BIOMATERIAL IMPLANTS IN BONE FRACTURES PRODUCED IN RATS FIBULAS

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ABSTRACT

Objective: To evaluate the importance of collagen and hydroxyapatite in the regeneration of fractures experimentally induced in the fibulas of rats. Method: 15 rats were used. These were subjected to surgery to remove a fragment from the fibula. This site then received a graft consisting of a silicone tubes filled with hydroxyapatite and collagen. Results: Little bone neoformation occurred inside the tubes filled with the biomaterials. There was more neoformation in the tubes with collagen. Conclusion: The biomaterials used demonstrated biocompatibility and osteoconductive capacity that was capable of stimulating osteogenesis, even in bones with secondary mechanical and morphological functions such as the fibula of rats.

Keywords – Durapatite; Fibula; Collagen; Osteogenesis

INTRODUCTION

The frequency of traumatic fractures has increased considerably over recent years, mainly as a result of vehicle accidents and diseases affecting bone metabolism(1). Thus, different orthopedic treatments for stimulating and accelerating bone regeneration have been widely investigated. Among these, the use of fundamental bone grafts in clinical cases of comminuted or explosive fractures in which there may be a need to use a graft because of the considerable loss of bone mass, according to the trauma energy or severity of the bone disease, has been highlighted.

As an alternative to repairing these fractures, with or without a possible association with autogenous bone grafts or other factors that induce osteogenesis, the use of biomaterials has also been highlighted because of their osteogenic properties and biocompatibility, along with the ease of construction, given the advances in tissue engineering that have be made. Thus, hydroxyapatite and collagen are among the various materials that have been receiving special attention in many studies that have sought synthetic implants that might be ideal for osteoconduction, biocompatibility and biomechanical resistance during the repair process on bone defects or in regeneration from fractures(2-10).

Hydroxyapatite has good bone conductibility, which influences its reabsorption speed and is regulated mainly by the porosity of the material(11). Direct stable contact between this biomaterial and the bone stimulates osteogenesis and therefore osseointegration of the biomaterial(12). Nandi *et al*(13) carried out a study to evaluate the efficiency of porous hydroxyapatite in bone defects that had been created in the diaphysis of the radius in goats, and observed good bone formation and revascularization in the area grafted with hydroxy-

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Declaramos inexistência de conflito de interesses neste artigo

apatite, thereby confirming the natural biological osteoconductive property of this material.

The indications for the use of hydroxyapatite are directed towards correction of cranial maxillofacial defects, traumatic events and congenital deformities, and may also be used in plastic surgery[14,15]. Other substances that deserve attention are natural polymers, which have been used in many applications[16].

Natural polymers like collagen not only are biocompatible but also participate in controlling the structure of the tissue and in regulating the cell phenotype, thus simulating the extracellular matrix. Collagen is the most abundant fibrous protein in the human organism, representing 25 to 30% of the total protein mass in mammals. Since collagen is the main organic compound in bone tissue, it has been widely used for manufacturing biomaterials[17].

The biocompatibility and stability of collagen, which are due to its biological characteristics of biodegradability and bioabsorbability, its antigenic debility and its capacity for easy manipulation into different forms, make it a fundamental resource for medical application[18]. Takaoka *et al*[19] used collagen from demineralized bone together with hydroxyapatite for treating congenital and acquired orthopedic defects. From their results, they noted that collagen from demineralized bone grafted in combination with hydroxyapatite was an excellent osteoinductive material in association with bone morphogenetic protein (BMP).

The aim of the present study was to evaluate the osteoconductive capacity of hydroxyapatite and collagen in the bone repair process in defects caused by removal of part of the middle third of the fibula in rats.

METHODS

Animals

Fifteen adult albino Wistar rats (Rattus norvegicus) were used, which came from the vivarium of Jundiaí School of Medicine. The animals were divided as follows:

Group TS: animals that received an empty silicone tube in the defect that was created in the fibula;

Group TH: animals that received a silicone tube filled with hydroxyapatite in the defect that was created in the fibula; and

Group TC: animals that received a silicone tube filled with collagen in the defect that was created in the fibula.

Surgical procedure

Firstly, the animals were weighed and anesthetized with a solution of ketamine (Francotar) and xylazine hydrochloride (Virbaxyl 2%), in proportions of 1:1 and at a dose of 0.10 ml/100 grams of body weight, intramuscularly. The animals were placed in dorsal decubitus and a longitudinal incision was made in the skin of the anterolateral region of the left leg. The musculature was moved aside in order to expose the fibula. With the aid of surgical materials, a defect was produced by removing approximately 2 mm from the middle third of the fibula. Silicone tubes were placed in this site.

