

# Effect of Low Level Laser Therapy on the Repair of Bone Defects Grafted with Inorganic Bovine Bone

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The aim of this study was to assess histologically the effect of LLLT ( $\lambda$  830 nm) on the repair of standardized bone defects on the femur of *Wistar albinus* rats which were grafted with inorganic bovine bone Gen-ox<sup>®</sup>. Three randomized groups were studied: group I (control, n=6); group II (Gen-ox<sup>®</sup>, n=9) and group III (Gen-ox<sup>®</sup> + LLLT, n=9). The animals were irradiated every 48 h during 15 days; the first irradiation was performed immediately after the procedure. The animals were irradiated transcutaneously at four points around the defect. At each point a dose of 4 J/cm<sup>2</sup> was given ( $\varnothing$ ~0.6 mm, 40 mW) and the total dose per session was 16 J/cm<sup>2</sup>. The animals were killed by an overdose of general anesthetic 15, 21 and 30 days after surgery. The specimens were routinely processed by embedding in paraffin, serially cut and stained with H&E and Picosirius and analyzed under light microscopy. The results showed evidence of a more advanced repair in the irradiated group when compared to the non-irradiated groups. The repair of the irradiated group was characterized by both increased bone formation and amount of collagen fibers around the graft within the cavity from the 15th day after surgery, also considering the osteoconductive capacity of the Gen-ox<sup>®</sup>. We conclude that LLLT had a positive effect on the repair of bone defects implanted with inorganic bovine bone.

Key Words: LLLT, bone repair, inorganic bovine bone.

## INTRODUCTION

Bone loss may be a result of various pathologies, trauma or a consequence of surgical procedures. This has led to extensive studies on the process of bone repair worldwide. Several techniques for the correction of bone defects have been proposed, amongst them, the use of several types of grafts, membranes, or the association of both techniques.

The use of inorganic bovine bone is not a new tool to improve bone repair as there are reports as early as 1889 (1). This material was used previously for the reconstruction of alveolar ridge (2), bone loss associated to both periodontal and periapical lesions (3), on

bone defects (4) and as a hemostatic agent (1).

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 (6,7), there are no previous 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.

## MATERIAL AND METHODS

Twenty-four young adult healthy male and female *Wistar albinus* rats (weight 270-320 g) were kept

under natural conditions of light, humidity and temperature at the Animal Experimentation Laboratory of the School of Dentistry of the Federal University of Bahia, Brazil. The animals were fed with laboratory diet and water *ad libitum*. The animals were divided into three groups: group I: control (N=6); group II: inorganic bovine bone (n=9); group III: inorganic bovine bone + LLLT (n=9). Each group was then divided into three subgroups (15, 21 and 30 days).

Under intraperitoneal general anesthesia (10% chloral hydrate, 0.4 ml/100 g), the right leg of the animals was shaved and the femur exposed. Standardized 3 mm<sup>2</sup> cavities were created on the superior third of the lateral side of the bone. In group I, the periosteum was repositioned and sutured with catgut and the skin closed with nylon. In groups II and III, the cavities were completely filled with inorganic bovine bone (Gen-ox<sup>®</sup>; Baumer S/A, Mogi Mirim, SP, Brazil). All wounds were routinely sutured as described previously. The animals of group III were submitted to 7 sessions of LLLT ( $\lambda$  830 nm, 40 mW,  $\varnothing$ ~0.60 mm, CW; Thera Lase, DMC Equipamentos, São Carlos, SP, Brazil), at 48-h intervals. The irradiation was performed transcutaneously and the first session was performed immediately after surgery. A dose of 4 J/cm<sup>2</sup> was applied to 4 points around the defect giving a total of 16 J/cm<sup>2</sup> per session and a total treatment dose of 112 J/cm<sup>2</sup>. This was based on previous studies (8) recommending doses ranging from 1.8 to 5.4 J/cm<sup>2</sup>. The animals were killed 15, 21 and 30 days after surgery by an overdose of general anesthetic. 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 (9).

