Brazilian Dental Journal (2015) 26(1): 19-25
http://dx.doi.org/10.1590/0103-6440201300055
ISSN 0103-6440

Repair of Surgical Bone Defects Grafted with Hydroxylapatite + β-TCP and Irradiated with λ=850 nm LED Light

Luiz Guilherme P. Soares[1], Aparecida Maria C. Marques[1,2], Milena G. Guarda[1], Jouber Mateus S. Aciole[1], Antonio Luiz B. Pinheiro[1,2,3], Jean Nunes dos Santos[4]

[1]Center of Biophotonics, Dental School, UFBA - Federal University of Bahia, Salvador, BA, Brazil
[2]National Institute of Optics and Photonics, Physics Institute of São Carlos, USP - University of São Paulo, São Carlos, SP, Brazil
[3]Camilo Castelo Branco University, São José dos Campos, SP, Brazil
[4]Laboratory of Surgical Pathology, Dental School, UFBA - Federal University of Bahia - Salvador, BA, Brazil

Correspondence: Prof. Dr. Antônio Luiz Barbosa Pinheiro, Av. Araújo Pinho, 62, Canela, 40110-150 Salvador, BA, Brasil. Tel.: +55-71-3283-9010. e-mail: albp@ufba.br

The treatment of bone loss due to different etiologic factors is difficult and many techniques aim to improve the repair, including a wide range of biomaterials and recently, photobioengineering. This work aimed to assess by histological analysis the repair of bone defects grafted with biphasic synthetic micro-granular HA + β-TCP associated with LED phototherapy. Forty rats were divided into 4 groups (Clot, LED, Biomaterial and LED + Biomaterial) each subdivided into 2 subgroups according to the time of animal death (15 and 30 days). Surgical bone defects were prepared on the femur of each animal with a trephine drill. In animals of the Clot group the defect was filled only by blood clot, in the LED group the defect filled with the clot was further irradiated. In the animals of Biomaterial and LED + Biomaterial groups the defect was filled by biomaterial and the last one was further irradiated (λ=850±10 nm, 150 mW, Φ ~ 0.5 cm^2, 20 J/cm^2 - session, 140 J/cm^2- treatment) at 48-h intervals for 2 weeks. Following animal death, samples were taken and analyzed by light microscopy. Using the degree of maturation of the bone by assessment of the deposition/organization of the basophilic lines in the newly formed bone tissue, the LED + Biomaterial group was the one in a more advanced stage of bone repair process at the end of the experiment. It may be concluded that the use of LED phototherapy was effective in positively modulating the process of bone repair of bone defects in the femur of rats submitted or not to biomaterial grafting.

Key Words: biomaterial, bone repair, hydroxyapatite, LED phototherapy.

Introduction

Bone loss is a frequent challenge for surgeons, as it may be caused by several etiologic factors (1) and the defects may be too large for spontaneous and physiologic repair. Autologous bone is the most common type of graft used to help the repair and it may be harvested from several parts of the skeleton. Biocompatibility and osseointegration, as well as substantial osteogenic potential characterize autologous bone grafts (2,3).

Recent studies have shown that LED phototherapy (LED-PT) induces a quicker repair process, with good quality newly formed bone. These features were observed in many studies in which this group used similar parameters to the ones carried out with Laser Phototherapy (LPT) or LED-PT (2-8). It seems that the beneficial effects of LED-PT are similar to those of the laser. It is possible that the mechanism involved is similar to the light absorption by the cytochrome-C-oxidase present in the mitochondrial membrane (9,10). Despite the increase of successful reports of applications of different phototherapies in many areas, their use in bone repair associated with bone grafting with biomaterials needs to be further studied (4-8).

The combination of HA + β-tricalcium phosphate (β-TCP) graft and phototherapies seems promising due to the fact that the biomaterial possesses osteoconductive properties and phototherapy influences function, proliferation, secretion of growth factors such as bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β) by different types of cells. This association may modulate the repair of bone defects in a manner similar to what is observed following the use of autologous bone graft, preventing its complications and limitations (2,4,5,8,11,12). However, the use of autologous grafts remains the gold standard for the treatment of bone defects (2,3).

The aim of this study was to assess, by light microscopy, the repair of bone defects grafted or not with biphasic synthetic micro-granular HA + β-TCP combined or not with LED phototherapy (λ=850±10 nm).