Radiological evaluation

Eight weeks after the implantation, the animals were sacrificed and the leg bones were subjected to radiography using the FUNK-X10 apparatus with a focal point of 0.8 x 0.8 mm and Kodak radiographic film measuring 4.4 x 3.3 cm.

Histological evaluation

The samples were subjected to the histological techniques of fixation, decalcification and slide production, with semi-serial longitudinal sections in the area of the bone defect filled with silicone tubes.

Morphometric study

The neoformed bone was quantified by means of stereology, in accordance with the Delesse principle (Mandarim de Lacerda, 1999). The following formula was used:

$V_V = P_P/P_T$ (%), where:

V_V = volume density or relative volume;

P_P = quantity of points (line intersections) over the neoformed bone; and

P_T = total number of points in the system.

By means of a quadrilateral grid of 100 points coupled to the eyepiece of a Carl Zeiss optical microscope, the density of the neoformed bone volume in the area of the implanted silicone tubes was calculated, starting from the extremity of the fibular fragment. This analysis was performed with the objective lens of the optical microscope standardized as a magnification of 4x.

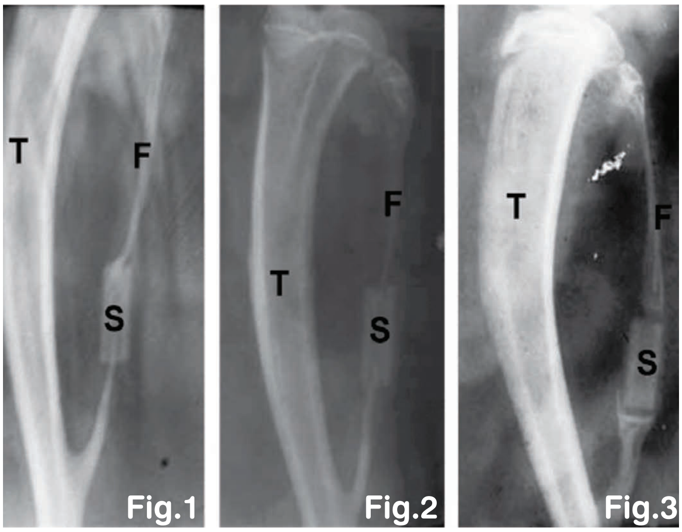
Statistical evaluation

The technique used for analyzing the morphometric data was evaluation of three independent samples and parametric means, using the Watson-Williams method.

RESULTS

Radiological evaluation

In the animals of the groups TS, TH and TC, it was seen that there was good interaction between the silicone tube and the surrounding tissue, given that there was a clear radiopaque image of the outline of the tube and no radiological sign of pathological abnormalities (Figures 1, 2 and 3).



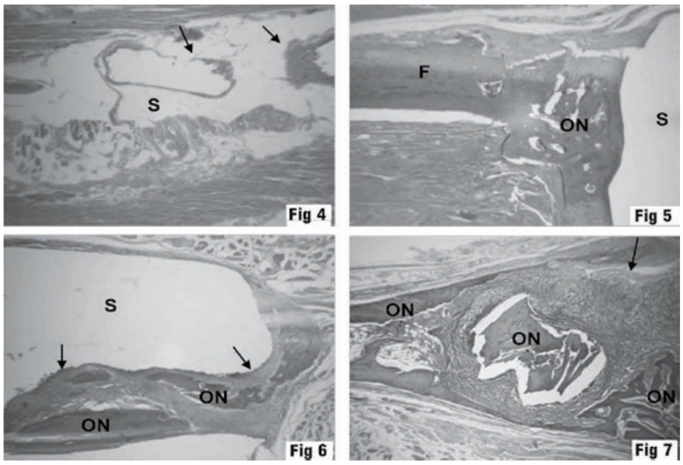
Figures 1, 2 and 3 – Groups TS, TH and TC, respectively. Note the radiopacity of the implanted silicone tube (S). Key: T = tibia; F = fibula.

Histological evaluation

In the animals of the group TS, it was noted that the interior of the silicone tube was partially filled with connective tissue, without indications of bone neoformation (Figure 4). In addition, there was a proliferation of bone tissue from the fibular fragment towards the end of the implanted tube (Figure 5). In the animals in the groups TH and TC, it was observed that as well as connective tissue, areas of bone neoformation were present inside the silicone tube, together with young bone growing from the end of the fibular fragment (Figures 6 and 7).

