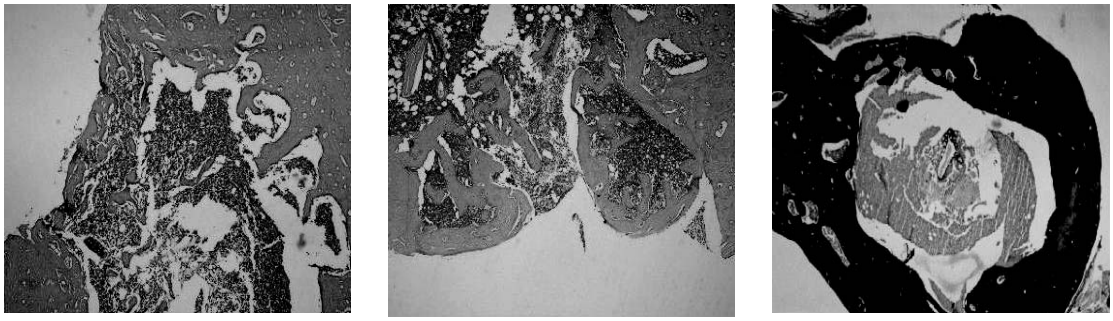

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FIG. 1. Photomicrograph of control specimens. **(A)** At day 15, beginning of the cortical bone repair could already be observed. **(B)** At 21 days after procedure, increased amount of cortical bone was present. **(C)** At day 30, total union of the cortical was observed. H&E, ~40× (A, B); Picrosírius, ~32× (C).

collagen fibers. Osteoblastic activity could also be observed at this stage, and well-organized bone trabeculae originated from the internal cortical area, which was permeated by cancellous bone. At the end of the experiment, the cavity was filled by well-organized bone trabeculae and cancellous bone and most of the graft was reabsorbed. Most of the cortical repair was complete (Fig. 3).

The use of the membrane associated to the Gen-ox®–Organic showed that, on day 15, the cavity was mostly filled by the Gen-ox® that was encircled by delicate bundles of collagen fibers. There was a discrete neoformation of osteoid tissue around some particles of the graft and the presence of granulation tissue filling the remaining space. Cortical repair was complete at this stage. On day 21, a chronic inflammatory infiltrate was present and a dense osteoid tissue was observed around the graft. Mineralized bone matrix was observed around the bundles of collagen fibers that were delicate and discrete on the area and dense around the particles. Cortical area was still incomplete. At the end of the experiment, there were vestiges of particles of the graft at the center of the defect. These remaining particles were encircled by thick bundles of collagen fibers and showed intense osteoblastic activity and typical bone neoformation by osteoinduction. Most of the defect did not show the presence of the graft that was replaced by bone trabeculae. Close to the cortical area, a large amount of collagen fibers and bone formation around remaining particles of the graft were seen. Complete cortical repair was not observed at this stage (Fig. 4).

When LLLT was added to Gen-ox®–Organic + Gen-derm®, it was observed that, on day 15, a discrete amount of particles of the Gen-ox® were encircled by osteoid tissue and delicate collagen fibers. Intense fibroblastic activity and large amounts of mature bone trabeculae and medullar tissue could be observed. Cortical repair was observed on most of the cavity. At the external cortical area, a discrete amount of granulation tissue could still be noticed. On day 21, the osteoblastic activity was less evident and most of the Gen-ox® was reabsorbed. A dense concentration of fibrous tissue was observed at the cortical area closing the aperture of the cavity. At the end of the experimental period, cortical repair was complete and the defect was mostly filled by well-organized mature bone trabeculae. The graft was mostly replaced by newly formed bone, except a small area rich in collagen fibers and osteoid tissue (Fig. 5).

