Assessment of bovine biomaterials containing bone morphogenetic proteins bound to absorbable hydroxyapatite in rabbit segmental bone defects[1]

Avaliação de biomateriais bovinos contendo proteínas morfogenéticas ósseas absorvidas a hidroxiapatita em defeitos ósseos segmentares em coelhos

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ABSTRACT

Purpose: To evaluate the osteo-regenerative capacity of two proprietary bone grafting materials, using a segmental defect model in both radial diaphyses of rabbits. **Methods**: The right defect was filled with pooled bone morphogenetic proteins (pBMPs) bound to absorbable ultrathin powdered hydroxyapatite (HA) mixed with inorganic and demineralized bone matrix and bone-derived collagen, derived from bovine bone (Group A). The left defect was filled with bovine demineralized bone matrix and pBMPs bound to absorbable ultrathin powdered HA (Group B). In both groups, an absorbable membrane of demineralized bovine cortical was used to retain the biomaterials in the bone defects, and to guide the tissue regeneration. The rabbits were euthanized 30, 90 and 150 days after surgery. Radiographic, tomographic and histologic evaluations were carried out on all specimens. **Results**: At 30 days, the demineralized cortical bone cover was totally resorbed in both groups. HA was totally resorbed from Group A defects, whereas HA persisted in Group B defects. A prominent foreign body reaction was evident with both products, more pronounced in sections from Group B. At 90 days, the defects in Group B exhibited more new bone than Group A. However, at 150 days after surgery, neither treatment had stimulated complete repair of the defect. **Conclusion**: The partial bone healing of the segmental defect occurred with low or none performance of the biomaterials tested.

Key words: Bone Morphogenetic Proteins. Biocompatible Materials. Hydroxyapatites. Rabbits.

RESUMO

Objetivo: Avaliar a capacidade osteo-regenerativa de dois biomateriais utilizando um modelo de defeito segmentar efetuado nas diáfises do rádio de coelhos. **Métodos**: O defeito direito foi preenchido com pool de proteínas morfogenéticas ósseas (pBMPs) e hidroxiapatita em pó ultrafina absorvível (HA) combinada com matriz óssea inorgânica desmineralizada e colágeno, derivados do osso bovino (Grupo A). O defeito esquerdo foi preenchido com matriz óssea desmineralizada bovina com pBMPs e hidroxiapatita em pó ultrafina absorvível (Grupo B). Em ambos os defeitos utilizou-se membrana reabsorvível de cortical bovina desmineralizada para reter os biomateriais no defeito ósseo e guiar a regeneração tecidual. Os coelhos foram submetidos à eutanásia aos 30, 90 e 150 dias após a cirurgia. Foram efetuados exames radiográficos, tomográficos e histológicos em todos os espécimes. **Resultados**: Aos 30 dias de pós-cirúrgico, o osso cortical desmineralizado foi totalmente reabsorvido em ambos os grupos. A HA tinha reabsorvido nos defeitos do Grupo A, mas persistiu nos do Grupo B. Uma reação de corpo estranho foi evidente com ambos os produtos, porém mais pronunciada no Grupo B. Aos 90 dias os defeitos do grupo B tinham mais formação óssea que os do Grupo A. Entretanto, aos 150 dias após a cirurgia, nenhum tratamento havia promovido o completo reparo do defeito. **Conclusão**: Os biomateriais testados contribuíram pouco ou quase nada para a reconstituição do defeito segmentar.

Descritores: Proteínas Morfogenéticas Ósseas. Materiais Biocompatíveis. Hidroxiapatitas. Coelhos.

