Journal of Photochemistry & Photobiology, B: Biology 163 (2016) 303–310

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effects of low-level laser therapy on bone healing of critical-size defects treated with bovine bone graft

Alvaro Francisco Bosco [a], Paula Lazilha Faleiros [a], Luana Rodrigues Carmona [a], Valdir Gouveia Garcia [a,b], Letícia Helena Theodoro [a], Nathália Januario de Araujo [a], Maria José Hitomi Nagata [a], Juliano Milanezi de Almeida [a,*]

[a] Univ. Estadual Paulista - UNESP, Division of Periodontics, Department of Surgery and Integrated Clinic, Dental School of Araçatuba, Rua José Bonifácio, n° 1193, CEP: 16015-050 Araçatuba, São Paulo, Brazil

[b] University Center of Educational Foundation of Barretos – UNIFEB, Department of Periodontics, Dental School of Barretos, Avenida Professor Roberto Frade Monte, n° 389, CEP: 14783-226 Barretos, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 3 June 2016
Accepted 25 August 2016
Available online 31 August 2016

Keywords:
Bone grafting
Bone regeneration
Calvaria
Low-level laser therapy

ABSTRACT

Objective: To histomorphometrically analyze the effect of low-level laser therapy (LLLT) on bone formation process in surgically created critical-size defects (CSDs) treated with bovine bone graft (BBG) and its influence over particles' resorption of BBG.

Methods: A 10-mm diameter CSD was surgically created in the calvaria of 64 male rats, which were distributed into 4 experimental groups: the C group (control), only blood clot; the LLLT group, LLLT (GaAlAs, 660 nm) and blood clot; the BBG group, CSD filled with BBG; the BBG/LLLT group, LLLT and CSD filled with BBG. Animals were euthanized at either 30 or 60 days post-operation. A histological analysis was performed. Additionally, the percentage of newly formed bone area (NFBA) and remaining particles areas (RPA) of BBG were histometrically evaluated and data statistically analyzed.

Results: The LLLT (5.82 ± 2.05; 7.34 ± 1.01) group presented significantly greater NFBA when compared to the C group (1.61 ± 0.30; 5.59 ± 0.94) at 30 and 60 days post-operation ($p < 0.05$). The BBG/LLLT group (7.39 ± 1.45; 9.44 ± 2.36) presented significantly greater NFBA than the BBG group (3.85 ± 1.56; 8.02 ± 0.63) at 30 and 60 days postoperation ($p < 0.05$). There was no significant difference in the mean percentage of implanted material RPA between the BBG and the BBG/LLLT groups.

Conclusions: LLLT can improve bone formation process in CSD filled or not with BBG in rat calvaria, but it is not able to accelerate particles resorption of this material in the interior of bone defect.

1. Introduction

Bone loss in the maxillofacial region presents a challenging clinical issue, especially in the case of large defects, where their physiological regenerative capability is exceeded [1]. A variety of bone grafting or substitutes [2,3,4,5] have been suggested in order to regenerate these defects [6].

Among the materials used for bone regeneration, autogenous bone has been considered the ideal graft material [7,8,9] because of its osteoinductive, osteoconductive, and osteogenic characteristics [10]. However, its collection is associated with significant donor site morbidity, including damage to anatomic structures [11], infections [11,12], pain [13,14], hematoma formation [12,15], and unpredictable graft resorption [7,8,16]. Obtaining bone tissue from donor site sufficient to fill the defect also becomes a challenge in some complex clinical conditions that require bone regeneration in large quantity, such as bone defects resulting from trauma, infection, tumor resection, skeletal abnormalities, atrophic non-unions and osteoporosis conditions [17]. Moreover, grafts are often resorbed before osteogenesis is finished in large defects [15].

Consequently, a search for bone biomaterials that could replace the autologous bone, with the advantages of unlimited supply and no need for a donor site [16], has taken place [10]. Nonetheless, these are not always graced with the advantages of osteogenesis and osteoinduction inherent of the autologous grafts [10,18]. Xenogeneic bovine bone grafts (BBG) are the most commonly used material [19,

* Corresponding author at: Departamento de Cirurgia e Clinica Integrada, Disciplina de Periodontia, Faculdade de Odontologia de Araçatuba, UNESP, Rua José Bonifácio, 1193, CEP: 16015-050 Araçatuba, São Paulo, Brazil.

