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Research Paper
Bone regeneration

Influence of the association between platelet-rich fibrin and bovine bone on bone regeneration. A histomorphometric study in the calvaria of rats

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Abstract. This study aimed to investigate the effects of platelet-rich fibrin (PRF) associated or not with Bio-Oss on bone defects in the calvaria of rats. A critical-size defect of 5-mm diameter was performed in the calvaria of 48 rats. These animals were divided into six groups of eight animals each, according to the treatment received: homogeneous clot, autogenous clot, autogenous PRF, homogeneous PRF, Bio-Oss, or Bio-Oss associated with PRF. The animals were euthanized after 30 or 60 days. Bone regeneration was evaluated by histomorphometric analysis. The highest mean percentages of new bone formation at 30 days (54.05% ± 5.78) and 60 days (63.58% ± 5.78) were observed in the Bio-Oss associated with PRF group; in particular, the percentage of new bone at 30 days was significantly higher than that of all of the other groups ($P < 0.01$). At 60 days, the Bio-Oss associated with PRF (63.58% ± 5.78) and Bio-Oss (57.34% ± 5.78) groups had similar results, and both showed a statistical difference compared to the other groups. PRF had a positive effect on bone regeneration only when associated with Bio-Oss.

Keywords: Fibrin; Platelet-derived growth factor; Bone graft; Bone transplantation; Bone regeneration.

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Introduction

With the advent of implantology, much has been discussed about the use of various bone substitute materials aimed at creating the bone conditions required to allow rehabilitation with dental implants. In this context, the use of platelet-derived concentrates to enhance the repair of oral tissues is of interest.[1,2] It is known that such materials are rich in growth factors and cytokines that are important in the inflammatory process leading to tissue healing, and their use may accelerate this process.[3,4] Nevertheless, evidence of the therapeutic efficacy of platelet concentrates remains controversial.[5]

The actual effectiveness of PRP is in doubt, as many studies suggest that due to the rapid release of growth factors, it produces an immediate and transitory effect that is insignificant for tissue repair.[6] A second generation of platelet concentrate has since emerged, called platelet-rich fibrin (PRF).[7] In PRF, the growth factors and cytokines are caught in the meshes of the fibrin network and therefore exert a controllable, gradual, and lasting effect on the tissue repair process since they are released slowly. Some authors have reported advantages with the use of PRF for the repair of oral tissues, including bone regeneration.[6]

There is also much speculation in the literature on the use of different grafts as bone substitutes. The ideal biomaterials should provide osteoconductive and osteoinductive features similar to autogenous bone grafts, which are still considered the gold standard in reconstructive bone surgery. However, there is no ideal biomaterial.[8,9] In this context, xenogeneic grafts, particularly Bio-Oss, have gained prominence as biomaterials for scaffolds, providing a site for the bone tissue to grow and attach biologically.[10] Thus, it is believed that the association of xenografts with PRF could have a promising effect on the healing of bone defects of critical sizes.

Materials and methods

Experimental model

The experimental protocol was approved by the ethics committee on the use of animals. Forty-eight male rats (*Rattus norvegicus*, Albinus, Wistar) were used, weighing 450 to 550 g; they were divided into six groups of eight animals each. Four animals from each experimental group were euthanized after 30 days and four after 60 days. The experimental groups were as follows: CA (autogenous clot), CH (homogeneous clot), PRF-a (autogenous PRF), PRF-h (homogeneous PRF), BO (particulate bovine bone (Bio-Oss; Geistlich Pharma AG, Switzerland)), and BOPRF (particulate bovine bone (Bio-Oss) associated with PRF). Each animal was kept in a separate cage and received a solid diet and water ad libitum.

Obtaining the platelet-rich fibrin

Intracardiac puncture with a needle attached to a disposable 5-ml syringe was performed directly into the left ventricle of the animal. The volume of blood removed was approximately 3.5 ml for the production of autogenous PRF from rats receiving the defect in the calvaria, and 10 ml for the homogeneous PRF from donor rats. The blood was then placed in 5-ml tubes and centrifuged immediately, only once, without anticoagulant, for 10 min at speeds of 3000 rpm (bench centrifuge NT 810; Novatécnica Equipamentos para Laboratório, Piracicaba, SP, Brazil), as recommended by Dohan et al.[11] The PRF, located in the middle portion of the tube, was isolated from the remaining blood components by clamping.