Introduction

Autografts and allografts are the traditional options for treatment of larger bone defects[1], however their use is associated with several disadvantages. Autografts are considered to be more effective but they necessitate an additional surgical procedure, are of limited availability and carry the possibility of donor site morbidity[1,2]. Allograft and xenograft bone substitutes have been studied to overcome the risks associated to autogenous bone, but their use is complicated by issues such as immunogenicity and consequent rejection, graft sequestration and infection; and the potential for disease transmission[1,2]. To overcome these limitations, considerable research has been directed towards developing clinically viable bone substitutes that are biocompatible, resorbable, and easy to handle, with physical and chemical properties similar to that of cancellous bone, free of pathological agents and cost-effective[2]. Essentially, bone consists of two components: a) mineralized matrix: crystalline calcium phosphate; b) organic matrix containing mainly type I collagen and traces of growth factors such as bone morphogenetic proteins (BMPs)[1]. Heat treatment of bone eliminates the organic matrix but preserves the architecture of mineralized bone, which possesses osteoconductive properties[3]. In contrast, the acid treatment removes the mineral content while maintaining the organic matrix with osteoinductive properties[4]. Bone morphogenetic activity was first described by Marshall Urist in 1965. He observed that demineralized bone matrix was able to produce ectopic bone formation when implanted in muscle of rabbits, rats, mice and guinea-pigs[5]. The BMP family now comprises approximately 30 members and has been implicated in a diverse range of biological activities including osteoinductive and osteogenic activities via the endochondral pathway[6,7]. BMPs are comparatively small proteins and diffuse easily through tissues. Therefore, to maintain strong localized activity, exogenous BMPs need to be ingrafted with suitable carriers that limit BMP release and diffusion[7]. Many bovine-derived bone substitutes are commercially available, such as Endobon™ (Merk KgaA – Merck Biomaterial Darmstadt, Germany); Keil Bone™ (Braun Milsungen, Germany); Bio-Oss™ (Geistlich Pharma, Swiss); Surgibone™ (Unlab Surgibone, Canada); and Gen-Pro™, Gen-Ox™ and Gen-Tech™ (Baumer SA, Brazil). *In vivo* studies are required to establish the potential clinical application and safety. This study was designed to compare two commercially available bone grafting material containing pooled fetal bone morphogenetic proteins (pBMPs) applied with a barrier membrane, in the healing of radial segmental bone defects in rabbits. The hypothesis addressed by the study is that the graft materials will exhibit similar osteo-regenerative effects.

Methods

In this study, two different graft substitutes were evaluated. The right forelimb defects were treated with Gen-Tech™ (Group A). The left forelimb defects were treated with Gen-Ox™ and Gen-Pro™ in combination (Group B). These proprietary graft substitutes are derived from bovine bones by Baumer S.A. (Mogi Mirim, Sao Paulo, Brazil). Gen-Tech™ is a composite constituting of four different materials: a) pooled fetal bone morphogenetic proteins (pBMPs) adsorbed to absorbable ultrathin powdered hydroxyapatite (HA); b) demineralized bovine bone; c) inorganic bovine matrix obtained from sintered bovine bone, and d) denatured bone-derived collagen. Gen-Ox™ is composed of demineralized bovine bone and Gen-Pro™ is comprised of pooled fetal bovine BMPs adsorbed to absorbable ultrathin powdered hydroxyapatite. The pooled bone morphogenetic proteins (pBMPs) were extracted from fetal bovine bone-demineralized matrix using caotropic agents. The pBMPs was adsorbed to absorbable ultrathin powdered hydroxyapatite. The ultrathin powdered hydroxyapatite is produced by wet pathway. It has high crystallinity, is 50 μm in size, and is not sintered. The demineralized bovine bone matrix is a natural biomaterial obtained from cancellous bone in granules (1000-2000 μm). After rigorous washings to eliminate blood, fat and any impurities, the bovine bones are chemically treated with sodium hydroxide and organic solvents, before demineralization. The inorganic bovine matrix is a natural biomaterial obtained from bovine cancellous bone in granules (1000-2000 μm) submitted to thermal treatment at 950^0C conferring to this material high crystallinity and low degradability. The resorbable membranes (Gen-Derm™, Baumer S.A., Mogi-Mirim, Brazil) were obtained from fully processed bovine cortical bone, free of cells, lipids and immunogenic proteins. Blocks of cortical bone were demineralized and sections of 400-600 μm were prepared.