Material and Methods

Ethics, Animal Model, Housing and Sampling

The Animal Ethics Committee of the Federal University of Bahia approved this work (Protocol 08.2010). Forty healthy adult male Wistar rats (~2 months old, average weight 295±25 g) were kept under natural conditions of light, humidity and temperature at the Laboratory of Animal Experimentation of the Dental School of the Federal University of Bahia during the experimental period. The animals were fed a standard laboratory diet (Labina®; Purina,

São Paulo, SP, Brazil) and water *ad libitum*. The animals were kept in individual micro-isolators and accommodated in ventilated shelves (Insight Equipamentos Ltda, Monte Alegre, Ribeirão Preto SP, Brazil). This system provides a controlled environment with decreased risk of infection and good sanitary condition. Controlled day/night light cycle and temperature was performed during the experimental period. The animals were randomly distributed into 4 groups and then divided into 2 subgroups according to the moment of animal death (Table 1).

Surgical Procedure

Prior to intramuscular general anesthesia, the animals were sedated (0.04 mL/100g of atropine subcutaneously) and 20 min later general anesthesia with 10% ketamine (0.1 mL/100g, Cetamin®, Syntec, São Paulo, SP, Brazil) and 2% xylazin (0.1 mL/100g; Xilazin®, Syntec) was carried out. The animals had their right leg shaved and a 3-cm-long incision was performed at the right tibia with a #15 scalpel blade. Skin and subcutaneous tissues were dissected down to the periosteum, which was gently sectioned exposing the bone and a standard partial thickness 2-mm round defect was surgically produced using trephine drill (SIN, São Paulo, SP, Brazil) mounted on a 16:1 reduction contra-angle handpiece (NSK; Utsonomya, Japan), maximum resistance of 35 N with low speed drill, 1.200 rpm, under refrigeration (Driller 600; SIN) in each animal (Fig. 1A) (4). Defects on animals in Clot and LED groups were filled only with the blood clot. Defects in Biomaterial and LED + Biomaterial groups were filled with biomaterial. The animals in LED and LED + Biomaterial groups were further irradiated. All wounds were sutured and the animals received a single dose of antibiotics (Pentabiotico; 0.2 mL; Fort Dodge Animal Health, Kansas City, MO, USA). A biphasic synthetic micro-granular HA + β-TCP (70%/30% respectively) completely filled the bone defects when indicated, as recommended by the manufacturer (Fig. 1B) (4).

Table 1. Distribution of study groups

Group (n = 10 animals)	Time of euthanasia (d)	Phototherapy	Biomaterial
Clot	15 or 30	-	-
Biomaterial	15 or 30	-	Yes
LED	15 or 30	Yes	-
LED + Biomaterial	15 or 30	Yes	Yes

Phototherapy Protocol

LED phototherapy was carried out using a LED device (FisioLED; MMOptics , São Carlos, SP, Brazil) λ=850±10 nm, 150 mW, $\Phi \sim 0.5$ cm^2, 20 J/cm^2 and light was transcutaneously applied over the defect at 48-h intervals, the first session carried out immediately after surgery. The animals were restrained with the use of a plastic apparatus designed to keep the animal immobile during irradiation, avoiding the use of sedation. Total energy delivered was 20 J/cm^2 per session and 140 J/cm^2 per treatment. Energy densities used were based upon previous studies carried out by the authors (1-8,11,12). LED output power was confirmed by using a calibrated power meter (Thorlabs PM30-121; Thorlabs GmbH, Munich, Germany).

Animal Death and Sample Manipulation

Following animal death, the samples were longitudinally cut under refrigeration (Buehler Isomet® 1000; Buehler, Markham, Ontario, Canada) and the specimens kept in 10% formalin solution for 24 h. The specimens were routinely processed to paraffin, cut and stained with hematoxylin and eosin and Sirius red, and underwent histological analysis (Table 2) at the Laboratory of Surgical Pathology of the Dental School of the Federal University of Bahia by an experienced pathologist in a blind manner using a light microscope (AxioStar®, Zeiss, Jena, Germany).