E-mail addresses: afbosco@hotmail.com (A.F. Bosco), paulal.faleiros@hotmail.com (P.L. Faleiros), luanarcarmona@yahoo.com.br (L.R. Carmona), vg.garcia@uol.com.br (V.G. Garcia), letheodoro@uol.com.br (L.H. Theodoro), nathaliajanuario@outlook.com (N.J. de Araujo), mjnagata@uol.com.br (M.J.H. Nagata), jumilanezi@hotmail.com (J.M. de Almeida).

http://dx.doi.org/10.1016/j.jphotobiol.2016.08.040
1011-1344/

20,21,22,23]. The literature reports its superior biocompatibility and osteoconductivity compared to other bone substitutes [19,22]. However, this material still lacks factors that promote osteogenesis and osteoinduction [10]. In turn, this increases healing time compared to autologous bone, which feature live cells and growth factors, fulfilling their osteogenic and osteoinductive potentials [18]. Such properties reflect positively on the time required for bone healing [24].

Low-level laser therapy (LLLT) has emerged as a strategy to accelerate the healing of bone defects treated with xenogeneic BBG [16,23] and others bone substitutes materials [25], since it can acts as an osteoinductive factor [26,27]. The exact mechanism of action of LLLT on bone healing is not well understood [23], but it has been reported that it can promote angiogenesis [28] and increase local blood flow (enhancing the supply of circulating cells, nutrition, oxygen, and inorganic salts to the bone defect) [29], stimulate cell growth such as fibroblasts (which are related to collagen production) [30], increase osteoblast proliferation and differentiation [31] and promote mitochondrial respiration and ATP synthesis [32]. Specifically regarding xenogeneic BBG, there is a report that LLLT can improve bone formation process and accelerate particles resorption in the interior of bone defects [16], since it can increases osteoblastic [33] and osteoclastic activity [34]. This is a valuable finding when particles fail to resorb and remain like a motionless body surrounded by the host bone [35,36].

Few studies have addressed the action of LLLT on the interaction of implanted biomaterial and tissue during bone healing process [37,38]. It has been shown that LLLT promotes bone healing and bone mineralization [39]. In the search for the optimal biomaterials tissue interaction, the effect of LLLT on these cells is an important field of investigation [39]. Thus, the purpose of the present study was to analyze histomorphometrically the effect of LLLT on bone formation process in surgically created critical-size defects (CSDs) treated with BBG and its influence over particles resorption of BBG.

2. Materials and Methods

2.1. Animals and Experimental Groups

After careful planning of a double-blind interventional animal study and an ethical approval by the Ethics Committee on Animal Use (protocol # 003162/2007) of the School of Dentistry, Araçatuba Campus, São Paulo State University, sixty four 3-month-old male rats (*Rattus norvegicus*, albinus, Wistar) weighing 250 to 300 g (UNESP, Dental School of Araçatuba, Animal Care Unit) were included in the study. This study conforms to ARRIVE (Animal Research: Reporting of In Vivo Experiments) [40]. The animals were kept in plastic cages with access to food and water ad libitum, in a room with a 12-h light/dark cycle and a temperature between 22 and 24 °C. Prior to surgical procedures, all animals were allowed to acclimatize to the laboratory environment for a period of 7 days. Following a table generated by a computer program, the animals were distributed into 4 experimental groups ($n = 16$): the C group (control), only blood clot; the LLLT group, LLLT and blood clot; the BBG group, CSD filled with BBG; BBG/LLLT group, LLLT and CSD filled with BBG.