Animals and surgical procedure

The use of experimental animals in this study was approved by the Ethics Committee from the Faculty of Veterinary Medicine and Animal Science, Unesp Botucatu. Fifteen female Norfolk rabbits of approximately 4.5 months of age with a mean body weight of 3 kg were used. Enrofloxacin (5mg/kg) was administered subcutaneously prior to surgery. The rabbits were sedated with intravenously administered acepromazine (0.5 mg/kg) and buprenorphine (0.03 mg/kg). After 15 minutes, rabbits were anaesthetized with an intramuscular injection of xylazine (1.5 mg/kg) and tiletamine/zolazepam (15 mg/kg). Anesthesia was maintained intravenously with tiletamine/zolazepam (3 mg/kg). Using a craniomedial approach, the radial diaphyses were exposed and 1-cm segmental defects were created in the middle third of both radial diaphyses using a circular bone saw. Thermal injury to the adjacent tissues was controlled by high-volume irrigation with 0.9% saline solution. The periosteum was removed along with the osseous fragment. This defect has been reported to form non-unions without therapeutic intervention, resulting in a pseudoarthrosis-like defect in rabbits[8]. The periosteum from the cut bone ends was elevated and the ulnar periosteum caudolateral to the defect site was also removed. A lyophilized resorbable membrane derived from demineralized cortical bovine bone (Gen-Derm™) was placed between the radius and ulna to enclose the segmental defect and prevent fibroblast colonization. The defect in the right radius was filled with a bone substitute compound Gen-Tech (Group A). The defect in the left radius was filled with Gen-Ox™

and Gen-Pro™ combination (Group B). The major difference is that Group A contains inorganic bone and bone-derived collagen, and consequently has less pBMP-HA than Group B. The Gen-Derm™ membrane was fixed to the periosteum using nylon 6-0 and the subcutaneous tissue and skin were sutured by a routine protocol using nylon 4-0. Postoperatively, enrofloxacin (5 mg/kg) was administered subcutaneously every 24 hours for five days and buprenorphine (0.03 mg/kg) every 12 hours for three days. The surgical wounds were cleaned daily with 0.9% saline solution and skin sutures were removed after 10 days post-surgery. Craniocaudal and mediolateral radiographs of both radii were obtained before and immediately after surgery, and at 30, 60, 90, 120 and 150 days postoperatively. Five rabbits were euthanized at 30, 90 and 150 days after surgery. The forelimbs were submitted for tomographic imaging with contiguous axial sections of 1-mm thickness (Tomograph Sytec 3000-i, General Electric). Three-dimensional coronal and oblique sagital reconstructions were generated from these axial images.

Histologic processing and histomorphometric analysis

The region of the defect was harvested and fixed in 10% phosphate buffered formalin for one week followed by decalcification in a 4.13% buffered EDTA solution (TRIPLEX III PA ACS ISSO, Merck KgaA, Germany). The decalcified specimens were dehydrated in ethanol, clarified in xylene and embedded in paraffin with synthetic resin (HISTOSEC, Merck KgaA, Germany). Longitudinal 5µm-thick semi-serial sections were obtained and stained with hematoxylin and eosin. Thirty six histological fields of each specimen were collected at 20x magnification using a Leica microscope (LEICA DMR, Leica Instruments, Nussloch, Germany) linked to a digital camera (Sony CCD – IRIS IRGB, Sony Co., Japan), for each specimen. In each field, the areas of persistent implanted material, inflammatory infiltrate, new bone formation, bone marrow and connective tissue were measured using KS 300, 3.0 software (Kontron Electronic GmbH, Carl Zeiss, Germany). The relative area for each quantified structure was calculated as a percentage of total area of the defect.

Statistical analyses

The morphometric results were analyzed by ANOVA, with the following independent variables: treatments, postoperative periods and interaction between treatments (Statistical Analysis System version 8.2, Cary, North Caroline, USA, 2001). The differences between means were tested using Student's t-test with probability level of 5%.

Results

Group A: defects treated with Gen-Tech

Radiographic results: Immediately after surgery, the radiographs showed that the defects were filled by radiopaque granular material interspersed with radiolucent areas (Figure 1A). After 30 days (Figure 1B), a radiolucent zone separating the implanted material and the new bone formed from cut bone ends and ulna was observed. Increase in radiopacity around the implanted material was more evident adjacent the osteotomy sites. Radiopacity due to periosteal bone formation was evident around the cut bone ends. At 60 days, bone bridging was mainly observed adjacent to the ulnar surface (Figure 1C). Progressive bone formation was evident at 90 (Fig. 1-D) and 120 days (Figure 1E) evaluations and it was associated with gradual loss of graft material radiopacity. At 150 days (Figure 1F) the defects were completely filled, but the granular graft material was still evident, predominantly in the cranial aspect of the defects suggesting failure of complete biomaterial incorporation.