Results

Clot Group

On the15th day, the defect was partially filled by newly

Table 2. Semi quantitative criteria used for light microscopy analysis

Criteria	Discrete	Moderate	Intense
Bone resorption	Presence of <25% of resorption of graft remnants and/or surgical bed	Presence of 25-50% of resorption of graft remnants and/or surgical bed	Presence of >50% of resorption of graft remnants and/or surgical bed
New bone formation	Presence of <25% of newly formed bone similar to adjacent untreated bone tissue	Presence of 25-50% of newly formed bone similar to adjacent untreated bone tissue	Presence of >50% of newly formed bone similar to adjacent untreated bone tissue
Inflammatory infiltrate	Presence of <25% of inflammatory cells on the area	Presence of 25-50% of inflammatory cells on the area	Presence of >50% of inflammatory cells on the area
Collagen deposition	Presence of <25% of collagen deposition in the area	Presence of 25-50% of collagen deposition in the area	Presence of >50% of collagen deposition in the area

formed bone, which was characterized by the presence of thin, interconnected or not, trabecular bone showing osteocytes and irregular basophilic lines within it and osteoblastic rimming. There was a moderate chronic inflammation and cartilaginous differentiation. At the end of the experimental period, the specimens of this group showed the defect completely filled by newly formed bone, but unlike the earlier period, the bone trabeculae were thick and basophilic lines were parallel to each other (Fig. 2A). Osteocytes within it were also frequent as well as sometimes a layer of rimming osteoblasts. The inflammation was chronic and ranged from moderate to severe in both experimental periods. The collagen deposition was intense and it was scored mostly as mature (Fig. 2B).

Biomaterial Group

On the 15th day, the defect was completely filled by newly formed bone characterized by a varied trabecular pattern, sometimes showing the presence of irregular osteocytes and rimming of osteoblasts. Often, these trabeculae were interwoven and surrounding the amphophilic material entrapped in small or large amounts, which was interpreted as being remnants of the biomaterial (Fig. 3A). In the area corresponding to the biomaterial, few layers of rimming osteoblasts were seen as well as foreign body reaction. It was characterized by presence of giant cells around the remnants. Discrete to moderate mixed inflammation permeated the entire specimen. Mature collagen was observed in the newly formed bone tissue, but it was absent in the remnants of the biomaterial. At the end of the experimental period most of the specimens of this group showed similar aspect to the previous period. The defect was completely filled by thick interconnecting trabecular bone. Sometimes in the form of globules, many of these trabeculae were seen surrounding the amphophilic material (Fig. 3B). The collagen deposition was intense and collagen was graded as mature in most specimens (Fig. 3C).

LED Group

After 15 days, the specimens of this group showed the defect partially filled by newly formed bone displaying thin interconnecting bone trabeculae characterized by osteocytes within and filled with red bone marrow (Fig. 4A). No signs of resorption were present, but a discrete to moderate inflammation was observed. The collagen deposition was intense and it was mature in the newly formed bone. At the 30th day, the specimens showed the defect either partially or completed filled by newly formed

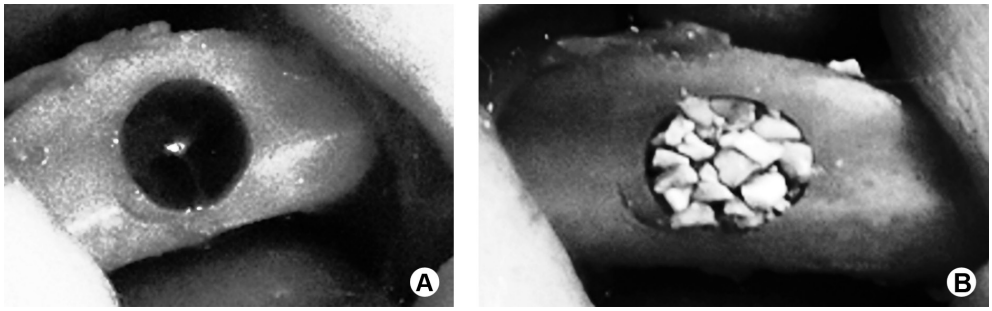

Figure 1. A: Clinical aspect of the surgical bone defect created on the right tibia of each animal. B: Clinical aspect of the surgical bone defect created on the right tibia of each animal filled with the biomaterial (4).

