5 - ORIGINAL ARTICLE
MODELS, BIOLOGICAL

Tomographic imaging of fragmented cortical bone heteroimplant and methylmethacrylate in segmental bone defect of rabbit tibia[1]

Silvio Henrique de Freitas[I], Renata Gebara Sampaio Dória[II], Fábio de Souza Mendonça[III], Marcelo Diniz dos Santos[III], Jair Rodini Engrácia Filho[IV], Atanásio Serafim Vidane[V], Alessandro Tadeu Corrêa Marques[VI], Carlos Eduardo Ambrósio[VII]

DOI: http://dx.doi.org/10.1590/S0102-86502014001900005

[I]PhD, Professor, Postgraduate Program in Animal Bioscience (PPBA), Faculty of Veterinary Medicine (FMV), University of Cuiaba (UNIC), Cuiaba-MT, Brazil and Postdoctoral Fellowship, Department of Veterinary Medicine (DMV), Faculty of Animal Sciences and Food Engineering (FZEA), University of Sao Paulo (USP), Pirassununga-SP, Brazil. Technical procedures, critical revision, approved the final manuscript.
[I]PhD, Professor, PPBA, FMV, UNIC, Cuiaba-MT, Brazil. Acquisition of data, supervised all phases of the study.
[II]PhD, Professor, PPBA, FMV, UNIC, Cuiaba-MT, Brazil. Technical procedures.
[IV]PhD, Researcher, Department of Pathology, Faculty of Agriculture and Veterinary Sciences, Sao Paulo State University Júlio de Mesquita Filho (UNESP). Jaboticabal-SP, Brazil. Analysis of data, manuscript preparation.
[V]Master, Fellow PhD degree, Postgraduate in Anatomy of Domestic and Wild Animals, Department of Surgery, Faculty of Veterinary Medicine and Animal Science, USP, Sao Paulo-SP, Brazil. Interpretation of data, manuscript preparation.
[VI]Master, Professor, Faculty of Odontology, UNIC, Cuiaba-MT, Brazil. Design of the study, interpretation of data.
[VII]PhD, Associate Professor, DMV, FZEA, USP, Pirassununga-SP, Brazil. Interpretation of data, manuscript writing, critical revision.

ABSTRACT

PURPOSE: To evaluate the performance of composites consisting of fragmented cortical bone heteroimplant in association with methylmethacrylate preserved in 98% glycerin, in segmental bone defect of rabbit tibia medial metaphysis.

METHODS: In this study were used twelve adult New Zealand rabbits, divided into three groups of four animals each: G30 (30 days), G60 (60 days) and G90 (90 days). The bone defects previously created in the tibia were filled with composites and both were evaluated by cone-beam computed tomography, immediately after surgery and after 30, 60, and 90 days.

RESULTS: The composites fulfilled and remained in the sites of bone defects in all cases and were not registered signals of infection, migration or rejection.

CONCLUSIONS: The implanted composites promoted the bone defects repair without signals of infection and/or rejection. The composites are one more option for bone defects repair.

Key words: Biocompatible Materials. Tomography. Tibia. Rabbits.

Introduction

The trauma leading to bone fractures, especially the comminuted fractures, play a key role in both clinical and surgical veterinary medicine routine, similar in human. Moreover, diseases such as osteomyelitis, non-unions, bone tumors, and others who require reconstructive orthopedics replacement of bone tissue are common[1-3]. The alternative treatment for bone defects is the spongious autograft, which involves the removal of spongious bone tissue from the patient, biologically compatible and accelerates bone repair. Nevertheless, the inconvenience of this procedure is that occurs in two stages, increasing patient morbidity by damaging normal structures, the pain, and prolongs the anesthetic and surgical period, and did not provides enough bone volume to repair large bone defects[3,4].

To prevent problems inherent to autograft, the current methods to treat fractures with large bone defects consist on biological or synthetic biomaterials, which are biocompatible and able to interact with the living system[4,5]. The bone tissue derived from the same species (aloimplats) or from different species (heteroimplants) are biological biomaterials that have been used with satisfactory results to repair bone defects[3,6,7].