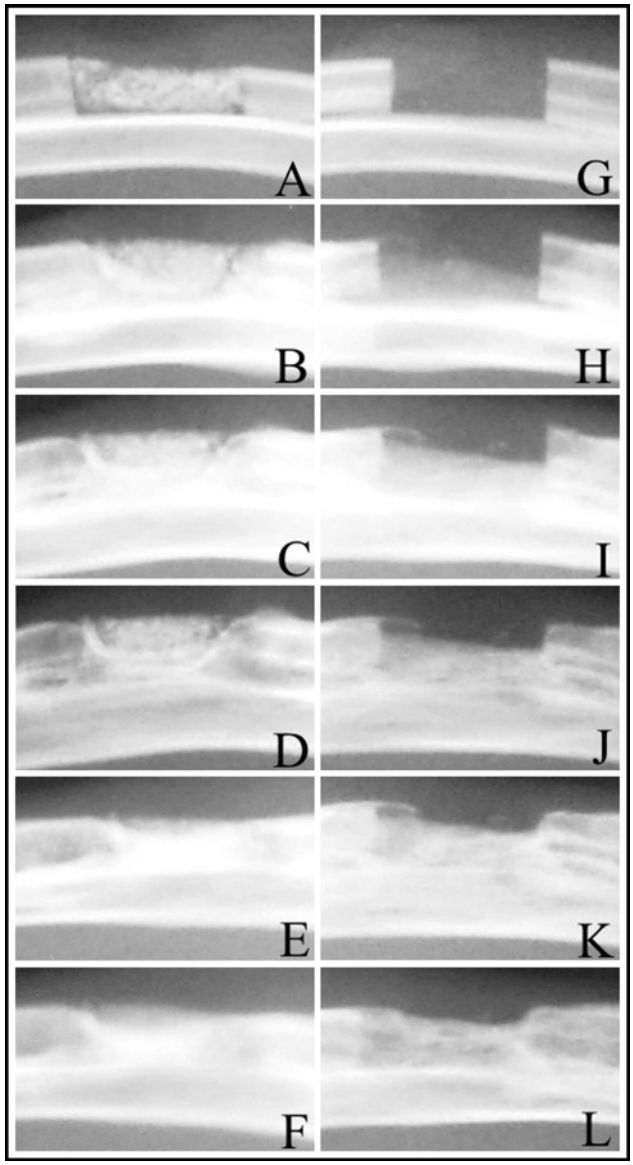

FIGURE 1 - Typical radiographic appearance of the defects of Group A, immediately after surgery (A) 30 (B), 60 (C), 90 (D), 120 (E), and 150 (F) days postoperatively; and of Group B, immediately after surgery (G), 30 (H), 60 (I), 90 (J), 120 (K), and 150 (L) days postoperatively.

Tomographic results

At 30 days after the surgery, the tomographic images showed segmental defects with approximately 7 mm in length, with new bone formation from both radial osteotomy surfaces extending into the defects. The defect itself was variably filled by new bone formed from the ulnar surface. Blocks of graft biomaterial were clearly evident, separated from the surrounding osseous surfaces by a distinct radiolucent zone. At the radial osteotomy surfaces, periosteal new bone and associated synostoses had developed between the radius and the ulna. At 90 and 150 days, the defects measured 6 mm in length and a 6 mm periosteal reaction was observed in the radial surface at the osteotomy sites. The synostoses between radius and ulna were remodeled and bone resorption occurred leaning toward a single medullary canal between the radius and the ulna. The same findings appeared in the coronal and oblique sagital reconstructions (Figure 2).

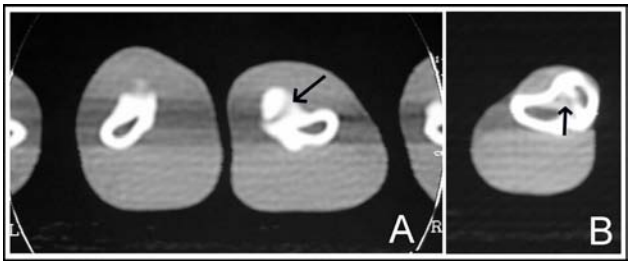

FIGURE 2 - Tomographic oblique sagital reconstruction of the forelimbs at 150 days after surgery. In A, notice the block of graft biomaterial (Gen-TechTM), and areas of bone resorption in the synostosis area between the radius and ulna leaning toward a single medullary canal (arrows). In B, notice the bone formation from the osteotomy sites and from the ulnar surface, filling the defect partially. Also, notice the areas of bone resorption (arrows)

