

Rat Subcutaneous Tissue Response to Macrogranular Porous Anorganic Bovine Bone Graft

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The ideal bone graft must present biocompatibility, osteoconductive and osteoinductive properties, resistance and plasticity. Xenogenic grafts of bovine cancellous bone origin are particularly interesting due to their biologically designed porous structure that enhance both cellular and vascular invasion. The purpose of this study was to evaluate the tissue response induced by bovine macrogranular porous anorganic bone implanted in rat subcutaneous tissue. Forty rats were assigned to 2 groups, as follows: the control group received empty collagen capsules and the test group received subcutaneous implants of the test material. Samples were collected after 10, 20, 30 and 60 days and processed histologically. Histological analysis showed at 10 days a granulomatous inflammatory infiltrate, rich in multinucleated giant cells and free of lymphocytes or plasma cells, similarly to mineralized allograft implanted in rat subcutaneous. In later periods, there was a significant decrease in the inflammatory infiltrate and an increase in fibrosis around graft particles. In conclusion, the test material induced a foreign body-type granuloma with subsequent fibrosis around the graft particles implanted in rat subcutaneous and did not elicit any immune response, thus being considered biocompatible.

Key Words: xenogenic graft, bovine cancellous bone, biocompatibility, subcutaneous tissue.

INTRODUCTION

Autogenous bone has been widely accepted and used as a pattern for the treatment of bone loss (1). Nevertheless, the limitations of autogenous grafts are availability, morbidity of the donor site and unsatisfying biomechanical properties (2). Consequently, there has been a continuous search for other alternatives such as allografts (3), xenografts (4) and aloplastic materials (5). Several companies have developed bone substitute materials of bovine origin, such as, Schering (Luboc™), Sulzer (Ne-osteo™), Geislich (BioOss™) and Baumer (Gen-Ox™). It is possible to obtain biomaterials from bovine long bones, such as deproteinized anorganic cortical or cancellous bone (6) and demineralized cor-

tical or cancellous matrix (7), in granules or blocks, and also demineralized resorbable membrane (4).

These biomaterials represent an important alternative to autogenous and allogeneous grafts because they have biocompatibility, due to appropriate mechanical and chemical treatments (4,6), and osteoconduction property due to its architecture and surface structural characteristics similar to original human bone. This enhances cell proliferation, migration and maturation into the repairing tissue. A previous *in vitro* study (8) showed that cultured rat osteoblasts interact with the surface of anorganic porous bone, promoting cell adhesion, proliferation and maturation. Regarding demineralized xenografts, they may still present a property of osteoinduction due to presence of bone morphogenetic

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proteins similarly to allografts (9).

This study evaluated the tissue response induced by bovine macrogranular porous anorganic bone implanted in rat subcutaneous tissue.

MATERIAL AND METHODS

The tested material was macrogranular porous anorganic bone (1-2 mm granules) of bovine cancellous bone origin, deproteinized at 100°C (Gen-Ox™; Baumer S.A., Mogi Mirim, SP, Brazil). The material was placed into clear collagen capsules (0.1 g *per* capsule) for implantation in the animals.

Forty male Wistar rats (weight 160 g) obtained from the Vivarium of the School of Dentistry of Bauru were randomly assigned to 2 groups (n=20), as follows: control group: subcutaneous implantation of empty collagen capsules; test group: implantation of collagen capsules containing 0.1 g of graft material.

The animals were anesthetized with an intramuscular dose of Dopalen® (AgribRANDS do Brasil Ltda, Paulinia, SP, Brazil; 0.5mL/kg body weight) and Anasedan® (AgribRANDS do Brasil) at a 1:1 (v:v) ratio. After shaving and antisepsis of the dorsal region, a linear incision was made in the skin, the subcutaneous tissue was dissected and the capsule implanted. Suture of the surgical wound and antisepsis were done thereafter.

The rats received a normal diet and water *ad libitum* throughout the study. After 10, 20, 30 and 60 days, 5 animals of each group were sacrificed *per* period by anesthetic overdose. Samples were immediately removed, fixed in 10% phosphate buffered formalin for 24 h, dehydrated in ethanol, clarified in xylol and embedded in Histosec® (paraffin + synthetic resin; Merck, Darmstadt, Germany). Alternate 5-µm-thick sections were cut and stained by hematoxylin and eosin (HE). The study was conducted following the guidelines of the Brazilian College of Animal Experimentation.

Inflammatory infiltrate and fibrosis intensity around the graft particles was analyzed semi-quantitatively under light microscopy, attributing scores according to the level of inflammatory reactions (inflammatory infiltrate) and reparative processes (fibrosis, angioblastic and fibroblastic proliferation).