Furthermore, the heteroimplants have osteoinductive properties, which occur by bone tissue formation from osteoprogenitor cells, derived from primitive mesenchymal cells under the influence of one or more inductive factors from bone matrix. Moreover, promotes the bone growth by affixing the subjacent bone tissue in the presence of bone or undifferentiated mesenchymal cells, featuring the osteoconductive properties. In addition, they must be biocompatible, non-carcinogenic, non-toxic, non-antigenic and not stimulate inflammatory processes or favor the infection[4,8].

The orthopedic disorders in bone defects may also be efficiently fulfilled by synthetic biomaterials, such as calcium phosphate cement, hydroxyapatite, lactic glycolic copolymer, methylmethacrylate, and others[9-11,20]. The methylmethacrylate has bioinert and biotolerable properties, can be molded to obtain a more adequate form, therefore is currently widely used in orthopedic surgical procedures to repair large bone defects[2,12,18].

A new approach for large bone defects repair would be a composites consisting of biological biomaterials (heteroimplants) and synthetic biomaterials (methylmethacrylate) with osteogenic, osteoinductive and osteoconductive properties, not favoring bacterial colonization or infection, which would provide mechanical stability, easy acquisition cost, did not require a specialized environment for their preservation, and completely fulfill the bone defect, eliminating thereby the problems inherent to autograft[3,13,19].

Besides radiographic examination, the biomaterials behavior can also be assessed by computed tomography (CT), which allows a visualization of all the three-dimensional layered structures, in particular the mineralized tissues. The imaging method allows a playback of a tissue cut section in any one of the three spatial planes. While the conventional radiographic technique which projects in a single plan all structures penetrated by x-radiation, the CT technique allows the evidence of depth structural relationships, showing images of tissues in serial sections (slices) in high definition. It allows evaluating, defining, limiting and quantifying the bone tissue reactions[4,14-16].

Therefore, is proposed in this study, the evaluation of cortical bone fragmented heteroimplant (HOCF) behavior, associated with methylmethacrylate in segmental bone defects in rabbit tibiae through cone beam computed tomography.

Methods

This study was approved by the University of Cuiaba-INIC Ethical Committee in animal research (2010-049), according to Resolution 196/96 of the National Health Council.

Twelve adult New Zealand rabbits, weighing an average of about 3kg, divided into three groups of four animals each were used as recipients: G30 (30 days postoperative), G60 (60 days) and G90 (90 days).

The cortical bone heteroimplant (HOC) was collected aseptically from canine tibial diaphysis, clinically healthy, with a history of traumatic death. The smooth tissues adjacent to tibia, epiphysis and bone marrow were removed, then, the diaphysis was collected, washed with 0.9% saline solution (0.9% saline solution®, JP Pharmaceutical Industry S/A, Brazil) and conditioned in 98% glycerin (glycerin®, VIC Pharma Industry and Commerce Ltda, Brazil), for a period not less than 30 days, at room temperature. For its use, the cortical bone hetroimplant (HOC) was hydrated in 0.9% saline solution for 10 minutes, divided into small particles approximately 2mm using the orthopedic nibbler, and finally dehydrated at room temperature. The cortical bone fragmented heteroimplant (HOCF) was mixed with methylmethacrylate polymer powder (1:1) and the liquid monomer (Vipi Flash®, Vipi Industries, Trade, Import Export, Brazil) was added until the pasty consistency was reached. The implant was then molded to the template of 6mm diameter and 2mm thickness. The generated composites were conditioned in 98% glycerin at room temperature for at least 30 days.