Histological results

The histological findings at 30 days showed small bone neoformation originated from the radial osteotomy sites and intensive bone and/or cartilaginous formation in the syndesmoses areas, promoting cortical fusion between radius and ulna. The ulnar surfaces showed bone neoformation that partially filled the defects. The defects were nearly filled with bovine inorganic bone particles of the bone substitute material, wrapped with connective tissue, and exhibited a large number of cells and inflammatory areas (Figure 3). Some of these inorganic bone particles, principally near of the cut bone ends, showed bone deposition in their surfaces. In the cranial aspect of the defects, a dense wall of fibrous tissue with some areas of macrophages-rich infiltrate was seen surrounding the particles. Multinucleate giant cells were also observed in some specimens. None of the specimens showed presence of the membrane (Gen-Derm^{TM}) and HA particles, but traces of organic matrix particles were detected. At 90 days, bone remodeling with islands of bone marrow was seen in the synostoses areas (Figure 4A). At 150 days, intensive bone remodeling in the synostoses and cortical resorption were seen in three samples. All specimens showed fatty tissue, in most cases, contiguously with fatty bone marrow. The implanted material was concentrated in the cranial aspect of the defects, externally surrounded by fibrous tissue originated from the remaining periosteum. The inorganic bone particles of the implanted compound were wrapped by connective tissue or showed bone neoformation in their outer surface.

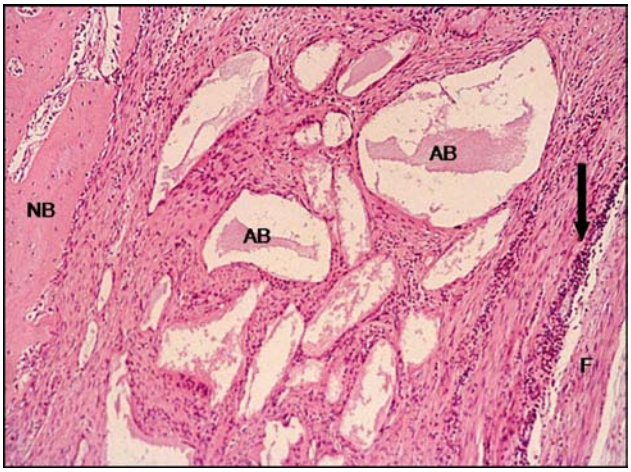

FIGURE 3 - Photomicrograph of a defect of Group A at 30 days after surgery. Notice the particles of bovine inorganic bone (AB) surrounded by connective tissue with a large number of cells and some inflammatory reaction areas. Observe the presence of a fibrous wall (F) cranially to the defect, presence of inflammatory infiltrate (arrow) and neoformed bone (NB). HE, 10x

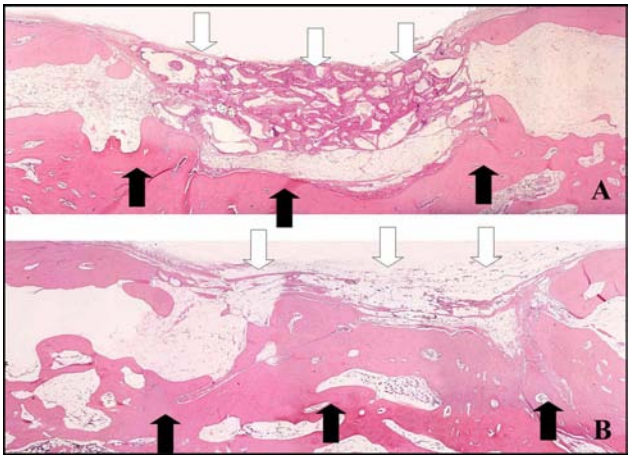

FIGURE 4 - Photomicrograph of a defect of Group A (A) and of Group B (B) at 90 days after surgery. The neoformed bone areas are indicated by black arrows and the graft biomaterial by the white arrows. HE, 4x

Group B: defects treated with Gen-Ox and Gen-Pro combination

Radiographic results

Immediately after surgery, the defects were radiolucent (Figure 1G) differently in Group A where the material ingrafted was seen. Increase in radiopacity near the radial osteotomy sites and the ulnar surface was observed at 30 days after surgery (Figure 1H). At 60 days, bone bridging was seen on the ulnar surface (Figure 1I). Progressive bone formation was observed at 90 (Figure 1J) and 120 days (Figure 1K). At 150 days (Figure1L) approximately 85% of the defects were filled with radiopacity similar to the contiguous bone.