Scores were given as follows: absent (0), mild (1), moderate (2) and intense (3). Data of both groups were analyzed by Kruskal-Wallis non-parametric test at 5% significance level.

RESULTS

The results are illustrated in Figure 1 (graphic presentation) and Figure 2 (histologic panel).

10 Days

In the control group (Fig. 2A) it was possible to observe a granulation tissue rich in fibroblasts and blood capillaries and also the presence of large macrophages with hyaline material in the cytoplasm, resembling fragments of phagocytosed collagen capsules. In the test group, a moderate to intense inflammatory infiltrate characterized by the presence of a large number of macrophages and inflammatory multinucleated giant cells (IMGC) (Fig. 2B) was seen around the graft particles in addition to a discrete presence of polymorphonuclear leukocytes and lymphocytes. Moderate fibroblastic and angioblastic proliferation and mild fibrosis were detected around the grafts particles (Fig. 1). The more peripherally situated particles were encapsulated while the more central ones were involved by a larger number of inflammatory cells.

20 Days

A moderate inflammatory infiltrate as well as

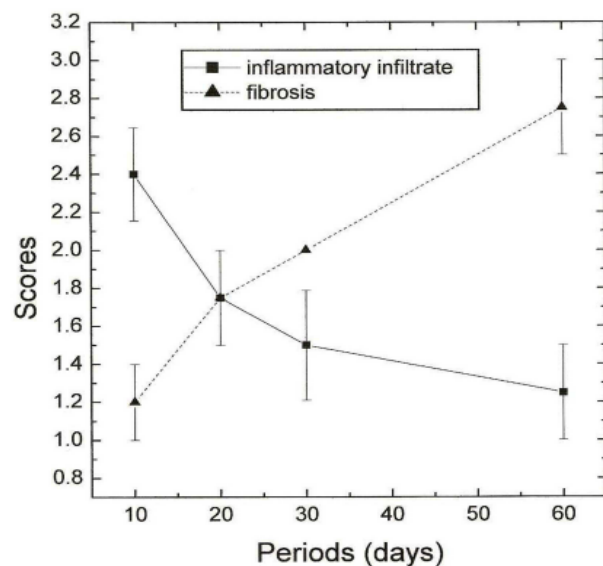


Figure 1. Test group. Comparison between inflammatory infiltrate and fibrosis level along the experimental periods. Scores: absence (0), mild (1), moderate (2) and intense (3).

angioblastic and fibroblastic proliferation were observed in the control group. Small foci of macrophages containing phagocytosed material were seen, similarly as in the 10-day period. There was a decrease in the intensity of the inflammatory infiltrate (moderate to mild) and an increase in the fibrosis around the graft particles in the test group. A capsule was formed in the outer limit exhibiting similar characteristics to the original connective tissue, with fat cells and blood vessels. The number of polymorphonuclear leukocytes and lymphocytes was reduced significantly and the number of macrophages and IMGC remained similar to the previous period.

30 Days

There was an increase in collagen fiber density

and angiogenesis compared to previous control periods. In the test group (Fig. 2C), there was invasion of graft particle pores by connective tissue and endothelial cells. Moderate fibrosis and mild inflammatory infiltrate was also detected. IMGC were the most abundant cell type in contact with the surface of the particles despite the reduction in the number of macrophages and the increase in the number of blood vessels.

60 Days

Scarce cells and discreet granulation tissue rich in blood vessels and fibroblasts were observed in the control group. Collagen fibers were as dense as in the original tissue. Intense fibrosis around the graft particles was seen in the test group (Fig. 2D). A mild inflammatory infiltrate with abundant IMGC, though in a lesser