Preoperatively the trichotomy of medial proximal region of the left tibia was performed, and the animals were anesthetized with combination of acepromazine (0.1 mg / kg) (0.2% Acepran®, Univet S/A, Brazil) and tiletamine / zolazepam (20mg/kg) (50 Zoletil®, Virbac's Brazil Industry and Commerce Ltda, Brazil), via intramuscular, followed by local infiltration anesthetic using 0.4 mL of lidocaine 2.0% (Lidovet®, Bravet, Brazil).

In dorsal decubitus under padded metal gutter, the antisepsis was performed using povidone-iodine (Topic Riodeine®, Pharmaceutical Riochemistry Ltda, Brazil), and then was made an incision on the skin and the proximal medial cortex of the left tibia was exposed. Using a trephine drill (Trephine Drill®, Dental Aragon, Brazil) attached to an autoclavable low speed electric drill (Autoclavable Electric Drill®, Caomedica, Brazil), was created a bone defect in this region, by removing a corticoperiosteal segment (6mm diameter). The bone defect was fulfilled with composite previously hydrated in 0,9% of saline solution, the periosteum and the subcutis were approached with 3.0 polyglycolic acid (Medcryl®, Med Goldman, Manaus, Brazil), and the skin was positioned with 3.0 polyamide yarn (Nylon®, Brasmedica, Brazil). Five consecutive applications of enrofloxacin (10mg/kg) (2.5% Flotril®, Intervet - Schering-Plough, Brazil) were performed subcutaneously every 24 hours; three consecutive applications of tramadol hydrochloride (4mg/kg) (Tramadol hydrochloride®, Hipolabor, Brazil) subcutaneously every 8 hours, and two daily dressings with rifampicin (Rifocina spray, Pharmaceutical Laboratory of Pernambuco, Brazil) until the tenth day, when the wound stitches were removed. Postoperatively, the animals were housed individually in cages, climate-controlled environment, fed with commercial diet (Feed Labina®, Purina, Brazil) and water *ad libitum*.

After each postoperative estimated time: 30, 60 and 90 days, the animals were euthanized using the anesthetic protocol previously described, followed by cardio-respiratory arrest with propofol (Propovan®, Cristalia, Brazil) and 10% of potassium chloride (Potassium Chloride 10%®, Alexistar, Brazil) intravenously. The implanted tibias with the composites were evaluated by cone beam computed tomography - Cone beam (60kV - 2.5 mA - 10.8s, KODAK 9000 3D model, Carestream Health, France).

Results

All animals supported the operated limbs immediately in the postoperative period, showing that the tibia structures were not compromised, and the surgical wounds healed in an average period of 15 days without signs of infection and/or reaction that would suggest rejection.

In the immediate postoperative period, all implanted tibias were evaluated by cone beam computed tomography, to confer the real position of implants in the receptors patients (Figure1).

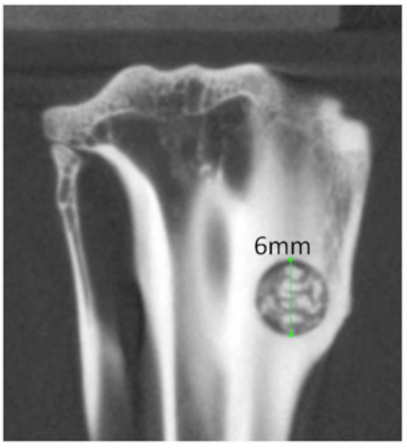

FIGURE 1 - CT scan of the proximal region of rabbit tibia in the immediate postoperative period. The composite in the recipient implanted site, showing radiolucent areas (methylmethacrylate) and radiopaque (HOCF). Sagittal section, 0.8 mm thickness.

The computed tomographic evaluations, at the coronal and axial sections in the rabbits tibias from each group G30 (Figures 2A and B), G60 (Figures 2C and D) and G90 (Figure 2E and F), revealed that all the composites remained in the implanted sites, no sign of proliferation or bone lysis, or reactions was reported.

Moreover, the CT scan also revealed heterogeneous densities of all composites, consisting of radiolucent areas, corresponding to methylmethacrylate, and radiopaque areas, corresponding to HOCF, during all the evaluation period (Figures 2B, 2D and 2F).