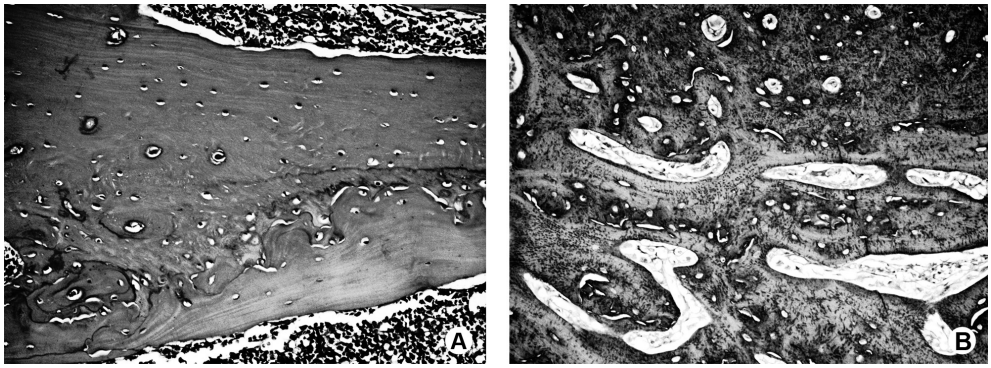

Figure 2. A: Photomicrograph of control specimen evidencing the defect completely filled by thick and mature newly formed bone showing osteocytes and parallel basophilic lines (30 days - HE). B: Photomicrograph of control specimen evidencing newly formed bone with mature collagen throughout its length (30 days - Picrosirius).

interconnecting trabecular bone of varied thicknesses, characterized the presence of osteocytes and nonparallel basophilic lines. Sometimes the trabeculae showed rimming osteoblasts (Fig. 4B). There were no areas of bone resorption. Cartilaginous differentiation was also evident as well as the presence of chronic inflammation that ranged from discrete to moderate. Collagen deposition in the bone trabeculae was intense and mature (Fig. 4C).

LED + Biomaterial Group

At the 15th day, the specimens showed a small surgical defect filled by newly formed bone, characterized by the presence of globules or thin interconnecting bone trabeculae and the presence of osteocytes and parallel basophilic lines permeated by moderate chronic inflammation (Fig. 5A). Cartilaginous differentiation was also present, as well as rare presence of focal areas of an amphophilic material, interpreted as remnants of the biomaterial, and foreign body giant cell reaction. The collagen was moderately mature. At the end of the experimental period, the specimens of this group showed the defect completely repaired in which new bone formation was observed with trabecular bone tissue of variable thickness, sometimes in the form of globules, showing few osteocytes and basophilic lines parallel to each other. Many of these trabeculae were seen surrounding amphophilic material interpreted as remnants of the biomaterial. Chronic inflammation was observed and ranged from discrete to moderate (Fig. 5B). Resorption was observed in only one specimen. The collagen was mature and intense in all samples (Fig. 5C).

Discussion

The reason for combining HA + β-TCP graft and LED light in this study was due to the osteoconductive properties of the biomaterial and previous reported positive effects of the LED-PT on both function and proliferation of cells. These effects are observed when the techniques are used in isolation and have been shown effective in accelerating the repair process in various experimental models. It was hypothesized that combination of both techniques would modulate the repair of bone defects grafted with a biomaterial and irradiated with LED light to a pattern

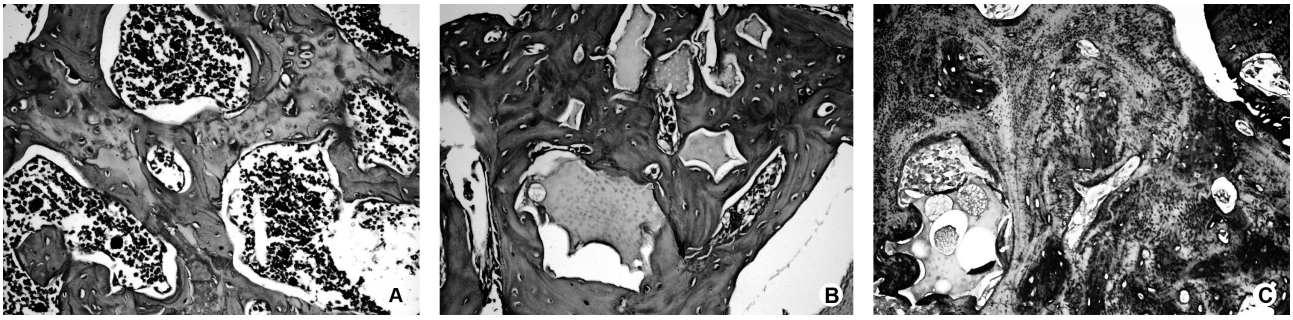

Figure 3. A: Photomicrograph of specimen from Biomaterial group showing on the right side the remaining bone from the surgical bed from which grows thin interconnecting trabecular bone with osteocytes inside and imprisoned remnants of the biomaterial (15 days - HE). B: Photomicrograph of specimen from Biomaterial group showing new bone formation characterized by predominantly thick trabecular bone with osteocytes inside and basophilic lines parallel to each other. Note the remaining biomaterial imprisoned by newly formed bone and chronic inflammation (30 days - HE). C: Photomicrograph of specimen from Biomaterial group showing on the right side bone from the surgical bed from which depart newly formed bone trabeculae with collagen of similar aspect to the surgical bed, but unevenly distributed in the biomaterial (30 days - HE).