DISCUSSION

Although bone tissue shows good regeneration, restoring both its structure and mechanical properties, this capacity of repair may be impaired by poor blood supply, mechanical in-

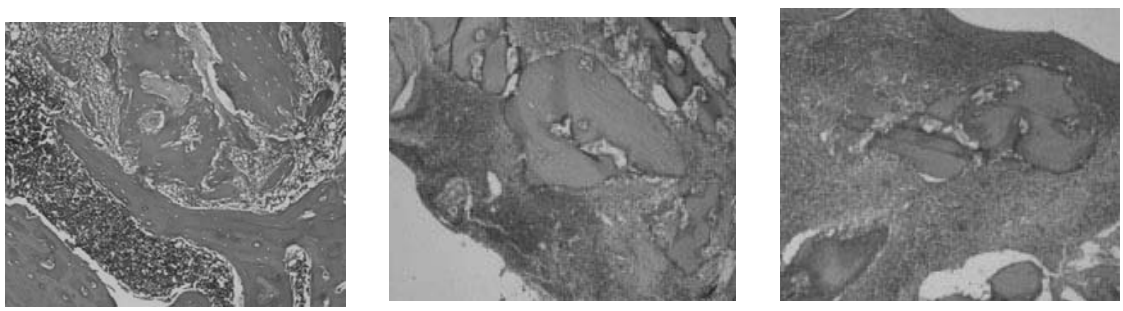

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FIG. 2. Photomicrograph of Group II. **(A)** At day 15, osteoinduction activity of the graft (arrows) was observed. **(B)** At 21 days after procedure, irregular surface on the graft particles was noticed. **(C)** At day 30, deposition of mineralized bone matrix encircling the graft could be seen. H&E, ~100× (A,B); ~40× (C).

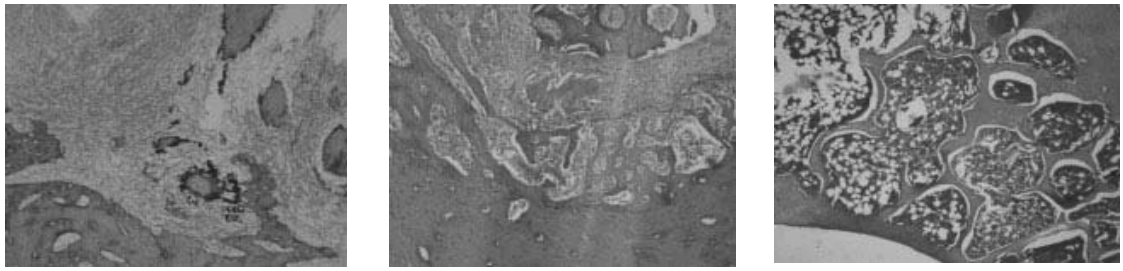

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FIG. 3. Photomicrograph of Group III. **(A)** At day 15, large amounts of collagen fibers were seen filling the cavity. **(B)** At 21 days after treatment, osteoinduction and cortical bone neoformation were evident. **(C)** At day 30, no graft particles could be seen at this time and mature bone trabeculae were observed. Picrosírius, ~40× (A); H&E, ~100× (B,C).

stability, and the presence of other tissues with higher proliferative activity. Large bone losses result in large defects, which are too big for routine bone repair. As a means of improving the recovery of large bone defects, the use of desmineralized bone matrix has been extensively studied, since it acts as both osteoinductor and osteoconductor factor of bone tissue regeneration. The osteogenic potential of this material is mainly related to the presence of bone morphogenic proteins (BMP) and growth factors (TGFb and VEGF).

Several studies demonstrated that BMPs are very effective in improving bone formation.[3,28–30] The life span of BMPs is very brief on tissular environment and its association to a carrier is important to extend it allowing a longer time of activity and resulting in osteoinduction and accelerating the repair process.[6,30,31]

GTR is a technique used to prevent the migration of soft tissues, which has more pronounced proliferative activity, into the bone defect. GTR promotes bone formation by the use of a mechanical barrier such as membranes and these may be reabsorbable or non-reabsorbable and may also be associated or not to bone substitutes.[6–8, 32–40]

Biomodulation is an area of controversy as there are conflicting results that have been reported. However, several studies have suggested a positive effect of LLLT on bone healing either *in vivo*[13,14,16,17,20,22,41–51] or *in vitro.*[19,23,25,52–55] However, some others did not find any effect of LLLT on the repair of soft or mineralized tissues.[18,56–58] These negative results may be due to the use of inappropriate wavelength.