## RESULTS

Light microscopy showed that at day 15 the cavity of group I (control) was filled by bone marrow and a few bone trabeculae. The cortical defect was more pronounced 21 days after surgery and nearly closed at day 30 (Figure 1).

In group II (Gen-ox<sup>®</sup>), the graft filled nearly the entire cavity and was encircled by collagen fibers 15 days after surgery. Immature bone trabeculae were observed at the graft surface but no newly formed bone around the graft could be seen at this time. Up to day 30 after surgery, the graft particles were evenly distributed in the cavity and were partially or totally encircled by newly formed bone. Newly formed bone trabeculae were seen initiating repair of the cortical region (Figure 2).

Fifteen days after surgery, specimens of group III (Gen-ox<sup>®</sup> + LLLT) showed that the graft filled most of the cavity and was also encircled either by collagen fibers or mineralized newly formed bone. At the cortical region, some neoformed bone trabeculae around some particles of the graft and a dense amount of collagen fibers could be observed. Twenty-one days after surgery, an increased amount of neoformed bone could be seen and the graft was encircled by collagen fibers. At the end of the experimental period, the cavity was still filled by the graft and the area was densely filled by collagen fibers (Figure 3).

## DISCUSSION

It seems clear from the results that Gen-ox<sup>®</sup> has osteoconductivity because all grafted specimens showed

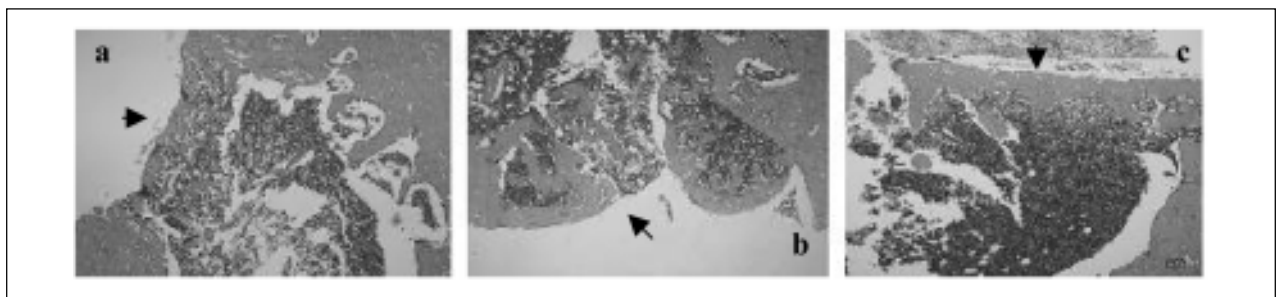


Figure 1. Photomicrography of control group. Left: At day 15, there was discrete bone formation at the cortical defect (arrow). Middle: At day 21, the deposition of new bone at the cortical region was more evident (arrow). Right: At day 30, there was a complete union of the cortical region (arrow). H&E. Original magnification: approximately 40X.

bone neoformation around the particles. It is interesting to observe that most specimens showed the particles of the graft encircled by collagen fibers and filling the center of the cavity at day 15. At day 21, these particles were unevenly distributed and were encircled by a variable amount of newly formed bone. The level of bone neoformation did not change much up to day 30 in most groups.

It is accepted that although this material has osteoconductivity, the repair of the defect may be slow because of the need for the graft to be reabsorbed, slowing down the process. In this study, the presence of macrophages and giant cells around the particles, as observed previously (2,10), may explain the reduction of the amount of particles throughout the experimental time. On the other hand, a previous report suggested that this type of material may also be incorporated into the newly formed bone (4,5).

It is clear that the use of the graft prevents the

formation of fibrosis of the lesion and also protects the cavity (4) and acts as a framework for the deposition of neoformed bone (11).

Although the biomodulatory effect of LLLT on bone regeneration was observed previously under different experimental conditions and the results observed in these studies (6,7) are aligned with the ones of the present investigation, others have found no effect (12,13). This may be due to several aspects of the design of the experiment.