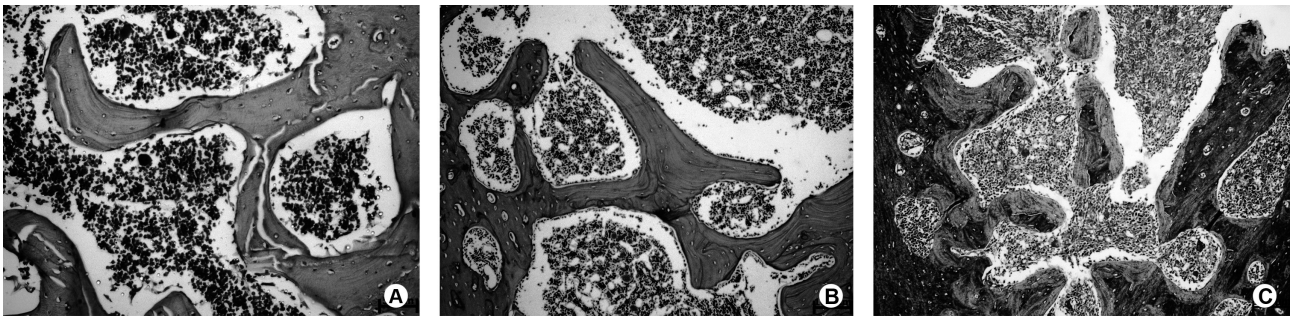

Figure 4. A: Photomicrograph of specimen from LED group showing discrete amount of newly formed bone characterized by thin trabecular bone with osteocytes and basophilic lines inside (15 days - HE). B: Photomicrograph of specimen from LED group showing discrete amount of newly formed bone characterized by thin trabecular bone with osteocytes, basophilic lines inside and rimming osteoblasts (30 days - HE). C: Photomicrograph of specimen from LED group showing new bone formation presenting mature collagen throughout its length (30 days - Picrosirius).

similar to what occurs when autologous bone graft is used as well as on preventing complications and reducing limitations. However, so far the use of autologous bone grafting remains the gold standard for the treatment of bone defects.

Despite being one of the most common synthetic bone graft and able to successfully regenerate bone in various osseous defect areas, β-TCP is also known for its rapid resorption rate which may precede the growth of new bone. Because of this, its association to HA has been used, since HA has low osteoconductive activity but a good space-maintaining capacity, whereas β-TCP is more bioresorbable and is rapidly replaced by new bone material. This association makes one part of the graft to be rapidly resorbed (TCP) and the other (HA) remain *in situ* for a longer period. Possibly higher HA/β-TCP ratios yield more replacement of biomaterial by new bone (13).

There is no full agreement on regards the most effective ratio of HA/β-TCP. It was suggested that 60% HA and 40% β-TCP seemed to provide the optimal bone conductive properties. For periodontal defects, a ratio of 85/15 has been suggested as optimal for repairing surgically created bone defects (13). However, a ratio of 60/40 was mentioned most suitable in a previous animal study (13). In the present study the HA/β-TCP ratio was 70:30, which could be considered within the range for optimal tissue response. It has been suggested that higher ratio of β-TCP could theoretically increase the degradation of the particles of the biomaterial and that biphasic HA/β-TCP composite powders exhibit a solubility between those of HA and β-TCP the dissolution rate of the Ca-P being strongly dependent on the content of β-TCP. It was initially thought that different ratios could induce different outcomes as regards bone formation and biodegradation. However, previous reports failed to find different rates of new bone formation when using different ratios but found as regards resorption of the material (13).

The authors have recently discussed that the utility of the of residual bone graft materials may vary with the type of defect where the materials are applied and that the biomaterials used shall allow osteoblasts to build bridges between its granules and integrate with other osteoblasts providing support for both proliferation and differentiation at earlier phases of the repair. This will then result in intrinsic stimulation of new bone formation (13).

The biomaterial used on the present study is granular and porous and besides that, it contains Ca and P that favor the early phases of repair. The presence of β-TCP in the graft used on the present investigation has shown to influence the mechanical stability of the graft and makes possible its rapid degradation. This aspect results in some volume instability of the graft that does not allow new bone formation to keep the original volume. In addition to the several advantages of using this type of biomaterial, it also present disadvantages that include poor mechanical properties, lack of an organic phase, the possibility of the presence of impurities and non-homogenous particle size and shape. Another aspect that influences the results of studies using such type of biomaterials is that the Ca/P ratio may differ according to the manufacturer (13).