Tomographic results

At all time points, the tomographic findings were, for each specimen, similar to those seen in the opposite limb. The only difference was the absence of a radiopaque area due to the blocks of graft biomaterial that were observed in the defects of Group A.

Histological results

In the same manner as in Group A, at 30 days after surgery, neoformed bone from the osteotomy sites and from the ulnar surface partially filled the defect. In the syndesmoses, cortical fusion between the radius and the ulna were seen near the osteotomy sites. Small amounts of the implanted material were found in the cranial aspect of the defects. The demineralized bone graft particles were being reabsorbed and were surrounded by eosinophilic cells. The remaining areas of the defects had a great number of hydroxyapatite crystals, surrounded by intense inflammatory macrophage-rich infiltrate, which were not seen in the defects of Group A. Aggregates of these micro-crystals surrounded by multinucleate giant cells were seen in some specimens (Figure 5).

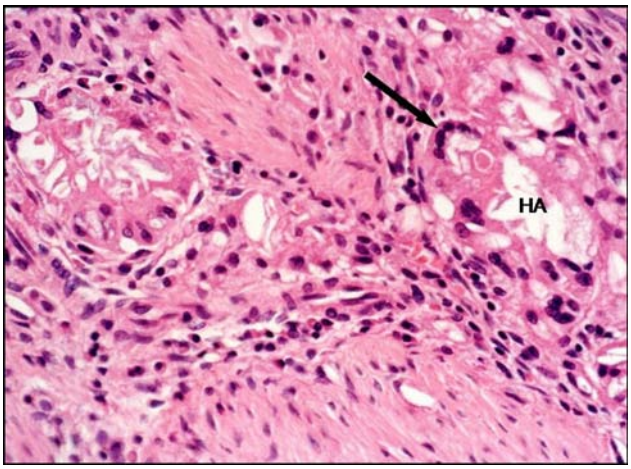

FIGURE 5 - Photomicrograph of a defect of Group B at 30 days after surgery. Observe the aggregates of micro-particles (HA) surrounded by giant cells (arrow) HE, 40x

Fibrous walls, thicker than those observed in Group A, were observed cranial to the persistent graft material and reactive tissue. Some areas of these fibrous walls showed inflammatory infiltrate with eosinophils. The membranes (Gen-Derm™) were not seen in any of the specimens. At 90 and 150 days, traces of hydroxyapatite crystals were still detected, but the demineralized matrix particles were not. As in Group A, intense remodeling of the neoformed woven bone accompanied by bone marrow formation was observed in the areas of synostosis (Figure 4B). At 150 days, cortical bone resorption with an appearance of a single medullary canal filled by bone marrow was seen in some specimens. The bone neoformation from the cut bone ends and the ulnar surface partially filled the defect, and the remaining spaces were filled by soft connective tissue and adipocytes.

Comparison between morphometric results

The persistence of implanted material was significantly higher in the defects of Group A at all times of the experiment ($p<0.05$). The presence of inflammatory infiltrate was higher ($p<0.05$) in Group B at 30 postoperative days. The areas of bone and bone marrow showed a statistically significant increase between 30 days and 90-150 days ($p<0.05$) in both groups, and Group B had significantly more new bone formation than Group A at 90 postoperative days ($p<0.05$) (Figure 6). The connective tissue showed statistically significant differences between 30 days and 90-150 days ($p<0.05$), but no difference was observed between treatments.