The radiopacity between composite interfaces increased in the animals of G60 (Figure 2C) and G90 groups (Figure 2E),

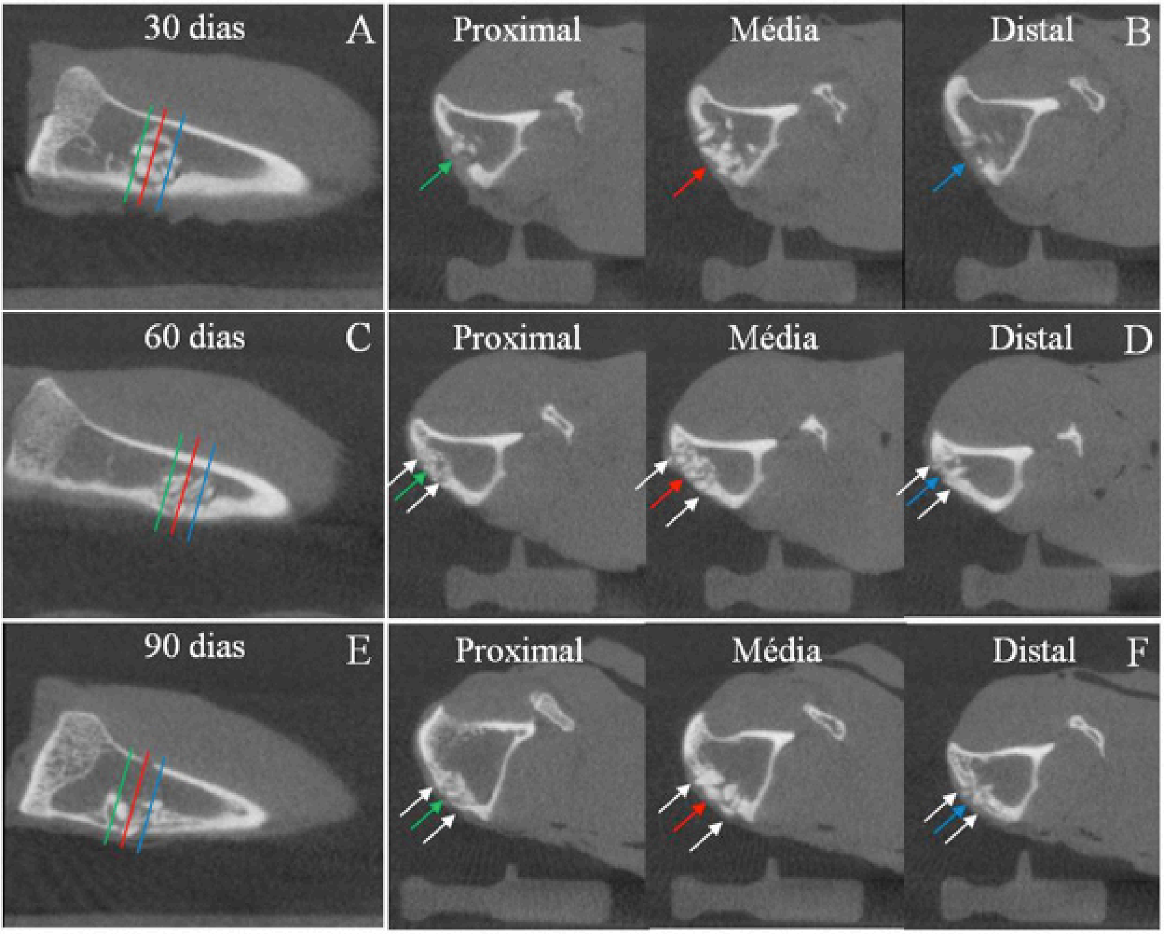

FIGURE 2 - CT scan of the proximal region of rabbit tibia at 30, 60 and 90 days. A, C and E: The composite in the recipient implanted site - green, red and blue lines (coronal section in anteroposterior position). B, D and F: The absence of reactions in the following portions: proximal - green arrow, medium - red and distal arrow - blue arrow (axial section). C and E: The high radiopacity in the composite - recipient interfaces (white arrows). Sections: 1.1 mm interval and 0,076 mm thickness.

due to increased density characterized by apposition of bone tissue and composite incorporation.