In a previous study it was also mentioned that a usual concern when using granular biomaterials is granule-mediated inflammatory reaction (13). In the present study, inflammatory reaction was well documented in all groups where the biomaterial was used. In the present study no sign of fibrosis was seen.

For evaluation of bone repair were chosen two experimental periods, 15 and 30 days. During the initial stages of bone repair, the cellular component (mainly fibroblasts and osteoblasts) is more prominent and more prone to be affected by light. At 30 days, the repair process is in an advanced stage and is widely used for evaluation of bone healing in several published studies (1-3,6,7,14-16).

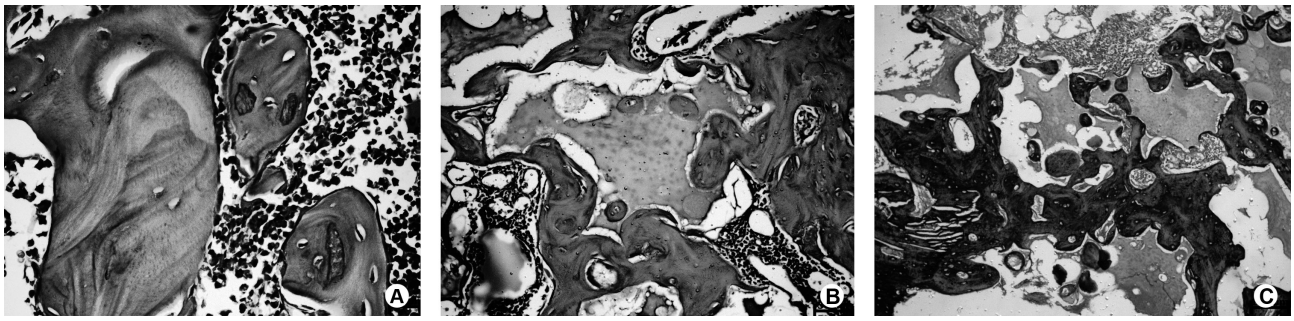

Figure 5. A: Photomicrograph from LED + Biomaterial group showing new bone formation of globular aspect with biomaterial imprisoned amidst chronic inflammation (15 days - HE). B: Photomicrograph of specimen from LED + Biomaterial group showing new bone trabeculae and globules evidencing the osteocytes and parallel basophilic lines inside. Notice presence of the biomaterial in the remaining central region and remaining red bone marrow (30 days HE). C: Photomicrograph of specimen from LED + Biomaterial group showing mature collagen (intense red color) unlike the aspect observed in the remnant of the biomaterial surrounded by the newly formed bone (30 days - Picrosirius).

In the present study, it was chosen to conduct a qualitative histological analysis, to describe the mechanisms involved in bone repair of defects treated or not with LED phototherapy associated or not with biphasic HA + β-TCP. The description followed the guidelines established in the methodology.

The phenomena observed in non-grafted groups were similar to the previously reported according to the used methodology. Therefore, no major differences were observed histologically in the repair process groups, Clot and LED in both experimental periods. Therefore, it appears that the irradiated groups showed a more advanced repair process and quality than observed in the non-irradiated group (Clot) as may be seen in Figures 2-5 (7,8,17).

The observation of inflammatory response and, in some cases, the presence of foreign body reaction around the biomaterial is an expected finding. No matter how the biomaterial is biocompatible, it will continue to be a foreign material. It is interesting to observe that in a previous study using another biomaterial (MTA - mineral trioxide aggregate), this reaction was detected in a more striking and intense manner than observed in the present study. This may be an indication that the material used in this work is less irritating to the tissue that the MTA (7). Besides, the fact that inflammation was limited it surely caused a physiological effect that would in some extent be deleterious to the repair process resulting in complications like fibrosis. However, in the present study no sign of fibrosis was seen. At the end of the experimental period, there was an intense inflammatory response in the Clot group, where no additional treatment was used, different from what was observed in all other groups. This can be explained by previous studies which indicated that the persistence of the inflammatory response in the later stages of bone healing might be the result of phlogistic activity caused by the remnants of blood clot (8).

For all parameters in this study, the use of the Biomaterial + LED showed the best results. This has been described in previous studies in which the use of LED was associated with increased proliferation of fibroblasts, chondroblasts and osteoblasts and hence increased deposition of collagen, an important precursor of the mineral matrix deposition (7,8,17). Thus, the increase in bone formation is probably closely linked with both the increased numbers of osteoblasts and their secretory activity.

