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Guided Bone Regeneration with Subperiosteal Implants of PTFE and Hydroxyapatite Physical Barriers in Rats

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Regeneration of periodontal and alveolar ridge defects utilizing membranes is a well-established procedure in reconstructive surgery. Biomaterial characteristics and membrane design employed in guided tissue regeneration (GTR) techniques play an important role in good results. The purpose of this histologic experimental study in rats was to compare the use of two physical barriers in the osteopromotion by using GTR principles in bone defects created in tibias. Fifteen animals divided into 3groups were used: group I (non-porous polytetrafluoroethylene (PTFE) barrier), group II (coral hydroxyapatite (HA) blocks), and group III (defects that received no physical barrier). Histological examination showed varied amounts of newly formed bone beneath both types of barriers. The non-porous PTFE barrier showed better results than the HA group. The results of this study suggest that bone regeneration can be successfully enhanced by a submerged membrane technique.

Key Words: guided bone regeneration, wound healing, polytetrafluoroethylene membrane, hydroxyapatite.

INTRODUCTION

Several studies and case reports have shown the use of guided tissue regeneration (GTR) in the treatment of localized atrophy of the alveolar edge in post-extraction socket to prevent bone deformities, in the treatment of bone defects associated with osseointegrated implants, and in several types of bone defects as a consequence of periodontal diseases, endodontic lesions, cysts and tumors (1-3).

The treatment of local atrophies of the alveolar edge with mucous membrane autogenous graft and HA implant presents some disadvantages and technical problems. The use of autogenous bone grafts has the inconvenience of adding a surgical area for the removal of an adequate quantity of donor tissue.

Murray et al. (4), Hurley et al. (5), Linghorne (6) and Melcher & Dreyer (1) were the first to use physical barriers creating an acceptable environment for osteogenesis through the exclusion of cellular elements of connective tissue in the repair area of a surgical wound. Experimental bone defects created in mice jaws and covered with polytetrafluoroethylene membrane showed bone regeneration (2). The biological principles of guided bone regeneration (GBR) can be used to increase or to reconstruct aesthetically and functionally the thickness and height of the atrophic alveolar edge and calvarial defects (7).

More recent experimental studies have demonstrated bone neoformation with the use of other types of membranes that act as physical barriers to exclude non-osteogenic soft tissue, so that the spaces created by the material were repopulated by cells able to produce bone tissue (8,9). In addition, an appropriate space is created

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where the natural biological potential can be expanded to assist the regeneration as desired (10).

Based on previous research (1,2,4,6), this work was an attempt to histologically evaluate the response of bone tissue and its regenerative potential after subperiosteal implantation of PTFE and HA physical barriers in the restoration of bone defects created in the rat femur.

MATERIAL AND METHODS

Physical barriers

Two different types of non-absorbable material were used in this study as physical barriers according to GBR biological principles. One is manufactured as a non-porous PTFE film (Tecnoflon & Brasflon, São Paulo, SP, Brazil) (11) and the other as small blocks of coral hydroxyapatite (HA; Faculty of Chemistry of Lorena, Lorena, SP, Brazil) (12). Both materials were cut to cover defects, extending for 2 to 3 mm beyond the margins of the bone defects.

Surgical procedure

A total of 15 Albinus male rats, weighing 250-300 g, were used and were fed a commercially prepared solid diet and water *ad libitum*.