It is important to also consider the systemic effect of LLLT (14), which was not considered in some previous reports in which no effects of LLLT were found (16). Other reports used very low doses (12). The use of inappropriate wavelengths may also result in negative results (12). Higher wavelengths are more resistant to dispersion than lower ones and deeply penetrate the skin (15). It has been reported that 632.8 nm laser light penetrates 0.5-1 mm before losing 37%

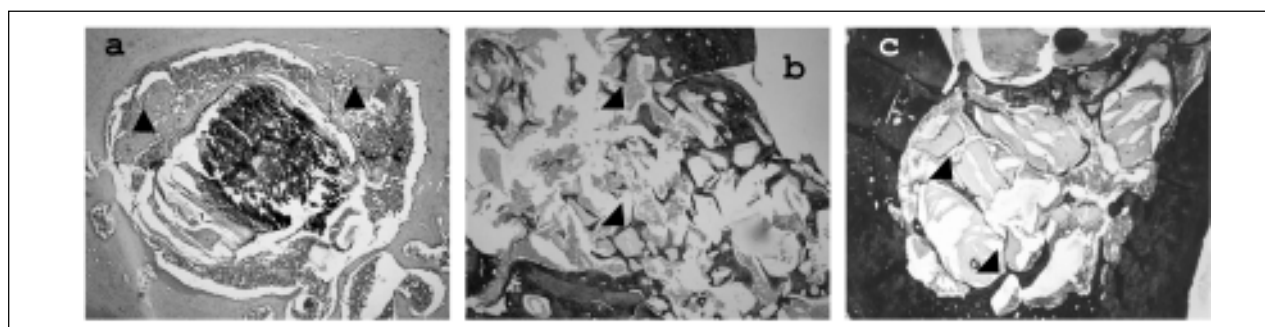


Figure 2. Photomicrography of group II. Left: At day 15, cortical repair was observed and also the presence of delicate newly formed bone within the cavity near the graft surface (arrowheads). At days 21 (middle) and 30 (right), newly formed bone could be seen around the graft (arrowheads). Left panel: H&E; middle and right panels: Picosirius. Original magnification: approximately 40X.

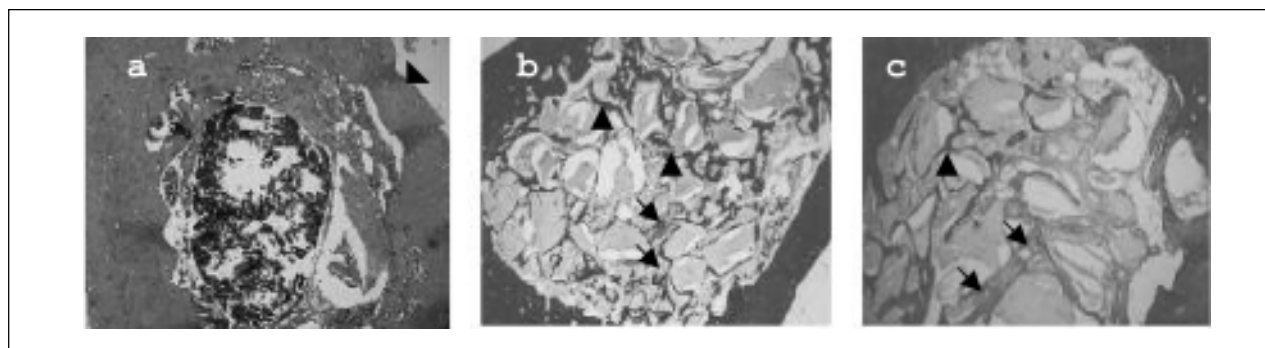


Figure 3. Photomicrography of group III. Left: At day 15, complete cortical repair was observed (arrowhead). At days 21 (middle) and 30 (right), newly formed bone could be seen (arrowheads) and also collagen fibers encircling the graft (arrows); Left panel: H&E; middle and right panels: Picosirius. Original magnification: approximately 40X.

of its intensity (16). On the other hand, infrared wavelengths penetrate 2 mm before losing the some percentile of energy. This is a clear indication for the use of infrared laser light on bone tissue. However, systemic effects may not be disregarded when visible laser light is used.