Discussion

All the materials and tools used directly for composite preparation were sterilized. Furthermore, the composites were stored at 98% glycerin over 30 days, period which, according to Vilela *et al.*[17] and Freitas *et al.*[2], is long enough for the bactericidal properties of this preservation medium destroy totally the all bacteria present in the implants. If these microorganisms colonize the surgical wound, they would occur the bone lysis and therefore, instability and migration of the composites in the site of transplantation

All animals supported the operated limbs immediately in the postoperative period, showing that the tibia structures were not compromised with osteotomy. Moreover, the analgesic effect reduced the pain and stress and also provided more comfort to the patient, and it may have contributed to the early use of the limb[3].

All animals (100%) had wounds healed within 15 days, exempt infection signal or tissue reaction, showing that the preoperatively and postoperatively care (antisepsis, asepsis and antibiotic therapy) were efficient[15].

When HOCF in association with methylmethacrylate (pasty consistency) are applied directly in the receptor bone defect, it could cause thermal necrosis and lysis of the bone tissue due to the exothermic reaction produced by the polymerization of methylmethacrylate. However, this was not observed in this study because before implantation, the composites were completely polymerized, therefore no exothermic reaction occurred at the time of its accommodation in the implantation site. According to Freitas *et al.*[2] and Moreira *et al.*[4], it was decisive for its full incorporation into the bone defect.

The composite incorporation and engraftment to the all recipient bone defect, is indicative of rejection absence and existence of osteoinductive properties, characterized by angiogenesis and fibroblast invasion involved with the bone tissue deposition, which also characterizes the osteoconduction[7].

The cone beam computed tomography allowed monitoring and satisfactory demonstration the composite behavior in the rabbit tibias in different evaluation phases. In this study, was possible to observe the bone tissue deposition and remodeling, since this technique also allows high resolution 3D tissue imaging. Therefore, in human odontology the cone beam CT is widely used to assess mineralized bone tissue and monitoring bone remodeling post-graft[4,15,16].

The CT scans of orthogonal and axial sections of rabbit tibia, in all groups G30 (Figures 2A and B), G60 (Figures 2C and D) and G90 (Figures 2E and F) revealed that the composite remained in the implantation site and were reported heterogeneous density of all composites consisting of radiolucent areas, corresponding to methylmethacrylate, and radiopaque areas, corresponding to HOCF, during all the evaluation period (Figures 2B, 2D and 2F). The radiopacity between composite interfaces increased in the animals of G60 (Figure 2C) and G90 groups (Figure 2E), characterized by apposition of bone tissue and composite incorporation. According to Moreira *et al.*[4] this findings occur due to composite osteoinductive and osteoconductive properties.

Conclusions

The composite of cortical bone heteroimplant fragmented (HOCF) and methylmethacrylate was successfully engrafted and promoted the repair of bone defects without signs of infection and/or rejection. Is biologically compatible, therefore, is effective alternative for bone substitute in bone defects healing.

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Correspondence:
Silvio Henrique de Freitas
Rua Siqueira Campos, 4380/casa 530
13631-018 Pirassununga – SP Brasil
Tel.: (55 19)99879-5297
shfreitas@terra.com.br

Received: Aug 18, 2014
Review: Oct 17, 2014
Accepted: Nov 20, 2014
Conflict of interest: none
Financial source: Faculty of Veterinary Medicine, UNIC

[1] Research performed at Laboratory of Experimental Surgery, Faculty of Veterinary Medicine, University of Cuiaba (UNIC), Cuiaba-MT, Brazil.