2.2. Creation of the CSD

For surgical procedures, the animals were anesthetized by intramuscular injection with ketamine (70 mg/kg) (Vetaset, Zoetis, Florham Park, NJ) and xylazine (6 mg/kg) (Coopazine, Coopers, São Paulo, São Paulo, Brazil). After aseptic preparation, a semilunar incision was made in the scalp in the anterior region of the calvarium, allowing reflection of a full thickness flap in a posterior direction. A 10-mm CSD was made with a trephine (3i Implant Innovations Inc., FL, USA) in a low-speed hand piece under continuous sterile saline irrigation. Extreme care was taken not to damage the dura mater during the creation of the CSD. The defect included a portion of the sagittal suture. The CSD of each animal was filled with particles of 250 to 1000 μm of BBG (Gen-Mix Baumer S.A., São Paulo, SP, Brazil) using a 6 mm^3 measuring cup [6]. The soft tissues were then repositioned and sutured (4-0 Silk; Ethicon, São Paulo, SP, Brazil) to achieve primary closure. Each animal received post-surgical intramuscular injections of 24.000 IU of penicillin G-benzathine (Fort Dodge, Saúde Animal Ltd., Campinas, SP, Brazil).

2.3. LLLT Protocol

In the LLLT and BBG/LLLT groups the LLLT was used after the displacement of the total retail and clothing of the surgical defect. The laser used in this study was gallium aluminum-arsenide (Bio Wave; Kondortech Equipment Ltd., São Carlos, São Paulo, Brazil), with a wavelength of 660 nm, power of 35 mW, and spot size of 0.07 cm^2. LLLT was performed once in eight points around the CSD, in contact with the bone tissue, and also in a central point of the CSD in the scanning procedure [41]. The treatment laser was emitted with power of 0.03 W during 72 s/point, irradiance of 0.42 W/cm^2, and fluency of 30.85 J/cm^2/point. The area received a total energy of 19.44 J.

2.4. Tissue Processing

Eight animals from each group were euthanized at 30 or 60 days post-operation. The area of the original surgical defect and the surrounding tissues were removed in block. The blocks were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48 h, rinsed with water, and then demineralized in a solution of 10% EDTA. After decalcification, they were processed and embedded in paraffin. Serial 6 mm-thick sections were cut in a longitudinal direction. The sections were stained with hematoxylin and eosin (H&E) for analysis under light microscopy. Two sections from the central area were selected for histological and histometric analyses.

2.5. Histomorphometric Analysis

Two histological sections, representing the center of the original surgical defect, were selected for histologic and histometric analyses to increase the reliability of the data used in the statistical analysis. These analyses were performed by an examiner blinded to the treatment rendered (LRC). The images of the histologic sections were captured by a digital camera (Olympus DP 10, Olympus Optical Co. Ltd., Tokyo, Japan) coupled to a light microscope (Olympus BX 50 *F*4, Olympus Optical Co. Ltd., Tokyo, Japan) with an original magnification of 32×. The digital images were saved on a computer. A composite digital image was then created by combining three smaller images, because it was not possible to capture the entire defect in one image at the level of magnification used. The composite image was created based on anatomic reference structures (such as blood vessels and bone trabeculae) within each of the histologic sections. The Imagelab 2000 software (Diracon Bio Informática Ltd., Vargem Grande do Sul, São Paulo, Brazil) was used for the histomorphometric analysis. The following criteria [42]

Fig. 1. Panoramic views of the surgical defects and detailed histological appearance of the edges and center of the surgical defect at 30 postoperative days. Photomicrographs showing the NFB close to the edges of the surgical defect; remnants of granules of BBG and formation areas of osteoid matrix in (A) C; (B) LLLT; (C) BBG and (D) BBG/LLLT. NFB restricted to areas close to the edges of the surgical defect in C; LLLT; BBG and BBG/LLLT– A(a)/(c), B(a)/(c), C(a)/(c) and D(a)/(c). Range of well-vascularized fibrous CT in C – A(b). Area of osteoid matrix with a large number of osteoblasts (asterisk) in LLLT – B(b). Granules of BBG encircled by areas of osteoid matrix with a large number of osteoblasts (asterisk) in BBG and BBG/LLLT - C(b) and D(b). (Hematoxylin and eosin staining; original magnification ×50 in A, B, C and D; original magnification ×100 in A(a)/(b)/(c), B(a)/(b)/(c), C(a)/(b)/(c) and D(a)/(b)/(c). Abbreviations: NFB, newly formed bone; BBG, bovine bone graft; CT, conjunctive tissue.