Preoperatively, the animals were anesthetized with an intramuscular injection of aqueous solution of chlorhydrate of 2-(2,6-xylidine)-5,6-dihydro-4H-1,3-thiazine (Rompun, Bayer, São Paulo, SP, Brazil) and ketamine (Francotar, Virbac, São Paulo, SP, Brazil), in the proportion of 1:0.5 ml in the dose of 0.1 ml/100 mg of body weight. After trichotomy and asepsis of the operative field, the lateral surfaces of the tibia, in both posterior paws, were exposed with removal of periosteal tissue to form concave cortical defects, approximately 3 mm in diameter, with spherical surgical drills at low rotation and constant irrigation with sterile saline. The defects were created making superficial wastes without perforations. In the right posterior paw, a PTFE barrier was placed to cover the defect, while in the left paw the defect was covered with small blocks of HA. Five mice were used as controls, and their defects were left to repair naturally. The incisions were sutured with 4-0 silk interrupted sutures (Ethicon-Johnson & Johnson, São José dos Campos, SP, Brazil), for a first intention repair.

Histological study

The animals were sacrificed with a high dose of the anesthetic at 7, 14, 21 and 30 days post-operatively. After sacrifice, fragments including the implanted material were placed in labeled bottles for fixation in 10% formalin solution, for a minimum of 48 h, and decalcified in 20% formic acid. The PTFE barrier was subsequently removed and the material was processed routinely for histological evaluation (5-µm thick sections and hematoxylin and eosin stain). The specimens were analyzed with an optic microscope.

RESULTS

The HA blocks were totally removed by the decalcification, with clear spaces remaining surrounded by connective tissue. These spaces were located close to the bone, eventually interposed among muscle fibers or in the subcutaneous connective tissue. In all experimental periods, septa of connective tissue were observed projecting into the spaces.

7 days

Control: Discrete local bone neoformation in the periphery of the cortical bone tissue was seen. This neoformation was continuous with compact bone tissue, in supracortical and subperiosteal disposition. The newly formed trabeculae were perpendicular to the cortical bone tissue, with an immature aspect, with several osteocytes included and large osteoblasts aligned along the trabeculae. The medullar spaces were filled by well-vascularized loose connective tissue. The periosteum was very cellular, in contact with the trabeculae, and more fibrous externally (Figure 1 top).

PTFE: The barrier was outlined by connective tissue rich in fibroblasts and fibrocytes, well-vascularized and with interstitial edema. Mono- and polymorphonuclear inflammatory cells were present close to the barrier, with fibrin deposit and remnants of necrotic tissue in some areas. Discrete formation of fibers was observed more externally. The muscle fibers close to the barrier were dissociated by edema, discrete inflammatory infiltrate and dilated and congested blood vessels. Subperiosteal bone neoformation was observed between the external surface of the bone and the internal surface of the barrier. The newly formed bone exhibited the same characteristics described for the control (Figure 1 middle).

HA: The HA fragments were surrounded by granulation tissue exhibiting young fibroblasts, newly formed blood vessels, and discrete and diffuse mononuclear inflammatory cell infiltrate. At the interface with the fragment, there were remnants of fibrin net, containing erythrocytes and polymorphonuclear neutrophils. More externally, there was a discrete fibrosis. Newly formed bone trabeculae, in general perpendicular to the cortical bone tissue, with radiated disposition, in supracortical and subperiosteal location were observed (Figure 1 bottom). The bone neoformation was seen mainly at the internal surface of the HA fragments; however, it was also observed in other external areas.

14 days

Control: The areas of bone neoformation showed a more mature aspect than observed at 7 days. The trabeculae were thicker, surrounded by large osteoblasts, and the medullar spaces were smaller. Basophilic lines indicating bone resorption followed by neoformation were observed below this area.

PTFE: Discrete fibrosis was observed around the barrier, with a few inflammatory cells, especially macrophages and eosinophils. One of the specimens presented a foreign body reaction between the bone and the barrier. The interposition of muscle fibers between the barrier and the bone was observed in some areas. The newly formed tissue was thinner than that observed at 7 days, and was more mature with lamellar trabeculae.

HA: The newly formed bone tissue presented a more mature aspect than that observed at 7 days, with smaller medullar spaces.

21 days

Control: The newly formed bone trabeculae were lamellar, sometimes irregular and immature.