The presence of a cartilaginous precursor was observed only at 15 days, in Clot and LED groups + Biomaterial, which denote a reparative process in a more advanced stage than in other groups. It is interesting to observe that at the end of the experimental period only the LED group showed this.

The trabecular aspect also varied between groups. In the initial period of the repair, the trabeculae were thin in Clot, LED, and LED + Biomaterial groups and in a variable manner in the other groups. At the end of the experimental period, the trabeculae were thicker in Clot and Biomaterial groups and of varied thickness in the other groups. Interesting was the observation of the remodelling activity, which was still present at this time, as evidenced by the presence of basophilic lines. These lines were observed in most groups at the end of the experimental period and were sometimes parallel or not. These lines were not observed in Biomaterial group. In fact, this aspect seems to have influenced mainly the defects of Clot group in which the presence of cartilage seems to have influenced the results. It was the group in which the cartilage apparently progressed to a trabecular bone which varied from thin to thick along the observation time. The reason for this needs further clarification and can be related either to the presence of the biomaterial or to the light source, which may have accelerated or delayed the differentiation of cartilage in some way. This could be demonstrated in a study using intermediate times between the two this work, as well as use of specific markers for this tissue.

Regarding the basophilic lines present in bone tissue, they were observed initially (15 days) in all groups except in the Biomaterial group. However, only in the LED + Biomaterial group these lines were deposited in a regular pattern, parallel to each other, which may be indicative of a more mature bone already in the initial repair. At the end of the experimental period the previously observed pattern was maintained. The fact that the Biomaterial group did not show these lines may be indicative of a slower or even delayed remodelling process.

Using the degree of maturation of bone by assessment of the deposition/organization of the basophilic lines in the newly formed bone tissue, the LED + Biomaterial group was the one in a more advanced stage of bone repair process at the end of the experiment. It may be concluded that the use of LED phototherapy was effective in positively modulating the process of bone repair of bone defects in the femur of rats submitted or not to biomaterial grafting.

Resumo

O tratamento de perdas ósseas devido a diferentes fatores etiológicos é difícil e muitas técnicas têm por objetivo melhorar o reparo incluindo o uso de uma ampla gama de biomateriais e, recentemente, a fotobioengenharia. Este trabalho teve como objetivo avaliar, por meio de análise histológica, o reparo de defeitos ósseos enxertados com HA bifásica micro-granular sintética + β-TCP associada à fototerapia LED. Quarenta ratos foram divididos em quatro grupos (Clot, LED, Biomaterial e LED + Biomaterial), subdivididos no dois subgrupos de acordo com o momento da morte (15 e 30 dias). Defeitos ósseos cirúrgicos foram criados em um fêmur de cada animal com uma broca trefina. Em animais do grupo coágulo, o defeito foi preenchido apenas pelo coágulo sanguíneo, no grupo de LED o defeito foi preenchido pelo coágulo e irradiado. Nos animais dos grupos do biomaterial e LED + biomaterial, os defeitos foram preenchidos com biomaterial e o último foi adicionalmente irradiado (λ=850±10 nm, 150 mW, $\Phi \sim 0{,}5$ cm^2, 20 J/cm^2-sessão, 140 J/cm^2-tratamento) a cada 48 h

por duas semanas. Após a morte dos animais, amostras foram colhidas e analisadas por microscopia de luz, usando o grau de maturação do osso como marcador (deposição/organização das linhas basofílicas) no tecido ósseo neoformado. O grupo de LED + biomaterial apresentou processo de reparação mais avançado ao fim do experimento. Pode-se concluir que o uso da fototerapia LED foi eficaz na modulação positiva do processo de reparo ósseo de defeitos ósseos no fêmur de ratos submetidos ou não a enxerto com biomaterial.

Acknowledgements

We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for providing financial support for this project.