Morphometric and statistical evaluation

From quantification of the percentage of neoformed bone in the area of the implant, it was seen that the values were greater for the groups TH (10.2%) and TC (13.4%) than for the group TS (2.6%). Statistically, the values were different between the groups ($p < 0.05$) (Figure 8).



Figures 4 and 5 – Group TS; Figure 6 – Group TH; and Figure 7 – Group TC. In Figure 4, note the presence of connective tissue (arrows) inside the silicone tube (S). In Figure 5, the presence of neoformed bone (ON) from the fragment of the fibula (F) can be seen towards the end of the silicone tube, but without indication of neoformed bone inside these tubes. In Figures 6 and 7, note the connective tissue (arrows) and areas of bone neoformation (ON) inside the silicone tubes that are filled with biomaterials.

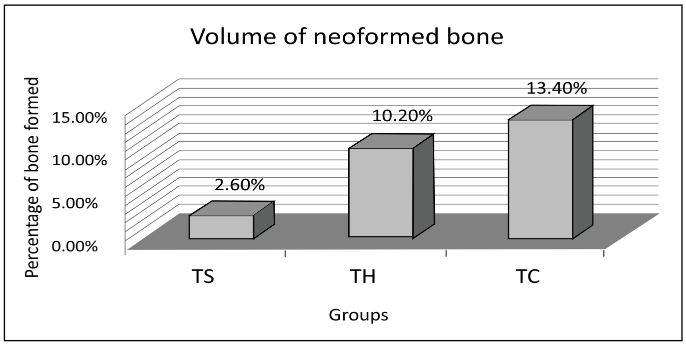


Figure 8 – Percentage of neoformed bone in groups TS, TH and TC.

DISCUSSION

The clinical limitations on the use of autogenous bone grafts in fractures with bone loss have led several studies towards advances in the field of tissue engineering and biomaterials, with the aim of manufacturing synthetic materials that would be capable of promoting fast osteogenesis and incorporation with bone tissue through osteoconductive and osteoinductive stimulation, without generating rejection complications associated with their use, as an essential biocompatibility factor, in addition to providing biomechanical resistance at the implant site[20]. Hydroxyapatite and collagen meet these requirements and have been receiving considerable attention within the fields of plastic surgery, orthopedics and dentistry[21].

Duarte *et al*[22] used synthetic hydroxyapatite in a defect in the alveolar process of the mandible of dogs and observed intense proliferation of osteoblasts and neovascularization in the presence of the implant. Camilli *et al*[23] implanted hydroxyapatite subperiosteally in the femur of rats and observed good bone neoformation in the area of the implant, as well as biocompatibility. Similar results were also described by Pinheiro *et al*[24] from implantation of hydroxyapatite in a bone defect created experimentally in the distal third of rats. Cunha *et al*[8] implanted collagen in defects in the femur of rats and noted that there was good closure of the area because large quantities of bone had formed. They concluded from biomechanical tests that the regenerated area presented good mechanical quality.

In addition to the importance that biomaterial implants should present biocompatibility and osteoconductive capacity for the bone regeneration process, the mechanical quality and type of embryological ossification of the bone are also fundamental. Camilli *et al*[23] observed that the femur, which is an endochondral bone, responded better to hydroxyapatite implantation than did the skull cap, which originates from membranous ossification. Raab *et al*[25] stated that the mechanical function of the bone influenced the resistance and formation of the bone tissue. Thus, it can be seen in the literature that most studies on biomaterials have used the femur and tibia of rats because of their good biomechanical capacity and endochondral origin, which is important for the osteogenic function of the bone[8-29].

Regarding the fibula of rats, it can be seen that it presents morphological peculiarities, since the axis of the distal diaphysis of the tibia fuses postnatally with the fibula. This process starts around the seventh day, with the formation of secondary cartilage that subsequently is replaced with endochondral ossification. Thus, the fibula presents low biomechanical quality and importance[30]. It is defined that the fibula presents a reciprocal role in regulating the growth of the tibia in rats. The low biomechanical influence of the fibula, even with the low action of gravity to which it is subjected, may interfere with the consolidation of fractures through its insufficient angiogenic and osteogenic function[31].