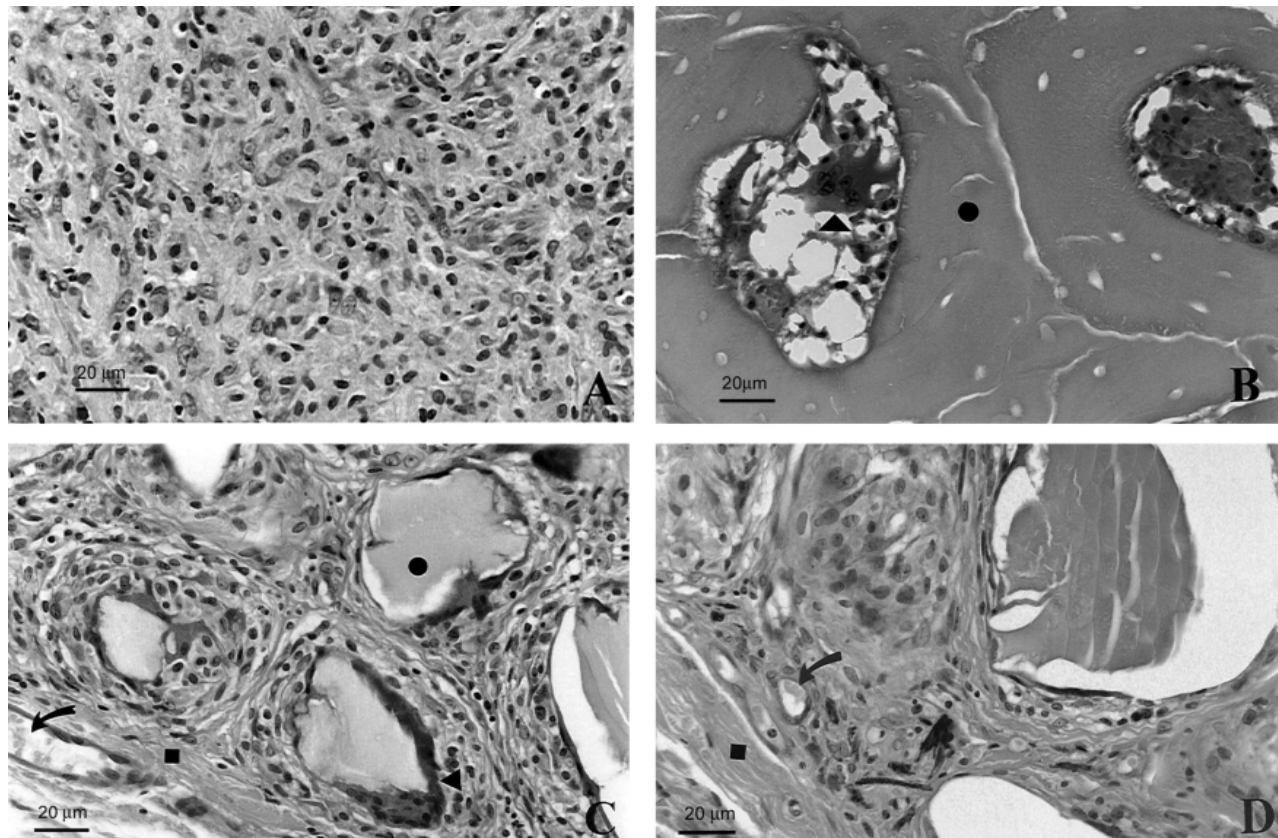


Figure 2. SEM micrographs of histological sections at different experimental periods. (A) Control group at 10 days: granulation tissue rich in mononuclear cells. (B) Test group at 10 days: implant particles (●) involved by inflammatory infiltrate showing polymorphonuclear leukocytes and IMGC (▲) inside the particles' pores. (C) Test group at 30 days: IMGC (▲) in close contact with implant particles (●); note the presence of blood vessels (arrow) and fibrosis (■). (D) Test group 60 days: fibrosis (■) and blood vessels (arrow) around particles were observed. HE staining.

amount than that of the previous period, was also observed and exhibited characteristics of slow-turn-over granuloma (foreign body-type reaction).

DISCUSSION

In the search for development of biocompatible materials to be used as bone substitutes, bovine bone has been continuously investigated as a reliable source, showing promising results both *in vivo* (4-8) and in clinical studies (10). Thousands of surgeries using bone grafts are made every year for the treatment of large bone defects, and autogenous bone remains the main bone graft used. In dentistry, a large number of cases demands this surgical procedure and, sometimes, the graft may be obtained from a donor area intraorally (11).

The shortcomings and risks of autogenous bone grafts (2) have stimulated the search for alternative bone substitutes that combine properties such as biocompatibility and osteoconduction. In this context, materials of xenogenic origin have been investigated as biomaterials since the 1960s (12).

Adequate mechanical, chemical and thermal processing used for the production of xenografts allows obtaining biocompatible bone substitute materials. The findings of a previous study (13) with porous anorganic bone grafting in experimental bone defects showed significant bone formation, attributing osteoconductive properties to this material. Another study (8) found an interaction of osteoblasts on the surface of these materials, enhancing cell adhesion and proliferation.