It is important to consider also the systemic effect of the LLLT,[42,59–61] which was not considered in previous reports that did not find effects of the LLLT.[62] Other reports used very low doses.[58,63] The use of inappropriate wavelengths may also result in negative results.[63,64] Higher wavelengths are more resistant to dispersion than lower ones and do penetrate deeply into skin.[65] Previous studies reviewed by Basford[66] mentioned that 632.8-nm laser light penetrates 0.5–1 mm before losing 37% of its intensity. On the other hand, IR wavelengths penetrate 2 mm before losing the some percentile of energy, which is a clear indication for the use of IR laser light on bone tissue. However, systemic effect may not be disregarded when visible laser light is used.

The doses used in this study are similar to those in previous reports, which suggested that 1–5 J/cm^2 is effective in inducing positive effects on both bone and soft tissues.[20,22,26,42,59,61,67–69] It is important to note that four points of irradiation were used to fractionate the total dose per session. The points of irradiation around the defect were chosen because the results of irradiation of the graft and or the membrane-grafted area were unclear. The presence of the particles and or the membrane would obstruct the diffusion of light into the tissues.

A total dose per session of 16 J/cm^2 is in accordance with the clinical parameters recommended by Pinheiro.[26] However, some reports have suggested higher doses.[14,16,18,26,42] The literature shows that biomodulatory effects are dose dependent.[14,16,18,26,42] The literature shows that biomodulatory effects are dose de-

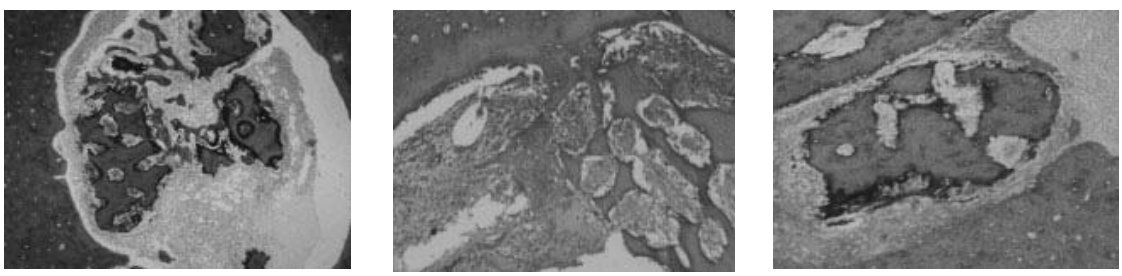

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FIG. 4. Photomicrograph of Group IV. **(A)** Ay day 15, advanced cortical repair and particles of the graft were seen within the defect. **(B)** At 21 days, osteoblastic activity and deposition of mineral bone matrix were observed. **(C)** At day 30, fragments of graft particles encircled by collagen fibers and deposition of mineralized bone matrix could be seen (arrow). Picrosírius, ~40× (A), 200× (C); and H&E, ~200× (B).