References

1. Pinheiro ALB, Gerbi MEMM. Photoengineering of bone repair processes. Photomed Laser Surg 2006;24:169-178.
2. Pinheiro ALB, Aciole GTS, Cangussú MCT, Pacheco MTT, Silveira Jr L. Effects of laser phototherapy on bone defects grafted with mineral trioxide aggregate, bone morphogenetic proteins, and guided bone regeneration: A Raman spectroscopic study. J Biomed Mater Res A 2010;95:1041-1047.
3. Weber JBB, Pinheiro ALB, Oliveira MG, Oliveira FAM, Ramalho LMP. Laser therapy improves healing of bone defects submitted to autogenous bone graft. Photomed Laser Surg 2006;24:38-44.
4. Pinheiro ALB, Soares LGP, Barbosa AFS, Ramalho LMP, dos Santos JN. Does LED phototherapy influence the repair of bone defects grafted with MTA, bone morphogenetic proteins, and guided bone regeneration? A description of the repair process on rodents. Lasers Med Sci 2012;27:1013-1024.
5. Pinheiro ALB, Soares LGP, Cangussú MCT, Santos NR, Barbosa AFS, Silveira Jr L. Effects of LED phototherapy on bone defects grafted with MTA, bone morphogenetic proteins and guided bone regeneration: a Raman spectroscopic study. Lasers Med Sci 2012;27:903-916.
6. Torres CS, Santos JN, Monteiro JSC, Gomes PTCC, Pinheiro ALB. Does the use of laser photobiomodulation, bone morphogenetic proteins, and guided bone regeneration improve the outcome of autologous bone grafts? An *in vivo* study in a rodent model. Photomed Laser Surg 2008;26:371-377.
7. Pinheiro ALB, Gerbi MEMM, Limeira Jr FA, Ponzi EAC, Marques AMC, Carvalho C, et al.. Bone repair following bone grafting hydroxyapatite, guided bone regeneration and infrared laser photobiomodulation: a histological study in a rodent model. Lasers Med Sci 2009;24:234-240.
8. Pinheiro ALB, Gerbi MEMM, Ponzi EAC, Ramalho LMP, Marques AMC, Carvalho CM, et al.. Infrared laser light further improves bone healing when associated with bone morphogenetic proteins and guided bone regeneration: An *in vivo* study in a rodent model. Photomed Laser Surg 2008;26:167-174.
9. Pinheiro ALB, Soares LGP, Aciole GTS, Correia NA, Barbosa AFS, Ramalho LMP, et al.. Light microscopic description of the effects of laser phototherapy on bone defects grafted with mineral trioxide aggregate, bone morphogenetic proteins, and guided bone regeneration in a rodent model. J Biomed Mater Res part A 2011;98:212-221.
10. Gerbi MEMM, Marques AMC, Ramalho LMP, Ponzi EAC, Carvalho CM, Santos RC, et al.. Infrared laser light further improves bone healing when associated with bone morphogenic proteins: An *in vivo* study in a rodent model. Photomed Laser Surg 2008;26:55-60.
11. Gerbi MEMM, Pinheiro ALB, Ramalho LMP. Effect of IR laser photobiomodulation on the repair of bone defects grafted with organic bovine bone. Lasers Med Sci 2008;23:313-317.
12. Pinheiro ALB, Gerbi MEMM, Limeira Jr FA, Ponzi EAC, Marques AMC, Carvalho CM, et al.. Bone repair following bone grafting hydroxyapatite, guided bone regeneration and infrared laser photobiomodulation: a histological study in a rodent model. Lasers Med Sci 2009;24:234-240.
13. Soares LGP, Marques AMC, Guarda MG, Aciole JMS, Santos JN, Pinheiro ALB. Influence of the λ780 nm laser light on the repair of surgical bone defects grafted or not with biphasic synthetic micro-granular hydroxylapatite + beta-calcium triphosphate. J Photochem Photobiol B: Biology 2014; 131:16-23.
14. Lopes CB, Pinheiro ALB, Sathaiah S, Silva NS, Salgado MC. Infrared laser photobiomodulation (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. Soares LGP, Magalhães Jr EB, Magalhães CAB, Ferreira CF, Marques AMC, Pinheiro ALB. New bone formation around implants inserted on autologous and xenografts irradiated or not with IR laser light: A histomorphometric study in rabbits. Braz Dent J 2013;24:218-223.
16. Soares LGP, Marques AMC, Barbosa AFS, Santos NR, Aciole JMS, Souza CMC, et al.. Raman study of the repair of surgical bone defects grafted with biphasic synthetic microgranular HA+β-calcium triphosphate and irradiated or not with λ780 nm laser. Lasers Med Sci 2014;29:1539-1550.
17. Pinheiro ALB, Santos NRS, Oliveira PC, Aciole GTS, Ramos TA, Gonzalez TA, et al.. The efficacy of the use of IR laser phototherapy associated to biphasic ceramic graft and guided bone regeneration on surgical fractures treated with miniplates: a Raman spectral study on rabbits. Lasers Med Sci 2013;28:513-518.

Received April 10, 2014
Accepted December 11, 2014