In the present study, the evaluation of the tissue response to a biomaterial produced from bovine cancellous bone by a Brazilian laboratory – particulate porous anorganic bone – implanted in rat subcutaneous tissue showed during the experimental periods, chronic inflammatory infiltrate rich in macrophages and multinucleated giant cells, and few polymorphonuclear leukocytes, plasma cells and eosinophils.

At 10 days post-implantation, the biologically designed pores of the graft particles were already filled with reparative tissue. Reduction of the inflammatory infiltrate and increase of the fibrosis around the graft particles were observed throughout the experiment.

It is well known that implants of anorganic xenografts and synthetic hydroxyapatite in subcutaneous tissue result in a foreign body-like granulomatous reaction of low turnover (6) with visible IMGCS. It must

be emphasized, however, that the presence of these cells occurs in an attempt of the organism to resorb the material and does not necessarily imply lack of biocompatibility. There are a couple of hypothesis that try to explain the nature of these IMGCS. Glowacki and Cox (14) identified these cells as osteoclasts, including a brush border, while other authors disagree (15). According to Baggi and Miller (16), two types of giant cells can be observed around mineralized particles: one, with IMGCS typical morphological characteristics, and the other, in a lesser extent, with osteoclastic morphology, although without its unique brush border. Despite the presence of IMGCS in contact with implant particles, brush border was detected here, which is in agreement with a previous study (15) that demonstrated IMGCS recruitment in response to implantation of particles, but without osteoclastic morphological aspects.

The porosity of the material certainly contributes for an increase in surface area, favoring the recruitment and posterior contact of a large number of inflammatory cells with the material. However, at 10 days post implantation, few cells were detected in the central area of the reactive tissue, probably due to the total volume and compaction of the graft particles. It should be noted that the interconnected biological pores might increase bone growth (17), as they provide a tunnel through which cells, blood vessels and growth factors may migrate. Aoplastic materials that mimic such characteristic have been developed as synthetic carriers for growth factors (18) and bioengineering (19).

Anorganic bovine bone matrix type is a natural, porous, xenogenic hydroxyapatite, approved by the Food and Drug Administration for the use as a filling material in bone defects (20). Thermal deproteinization allows elimination of protein contents while keeps the natural hydroxyapatite structure and surface. Its biologically structured surface allows cellular adhesion, proliferation and migration (8), thus enhancing terminal differentiation and maturation of new bone cells. Xenografts may be associated to bioactive molecules increasing their osteoconductive property or providing an osteoinductive potential for the treatment of bone defects (20).

Within the limitation of this study, it may be concluded that the xenograft of macrogranular anorganic porous bovine bone was well tolerated in rat subcutaneous tissue and did not elicit any immune response.

RESUMO

O enxerto ósseo ideal deve possuir características como biocompatibilidade, capacidade osteocondutora, osteoindutora, resistência e plasticidade. Dentre os implantes xenogênicos de origem bovina, os produzidos com o osso esponjoso revestem-se de particular interesse devido a sua arquitetura constituída de poros biologicamente desenhados que favorecem a invasão celular e vascular até o centro do defeito. O objetivo deste estudo foi avaliar a resposta tecidual ao material de osso inorgânico medular bovino macrogranular. Quarenta ratos foram divididos em 2 grupos (n=20): o grupo controle recebeu cápsulas de colágeno vazias, e o grupo experimental recebeu implante subcutâneo do material teste. As amostras foram coletadas após 10, 20, 30 e 60 dias de implantação e processadas histotecnicamente. A análise histológica mostrou aos 10 dias pós-cirúrgicos que o infiltrado inflamatório era do tipo granulomatoso rico em células gigantes multinucleadas, mas livre de linfócitos ou plasmócitos, quadro similar ao observado para aloenxertos mineralizados implantados em subcutâneo de ratos. Com o avançar do tempo experimental houve significante diminuição do infiltrado inflamatório inicial concomitantemente ao aumento no grau de fibrosamento ao redor das partículas implantadas. Concluiu-se que o material de enxerto testado em tecido conjuntivo subcutâneo de ratos induziu um granuloma tipo corpo estranho e fibrose ao redor das partículas implantadas, resposta semelhante do mesmo tecido aos aloenxertos mineralizados, e não desencadeou nenhuma resposta imune, sendo portanto biocompatível.

ACKNOWLEDGEMENTS

The authors wish to thank Ovídio dos Santos Sobrinho, Thelma Lopes da Silva, Daniele Santi Ceolin, Luiz Carlos da Silva and Erasmo Gonçalves da Silva for their technical assistance and to FAPESP (Process N. 02/03928-0) for financial support.

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Accepted August 29, 2006