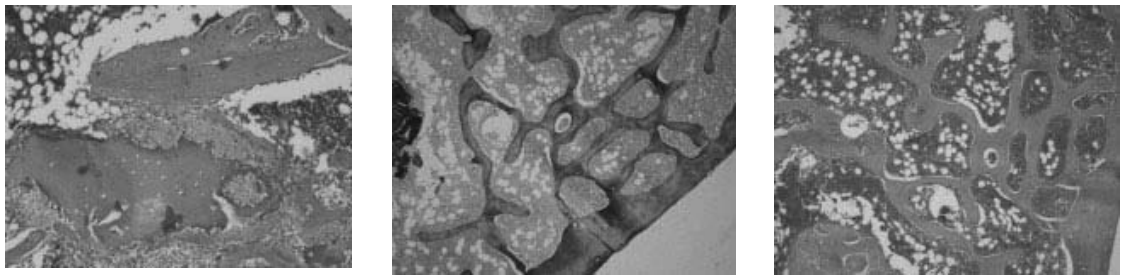

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FIG. 5. Photomicrograph of Group V. **(A)** At day 15, presence of bone trabeculi and deposition of mineralized matrix. **(B)** At 21 days, presence of giant cells near to remnants of the graft could be observed. **(C)** At day 30, presence of well-organized and distributed bone trabeculi. Picrosírius, ~40× (A), 200× (C); H&E, ~200× (B).

pendent.[16,19,42,54,70–72] It is also recognized that other factors such as the phase of cell growth,[19,73] and the frequency and number of sessions[16,22] can influence the final result of use of LLLT.

The results of the present investigation demonstrated that bone neoformation was increased and of better quality on the experimental groups when compared to their controls which throughout the experimental period showed only the presence of granulation and medullar tissues and discrete bone trabeculae. This aspect is aligned to several previous reports that showed positive effect of LLLT on bone neoformation.[13,16,19–22]

Although it was observed that the use of the Gen-ox® associated or not to the use of the membrane did not improve the speed or quality of bone formation in comparison to the control subjects; its osteoconductivity property could be noticed as bone growth was also observed originating from the particles of the graft towards the periphery as reported previously.[6–8]

The incorporation of the particles of the graft to the matrix of newly formed bone was also observed and no foreign body reaction was detectable during the experimental period. However, a few specimens showed the presence of giant cells near the particles 21 days after procedure that indicated a process of reabsorption of the graft. This was clearly seen at day 30 when the graft was mostly reabsorbed. Similar observations were reported previously.[6–8,30,74]

Histological analysis showed that, on day 15, all grafted defects were filled by particles of the implant. However, in the group in which the graft was associated to the membrane and LLLT, the particles were already being reabsorbed and discrete bone formation could be seen. In this group, it was collagen fibers were well distributed and well organized throughout the defect. As collagen fibers are precursors of bone matrix, this increased production in comparison to non-irradiated subjects shows a stimulatory effect of LLLT. Similar results were also previously reported.[13,16,19–22,43,46,47,75] These findings were further evident at the end of the experimental period as the subjects of the same experimental group showed better bone formation. It seems clear that LLLT effects, which were detectable early on day 15 on the osteoblastic activity, increased collagen formation and deposition of bone matrix improved bone repair. On the other hand, when the defect without graft was irradiated, on day 30, the differences were not so evident compared to the control and graft and membrane. This may indicate that the use of the Gen-ox® associated to LLLT resulted in better repair than the observed when the graft was used alone which was better than the control.

The effects observed on irradiated subjects might be a result of positive effects of laser irradiation on the cell membrane and mitochondria as reported previously.[60,73,74,76] Positive effects on the synthesis of DNA and RNA and on collagen synthesis and on its precursors were also reported[75] and on the level of prostaglandin and on phagocyte cytoplamatic granules[77] as well as on neovascularization and cell proliferation.[60,78] LLLT influences the production of ATP,[71,73,79,80] but LLLT seems ineffective on normal tissues.[16,53]

It seems that laser effects were due to increased levels of growth factors such as fibroblast growth factor also found on healing bone tissue that acts on differentiated cells increasing the rate of proliferation and stimulating maturation and secretion of bone matrix.[81] It is also accepted that acceleration of the repair may be a result of LLLT on the synthesis of bone matrix due to increased vascularization and early onset of the inflammatory response.[82]

The results of this study indicate that LLLT had a positive effect of bone healing following the use of organic bone graft and/or membrane. These effects may be noticed as early as 15 days after the procedure when increased amounts of collagen fibers, osteoblastic activity, and bone trabeculae formation are evident and result in a complete repair of bone defects treated with LLLT.

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