PTFE: The barrier was surrounded by fibrous connective tissue, containing some macrophages at the interface with the material. The newly formed bone tissue was mature, with lamellar trabeculae; however, in 2 specimens it presented wide medullar spaces, outlined by osteoblasts and containing numerous fatty cells.

HA: The newly formed bone tissue presented thick lamellar trabeculae and reduced medullar spaces.

30 days

Control: The newly formed bone exhibited remodelation aspects, with the presence of basophilic lines indicating resorption and bone neoformation (Figure 2 top).

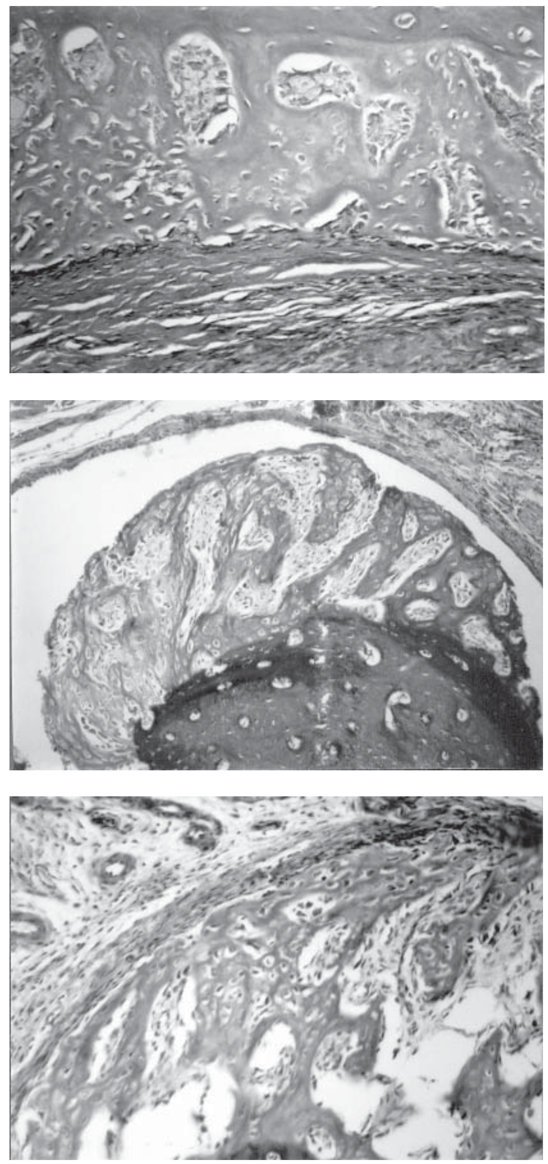

Figure 1. 7 days. *Top*: Control. Supracortical and subperiosteal discrete bone neoformation. H&E. Original magnification = 175X. *Middle*: PTFE. Large subperiosteal bone neoformation in the space created by the barrier. H&E. Original magnification = 100X. *Bottom*: HA. Supracortical and subperiosteal trabecullar bone neoformation. H&E. Original magnification = 140X.

PTFE: The barrier was surrounded by fibrous connective tissue with rare macrophages. The newly formed bone exhibited a mature aspect with concentric lamellae around vascular channels (Figure 2 middle).

HA: The HA fragments were surrounded by fibrous connective tissue, with discrete mono- and polymorphonuclear inflammatory infiltrate, especially in the septa of connective tissue that projected into the interior of the space. The newly formed bone tissue showed mature lamellar trabeculae and small medullar spaces. Newly formed cartilage was eventually observed contiguous with the HA fragments or with the connective tissue that surrounded the fragments (Figure 2 bottom).