Through the anatomical factors of the fibula mentioned earlier, we could see from our investigation that the amount of bone that formed inside the tubes with biomaterials that had been implanted in the bone defects of the fibula of the rats was a small quantity, compared with the results described in the literature using the femur and tibia. Moreover, there was no bone neoformation inside the empty tubes that were implanted. This may have occurred in view of the secondary biomechanical function of the fibula resulting from its fusion with the tibia and consequent low angiogenic and osteogenic function. These morphological characteristics of the fibula suggest that in the present study, the time for which the implant was left in place up to the time of sacrificing the animals was insufficient for the complete process of osteoconduction among the biomaterials to be achieved.

Despite the low amounts of bone neoformation in the area of the implant, we could see from the radiological data that there was no rejection of the type of biomaterial used. This suggests that the materials were biocompatible, as also described by other researchers who used the same implants[32-35].

CONCLUSION

The biomaterials used had osteoconductive capacity, even though the amount of bone neoformation in our study was low. However, other factors such as the embryology, ossification type, morphology and biomechanics of the bone that is studied are fundamental in the osteogenesis process. Thus, there is a need to draw up a better standardized and more scientifically based experimentation protocol in the cases of bones like the fibula of rats for which the biological qualities and mechanical parameters are not yet well defined, since these are factors that interfere directly in the expected results regarding the bone regeneration process.

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REFERENCES

1. Alonso JE, Lee J, Burgess AR, Browner BD. The management of complex orthopedic injuries. Surg Clin North Am. 1996;76(4):879-903.
2. Murata M, Huang BZ, Shibata T, Imai S, Nagai N, Arisue M. Bone augmentation by recombinant human BMP-2 and collagen on adult rat parietal bone. Int J Oral Maxillofac Surg. 1999;28(3):232-7.
3. Pinilla M, Ramírez-Camacho R, Salas C, González F, López-Cortijo C, Vergara J. Development of interface in hydroxyapatite implanted in the middle ear of

the rat: a light and scanning microscopy study. Otolaryngol Head Neck Surg. 2003;128(1):124-31.