Discussion

Many researchers have reported total healing of large segmental defects in eight to 12 weeks postoperatively, when recombinant or purified BMP was used[9,10]. Bovine purified BMP (bBMP), or even the crude extract, showed their osteogenic properties in other animal defects models [7,11,12] and in human clinical studies [6,7,13]. However, the present experiment using two commercially available compound bone substitutes, both containing pooled fetal bone morphogenetic proteins (pBMPs), failed in promoting total healing of rabbit radius segmental defects within 20 weeks after surgery. Bone healing was partial in both groups being more evident in Group B. Bone healing exclusively occurred due to bone formation from the osteoconductive surfaces of the osteotomy sites and ulnar surface. New bone formation was not observed in the central area of the defect near the implanted material, by radiographic, tomographic or histologic examinations. This suggests absence of bone formation by osteoinduction. The analyses showed that both materials were partially or totally isolated by a fibrous tissue. The components were resorbed with different rates, and the block of material was dislodged to the cranial aspect of the bone tissue being formed. Therefore, in both groups, the osteogenesis occurred with little or none involvement of the implanted materials. To discuss the factors that might have led to these negative results, it is necessary to analyze the biological characteristics of each compound, separately. According to manufacturer's information, Gen-Tech™ (Group A) is constituted by pooled fetal bone

morphogenetic proteins (pBMPs) adsorbed to an absorbable ultrathin powdered hydroxyapatite, mixed with inorganic bone particles and granulated demineralized bone matrix, and bone-derived collagen as agglutinant. The Gen-Ox™ and Gen-Pro™ (Group B) are constituted, respectively, by granules of demineralized bone matrix and pBMPs adsorbed to absorbable ultrathin powdered hydroxyapatite. The compounds differ by the presence of inorganic bone and bone-derived collagen, and consecutively less amount of pBMP-HA in Group A. The inorganic bone particles used in the first compound are natural ceramic of hydroxyapatite produced by deproteinization of cancellous bovine bone at high temperatures. This material is highly biocompatible and, due to its mineral surface being identical to the natural bone, supports adhesion of bone progenitor cells to its surface[1]. These cells proliferate and undergo to morphological maturation to become osteoblasts that are responsible by bone neoformation[1]. Therefore, this inorganic bone matrix exerts its function by its high osteoconductive property[3]. In recent years, the inorganic bone is one of the most used materials as bone graft, in dentistry. In the present study, the osteoconductive property of this material was verified next to the osteotomy sites, where the inorganic bone particles were surrounded by neoformed bone, leading toward their enclosure. Therefore, this component of Gen-Tech™ does not seem to be responsible for the incomplete bone healing verified in this study. The use of demineralized bone matrix, mainly the allogenic, in the treatment of bone defects is based on the fact that the organic matrix also possesses growth factors and BMPs in small amount that are biologically significant, i.e., with osteoinductive property[4,5]. The osteoinductive and osteogenic properties were verified by Einhorn et al.[14] in the treatment of critical segmental defect in femur of rats. These properties were also verified by Bolander and Balian[15], while comparing the use of organic matrix to autographs on healing of 2 cm bone defect in ulna of rabbits. Nevertheless, studies have failed in demonstrating the osteoinductive property of the demineralized bone matrix when used in heterotopic sites of different experimental species[16,17]. An absent or a nearly absent osteogenic response in non-genetically modified animals is due to an immunological interference in the host. The expression of the osteoinductive property in immunodeficient animals supports this assumption[17]. On the other hand, Taga et al.[18] using a demineralized bone matrix similar to one used in the present experiment, verified matrix resorption and new bone formation three months after the implantation. They used the demineralized bone matrix to treat critical-size calvarial defects of guinea pigs. Moreover, they observed total healing of the defects 6 months after the surgery. In the present study, both groups showed rapid resorption of the demineralized bone matrix. At 30 days, traces of demineralized bone matrix were seen associated to inflammatory reaction with presence of eosinophils, and no osteoinductive activity was verified. The presence of inflammatory cells surrounding the particles of demineralized bone matrix suggests that an immunological reaction against the xenogenic matrix could have occurred, inhibiting its osteinductive and osteogenic activities. Regarding the use of BMPs to stimulate osteogenesis on healing of bone defects, researchers have been shown that best results are obtained with use of human recombinant BMPs or bovine chromatographically purified BMPs[11,19]. In the present experiment, the BMPs presents in the two tested compound consisted of a non-purified guanidine hydrochloride extract from bovine fetal bones, i.e., this extract contains also others hydrophobic non-collagenous proteins. In this respect, Costa Filho et al.[12] testing a pool of bBMPs bound to a similar absorbable hydroxyapatite mixed with inorganic bovine bone in cylindrical titanium prototypes introduced into tibiae of rabbits, concluded that the pool of bBMPs containing other bone non-collagenous proteins had a inhibitory effect on osteogenesis. They also verified that the biomaterials without the *pool* of BMP formed a favorable substrate to bone formation. Thus, some of these proteins could induce immunological response [16,20], or the presence of osteogenic inhibitor proteins (OIPs)[21] could be responsible for inhibiting the bone neoformation, in both groups. Therefore, the pBMPs used in the present study may have impaired the effects of the two compounds on healing of rabbit segmental bone defects. However, we pointed out that none of the specimens, in both treatments, exhibited histologically significant number of lymphocytes and/or plasma cells around of the graft particles. Another fact to be considered is the carrier for the BMPs used in the two compounds tested here. According to the manufacturer's information the synthetic microgranular absorbable hydroxyapatite is used. Particles of BMP-HA were detected histologically at 30 days after surgery in higher amounts in the defects treated of Group B associated to intense macrophage-rich inflammatory infiltrate. A prominent foreign body reaction was observed around HA agglomerates, more so in Group B. Brandão et al.[22] evaluating the same hydroxyapatite, mixed or not with pBMPs implanted in rat alveolar wounds, achieved delay in wound healing in both groups, suggesting an absence of osteogenic properties in these materials. A similar finding was observed by Ferreira et al.[23] while using the same hydroxyapatite bound to pBMPs for treatment of critical size calvarial defects in rats. The authors showed occurrence of intense foreign body reaction around agglomerates of BMP-HA particles with high number of macrophages and multinucleate giant cells. The authors concluded that this type of hydroxyapatite was responsible for the deleterious effects of the biomaterial. We suggested that the same effects might have occurred here. Regarding the carriers, good results were described using other carriers such as polylactide and/or polyglycolide polymers[10], demineralized organic bone matrix[11] and collagen[9,19]. Besides, the chemical characteristics of the carrier such as their geometry with presence of pores and its interconnection might influence osteogenesis, independently of BMP stimulus[24]. Therefore, other carriers that produced better results must be used for testing the pBMPs studied here. The use of a membrane barrier associated or not associated with osteoinductive materials is a current procedure in dentistry[25,26]. Also, it has been used in orthopedic surgery[13,27], being a center of interest in this study. The e-PTFE membrane is one of the best and has produced good results in several studies[13,27]. However, because it is non-absorbable, there is a need for another surgery procedure for its removal. In the present