Surgical procedure

The animals were anaesthetized by intramuscular injection of xylazine (6 mg/kg, Rompum; Bayer do Brazil, Sao Paulo, Brazil) and ketamine (70 mg/kg, Dopalen; Vetbrands, Sao Paulo, Brazil). After preparation of the surgical site in the frontal–parietal region of the calvaria and antisepsis with 10% povidone–iodine (PI) with 1% active iodine, a U-shaped incision was performed with the caudal base in the rat calvaria and a full-thickness flap was folded posteriorly. Using a trephine drill of 5-mm diameter (Neodent, Curitiba, PR, Brazil) attached to a BLM 600 plus motor (Driller, Carapicuíba, SP, Brazil) and under abundant cooling with sterile saline, a critical-size defect was made in the rat calvaria. The defect included a portion of the sagittal suture. 'L'-shaped markings were made with a carbide conical drill bit 2 mm anterior and 2 mm posterior to the surgical defect margins; these were filled with silver amalgam, as recommended by Messora et al. (Fig. 1).[4] The major axis of each 'L' was located on an imaginary longitudinal cranial–caudal line that divided the surgical defect in half. These markings are useful for identifying the centre of the original surgical defect during laboratory processing, allowing the original bone margins of the defect to be located during histological analysis. The animals in each group had the defects filled with the respective materials, and the tissues were placed in their original position and sutured with silk (4–0 Silk; Ethicon, São Paulo, SP, Brazil).

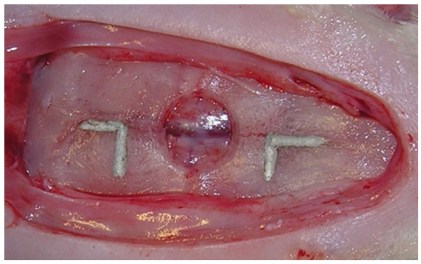

Fig. 1. Channels made 2 mm from both margins of the bone defect, filled with silver amalgam.

Processing of specimens

Specimens were placed in 10% neutral formalin, washed in running water, and decalcified in a solution of 18% ethylenediaminetetraacetic acid (EDTA). After initial decalcification, each specimen was divided longitudinally into two blocks, exactly over the centre of the original surgical defect, using the largest axes of each 'L'-shaped amalgam marking as a reference. Transverse sections touching the lower axis of both 'L'-shaped markings were also performed, so that each specimen was 9 mm long in the longitudinal direction. Therefore, it was possible to determine precisely the boundaries of the original surgical defect. After an additional period of decalcification, the specimens were processed and embedded in paraffin. Six-micrometer-thick serial sections in the longitudinal direction of the specimen were made, starting from the centre of the original surgical defect. The sections were stained with haematoxylin and eosin (HE) for microscopic analysis.

Histomorphometric analysis

The histological sections were selected so that the original surgical defect could be evaluated histomorphometrically throughout its length. Images of histological sections were captured with a digital camera (Axiocam MRc 1.4Mb; Carl Zeiss, Göttingen, Germany) connected to a binocular optical microscope (Axio Lab; Carl Zeiss, Göttingen, Germany) with an original magnification of 40× (objective lens N, Achroplan) and saved on a computer. Histomorphometric analysis was performed with the aid of AxioVision Rel. 4.8 software.

The following criteria, based on the studies of Melo et al.[12] and Messora et al.[4], were used to standardize the histomorphometric analysis of the scanned images: (1) the total area (TA) to be analysed corresponded to the total area of the surgical defect. This area was determined by identifying the internal and external surfaces of the original calvaria at the right and left margins of the surgical defect. These surfaces were connected with lines drawn following its curvature. Measurements of 2 mm were made from the ends of the specimens towards the centre of the defect, in order to determine the limits of the original surgical defect. The bone area (BA) was outlined within the limits of the TA. (2) TA was measured in μm^2 and considered 100% of the area being analysed. BA was also measured in μm^2 and calculated as a percentage of TA.