4. Bombonato-Prado KF, Brentegani LG, Thomazini JA, Lachat JJ, Carvalho TL. Alcohol intake and osseointegration around implants: a histometric and scanning electron microscopy study. Implant Dent. 2004;13(3):238-44.
5. Rammelt S, Schulze E, Witt M, Petsch E, Biewener A, Pompe W, Zwipp H. Collagen type I increases bone remodelling around hydroxyapatite implants in the rat tibia. Cells Tissues Organs. 2004;178(3):146-57.
6. Itoh S, Nakamura S, Kobayashi T, Shinomiya K, Yamashita K, Itoh S. Effect of electrical polarization of hydroxyapatite ceramics on new bone formation. Calcif Tissue Int. 2006;78(3):133-42.
7. Reikerås O, Johansson CB, Sundfeldt M. Hydroxyapatite enhances long-term fixation of titanium implants. J Long Term Eff Med Implants. 2006;16(2):165-73.
8. Cunha MR, Santos AR Jr, Goissis G, Genari SC. Implants of polyanionic collagen matrix in bone defects of ovariectomized rats. J Mater Sci Mater Med. 2008;19(3):1341-8.
9. Schwartz Z, Doukarsky-Marx T, Nasatzky E, Goultschin J, Ranly DM, Greenspan DC, et al. Boyan BD. Differential effects of bone graft substitutes on regeneration of bone marrow. Clin Oral Implants Res. 2008;19(12):1233-45.
10. Kawai T, Anada T, Honda Y, Kamakura S, Matsui K, Matsui A, et al. Synthetic octacalcium phosphate augments bone regeneration correlated with its content in collagen scaffold. Tissue Eng Part A. 2009;15(1):23-32.
11. Ravaglioli A, Krajewski A, Biasini V, Martinetti R, Mangano C, Venini G. Interface between hydroxyapatite and mandibular human bone tissue. Biomaterials. 1992;13(3):162-7.
12. Zaffe D. Interfacial study of some inert and active ceramics implanted in bone. In: Ravaglioli A, Krajewski A, editors. Bioceramics and the human body. London: Elsevier; 1991.
13. Nandi SK, Kundu B, Ghosh SK, De DK, Basu D. Efficacy of nano-hydroxyapatite prepared by an aqueous solution combustion technique in healing bone defects of goat. J Vet Sci. 2008;9(2):183-91.
14. Golec TS, Krauser JT. Long-term retrospective studies on hydroxyapatite coated endosteal and subperiosteal implants. Dent Clin North Am. 1992;36(1):39-65.
15. Hebert S, Xavier R. Ortopedia e traumatologia: princípios e prática. 3ª ed. Porto Alegre: Sarvier; 2003. p. 26.
16. Zoppi RA, Duek EAR, Coraça DC, Barros PP. Preparation and characterization of poly (L-lactic acid) and poly(ethylene oxide) blends. Mat Res [online]. 2001;4(2):117-25.
17. Kim BS, Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol. 1998;16(5):224-30.
18. Bernales DM, Caride F, Lewis A, Martin L. Membranas de colágeno polimerizado: Consideraciones su uso em técnicas de regeneracion tisular y osea guiadas. Cubana Invest Biomed. 2004;23(2):65-74.
19. Takaoka K, Nakahara H, Yoshikawa H, Masuhara K, Tsuda T, Ono K. Ectopic bone induction on and in porous hydroxyapatite combined with collagen and bone morphogenetic protein. Clin Orthop Relat Res. 1988;(234):250-4.
20. Mellonig JT. Porous particulate hydroxyapatite in a human periodontal osseous defect: a case report. Int J Periodontics Restorative Dent. 1991;11(3):217-23.
21. Ono I, Ohura T, Murata M, Yamaguchi H, Ohnuma Y, Kuboki Y. A study on bone induction in hydroxyapatite combined with bone morphogenetic protein. Plast Reconstr Surg. 1992;90(5):870-9.
22. Duarte TS, Borges AP, Lavor MS, Figueiras R, Tsiomis AC, Oliveira FL, et al. Osteointegração da hidroxiapatita sintética no processo alveolar da mandíbula de cães: aspectos histológicos. Arq Bras Med Vet Zootec. 2006;58(5):849-53.
23. Camilli JA, da Cunha MR, Bertran CA, Kawachi EY. Subperiosteal hydroxyapatite implants in rats submitted to ethanol ingestion. Arch Oral Biol. 2004;49(9):747-53.
24. Pinheiro TC, Santos FFC, Shirane HY, Cunha MR. Implantes de hidroxiapatita em falhas ósseas produzidas no fêmur de ratos submetidos ao tabagismo passivo. Rev Bras Ortop. 2008;43(10):433-41.
25. Raab P, Wild A, Seller K, Krauspe R. Correction of length discrepancies and angular deformities of the leg by Blount's epiphyseal stapling. Eur J Pediatr. 2001;160(11):668-74.
26. Du C, Cui FZ, Feng QL, Zhu XD, de Groot K. Tissue response to nano-hydroxyapatite/collagen composite implants in marrow cavity. J Biomed Mater Res. 1998;42(4):540-8.
27. Caiazza S, Colangelo P, Bedini R, Formisano G, De Angelis G, Barrucci S. Evaluation of guided bone regeneration in rabbit femur using collagen membranes. Implant Dent. 2000;9(3):219-25.
28. Wang YJ, Lin FH, Sun JS, Huang YC, Chueh SC, Hsu FY. Collagen- hydroxyapatite microspheres as carriers for bone morphogenic protein-4. Artif Organs. 2003;27(2):162-8.
29. Nishikawa T, Masuno K, Tominaga K, Koyama Y, Yamada T, Takakuda K, et al. Bone repair analysis in a novel biodegradable hydroxyapatite/collagen composite implanted in bone. Implant Dent. 2005;14(3):252-60.
30. Moss ML. A functional analysis of fusion of the tibia and fibula in the rat and mouse. Acta Anat (Basel). 1977;97(3):321-32.
31. Kirchen ME, O'Connor KM, Gruber HE, Sweeney JR, Fras IA, Stover SJ, et al. Effects of microgravity on bone healing in a rat fibular osteotomy model. Clin Orthop Relat Res. 1995;(318):231-42
32. Ruano R, Jaeger RG, Jaeger MM. Effect of a ceramic and a non-ceramic hydroxyapatite on cell growth and procollagen synthesis of cultured human gingival fibroblasts. J Periodontol. 2000;71(4):540-5.
33. Endres S, Landgraff M, Kratz M, Wilke A. [Biocompatibility testing of various biomaterials as dependent on immune status]. Z Orthop Ihre Grenzgeb. 2004;142(3):358-65.
34. Rücker M, Laschke MW, Junker D, Carvalho C, Schramm A, Mülhaupt R, et al. Angiogenic and inflammatory response to biodegradable scaffolds in dorsal skinfold chambers of mice. Biomaterials. 2006;27(29):5027-38.
35. Hedia HS. Effect of coating thickness and its material on the stress distribution for dental implants. J Med Eng Technol. 2007;31(4):280-7.