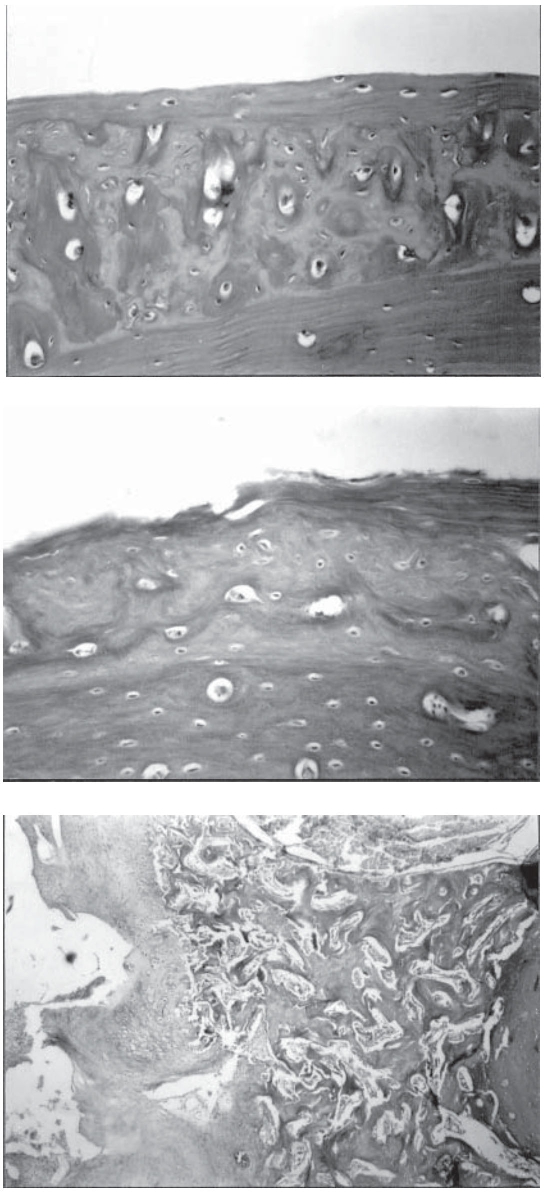

Figure 2. 30 days. *Top*: Control. Bone with remodelation aspect with resorption and remodelation. H&E. Original magnification = 200X. *Middle*: PTFE. Mature neoformed bone with concentric trabecullae that is distinguished from pre-existent bone. H&E. Original magnification = 250X. *Bottom*: HA. Neoformed bone with lamellar trabecullae, small medullar spaces, and cartilage presence. H&E. Original magnification = 45X.

DISCUSSION

Although this was not the main objective, this histological study confirmed the biocompatibility of physical barriers, previously reported by Macedo et al. (11,12). However, HA caused a more pronounced inflammatory response that persisted for the entire study period without effects on bone neoformation (13). Hydroxyapatite can attract circulating biocomponents to sites of tissue repair to promote bone repair (14,15).

The microscopic analysis demonstrated the location of the barrier of PTFE sometimes close to the bone and sometimes separated from the bone by muscle tissue. Around the barrier, there was an inflammatory reaction, initially characterized by edema, vascular congestion and mono- and polymorphonuclear inflammatory infiltrate. As already reported by Macedo et al. (11), the inflammatory reaction decreased its intensity with time, the infiltrate became mainly macrophagic and the formation of collagen fibers began. Up to 30 days, fibrous dense connective tissue was observed around the membrane, with rare macrophages in contact with it.

Bone neoformation was observed in the bone outer surface, located directly over the pre-existent cortical bone plate and under the periosteum. This bone tissue was initially immature and became more mature during subsequent periods, going from cancellous bone, with many cellular bone trabeculae that delimited wide medullar spaces, to compact bone tissue, with formation of osteons and parallel bone lamellae. As in the studies of Murray et al. (4) and Mecher & Dreyer (1), the bone neoformation, in the present study, showed a continuous protuberance that stood out from the adjacent bone profile.