study, an absorbable membrane derived from demineralized bovine cortical bone was used. The results showed that its presence was not seen histologically at 30 days after surgery in both treatments. Collagen membrane has shown satisfactory results in dentistry[25,26] and in treatment of non-segmental defects experimentally produced in flat bones of several laboratory animals[18,28]. Despite that, in segmental defects, there is a need for better controlled studies to establish the membrane influence in bone formation. It should be emphasized that in accordance with Zellin et al.[29], membranes developed originally for periodontal regeneration might not be suitable for promoting bone regeneration in segmental bone defects, and their physical characteristics, such as porosity and stiffness[9], are essential as well as their composition[30] for providing a membrane barrier. Based on the results obtained in this study, we concluded that the partial bone healing of the segmental defect created in the radii of rabbits, treated with two proprietary compounds containing pBMPs, especially occurred by the bone formation induced, in the osteoconductor surfaces of the osteotomy sites and ulnar surface, by surgical trauma; with low or none performance of the materials tested here. The carrier for pBMPs used in both compounds, generated a foreign body reaction that might have inhibited the osteoinductive effects of pBMPs, and the osteogenesis, delaying and/or interfering the bone healing in the defects.

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Conflict of interest: none
Financial source: FAPESP and CNPq

Received: July 05, 2006
Review: August 13, 2006
Accepted: September 18, 2006

How to cite this article:
Caporali EHG, Rahal SC, Morceli J, Taga R, Granjeiro JM, Cestari TM, Mamprim MJ, Correa MA. Assessment of bovine biomaterials containing bone morphogenetic proteins bound to absorbable hydroxyapatite in rabbit segmental bone defects. Acta Cir Bras. [serial on the Internet] 2006 Nov-Dec;21(6). Available from URL: http://www.scielo.br/acb.

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