The presence of large amounts of collagen fibers on irradiated specimens was the major finding in these groups and this may represent an early effect of the LLLT on repair as other studies demonstrated increased collagen production following LLLT (17). As collagen is an important component of the extracellular matrix of bone and increased amounts, as seen in this study, may indicate a positive effect of LLLT on bone healing despite the fact that in some specimens there was no difference in the amount of newly formed bone between the irradiated group and controls. It is necessary to consider that a large amount of collagen fibers will represent an increased bone neoformation after mineralization of the matrix. The mechanism by which LLLT interferes in collagen synthesis is not fully understood; however, it may be because of alterations in the genetic regulation or in the modulation of enzymatic activity involved in the metabolism of the collagen as suggested previously (17).

It is uncertain whether biomodulation of bone formation is an overall effect on mesenchymal cells or a direct stimulation of osteoblasts. It is possible that the observed results in irradiated specimens are due to an increased release of growth factors, mainly fibroblast growth factor, which is found in bone tissue and acts on differentiated cells increasing both cell proliferation and secretion of components of the matrix (18).

The doses used in this study are in agreement with several previous reports that suggested that 1-5 J/cm<sup>2</sup> induces positive effects on both bone and soft tissues (6-8,17). 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 would be uncertain. The presence of the particles would make the diffusion of light into the tissues more difficult.

A total dose per session of 16 J/cm<sup>2</sup> is in accordance with the clinical parameters recommended by Pinheiro et al. (8). The literature shows that biomodulatory effects are dose dependent (19). It is also recognized that other factors such as the phase of cell growth

(20) and the frequency and number of sessions (7) also influence the final result of the use of LLLT.

When groups II and III were compared, it was evident that bone neoformation was similar at day 15. However, at days 21 and 30, bone formation was more evident in both the core of the cavity and in the cortical region. Although irradiated specimens showed a dense deposition of collagen fibers around the particles in the core of the defect and at the cortical region, the same was not detected in control specimens, especially in the core of the defect.

We conclude that LLLT had a positive biomodulatory effect on the repair of bone defects implanted with inorganic bovine bone.

## RESUMO

O objetivo deste trabalho foi avaliar histologicamente a influência da radiação laser não-cirúrgica ( $\lambda$  830 nm) sobre o reparo ósseo de defeitos padronizados em fêmur de ratos *Wistar albinus* e submetidos a implante de osso bovino inorgânico Gen-ox<sup>®</sup>. Foram estabelecidos três grupos de animais: grupo I (controle, n=06); grupo II (Gen-ox<sup>®</sup>, n=09) e grupo III (Gen-ox<sup>®</sup> + LLLT, n=09). Os animais foram irradiados a cada 48 horas, sendo a primeira irradiação realizada imediatamente após a cirurgia. Os animais foram irradiados transcutaneamente em quatro pontos em torno da ferida. Cada ponto recebeu uma dose de 4 J/cm<sup>2</sup> ( $\varnothing$ ~0,6 mm, 40 mW) e a dose total por sessão foi de 16 J/cm<sup>2</sup>. Os sacrifícios foram realizados 15, 21 e 30 dias após a cirurgia. Os espécimes foram removidos e fixados para procedimento laboratorial, sendo corados com H&E e Picrosirius e analisados em microscopia de luz. Os resultados mostraram evidência de um reparo ósseo mais avançado nos espécimes irradiados em relação aos não irradiados. O reparo nos espécimes irradiados foi caracterizado por uma maior neoformação óssea, bem como por uma maior proliferação de fibras colágenas no interior do defeito já a partir de 15 dias após a cirurgia, também considerando a capacidade osteocondutiva do Gen-ox<sup>®</sup>. Conclui-se que a LLLT resultou num efeito de biomodulação positiva sobre o reparo do defeito ósseo submetido a implante de osso bovino inorgânico.

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