The importance of the blood coagulum and the use of physical barriers in bone defects in several clinical situations has been reported (1,5,7,12,16). In this study, physical barriers (PTFE and HA) on the bone defect filled out with blood coagulum that created conditions for the area of the surgical wound to be colonized by osteogenic cells of the adjacent bone tissue. The physical barrier protects the blood coagulum from moving away due to the mechanical stress that acts on the flap during the earliest phase of wound repair. Micromovements of the flap in the initial phase of repair are enough to modify the differentiation of mesenchymal cells from osteoblasts to fibroblasts (17). The displacement and the movement of the membrane affect the stabilization of the surgical wound and the blood coagulum would be colonized by connective tissue cells. Fibroblasts growing more quickly occupy the bone defect preventing osteogenesis.

In this study, the variation of the amount of newly formed bone was due to the fact that physical barriers were placed on the defects without the aid of sutures or microscrew fixation. This made their displacement possible, inducing variations in the shape and dimension of the space for bone growth. However, when the membrane remained in place, large amounts of bone neoformation were obtained, even during the initial periods of the study. The correlation between the maintenance of the space and the amount of bone neoformation in rats was shown. On the other hand, in the control group, in which no type of physical barrier was used, there was also a small amount of bone neoformation as a consequence of the absence of sutures in the deepest planes of the surgical wound, and probably periosteum stimulation due to the raising of the flap. In this case, tissues were not well adapted on the bone defect, creating a narrow space between soft and bone tissues.

Schmid et al. (18) and Weng et al. (19) reported that the periosteum is not that important for guided bone regeneration techniques. Our study demonstrated bone neoformation without suture of the deepest planes, and isolation of periosteum by the barriers.

The critical size of the bony defects constituted another important factor, and perhaps influenced the variation in bone neoformation found in this study (19,20). Bone defects smaller than 5 mm are critical to study the bone neoformation because a natural regeneration occurs. In this study, the creation of concave defects (no cavities) might have contributed for the osteogenic cells of the margins of the defect to supply elements for bone neoformation as a protuberance in continuity with the adjacent bone, in the space maintained by the physical barriers.

Unlike Dahlin et al. (2), this study also demonstrated that bone regeneration using the GBR biological principles can also be obtained with non-porous physical barriers, in agreement with the results of Schmid et al. (18). Thus, we can suggest the use of non-porous PTFE physical barriers in the treatment of periodontal disease bone defects, originating from endodontic and cystic lesions, osseointegrated implant associated defects, prevention and treatment of atrophy of the alveolar ridge, etc.

Thus, we conclude that: the physical barriers used in this study helped exclude the adjacent soft tissues, allowing the area of the bony defect to be preferentially repopulated by cells with osteogenic capacity; non-porous PTFE barriers fit better than HA in the GBR biological principles, and results can be extrapolated for the clinical treatment of several types of bone defects and the predictable osteopromotion in several clinical situations; the porosity and the integration of the physical barrier were not absolutely necessary for bone neoformation in the GBR technique; the amount of bone neoformation depended on the volume of the space created by the physical barriers.

RESUMO

A regeneração periodontal e do rebordo ósseo utilizando barreiras físicas são procedimentos bem estabelecidos em cirurgias reconstrutivas. As características do biomaterial e o desenho da membrana empregados na regeneração tecidual guiada desempenham um papel importante na obtenção de bons resultados. O objetivo deste estudo experimental histológico foi comparar o uso de dois tipos de barreiras físicas na regeneração óssea guiada em defeitos criados na tíbia de ratos. Quinze animais foram divididos em três grupos: grupo I (barreira não-porosa de politetrafluoretileno), grupo II (blocos de hidroxiapatita de coral) e grupo III (controle que não recebeu nenhuma barreira). A análise histológica demonstrou várias quantidades de osso neoformado com ambos os tipos de barreiras. A barreira de politetrafluoretileno mostrou melhores resultados do que a hidroxiapatita. Os resultados deste estudo sugerem que a regeneração óssea pode ser conseguida com a técnica de submersão da barreira física.

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