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Comparison of Two Composites Developed to be Used as Bone Replacement – PMMA/Bioglass 45S5® Microfiber and PMMA/Hydroxyapatite

Lourdes Cristina de Albuquerque Haach[1]*, Benedito de Moraes Purquerio[1], Nelson Ferreira Silva Jr[2], Ana Maria Minarelli Gaspar[3] and Carlos Alberto Fortulan[1]

[1]*Department of Mechanical Engineering, University of São Paulo, Av. Trabalhador São-Carlense 400, CEP 13566-590 São Carlos (SP), Brazil*
[2]*Laboratory of Bioengineering, University of São Paulo, Av. Trabalhador São-Carlense 400 CEP 13566-590 São Carlos (SP), Brazil*
[3]*Laboratory of Morfology, Universidade Estadual Paulista Júlio de Mesquita, Rua Humaitá 1680 CEP 14801-903, Araraquara, São Paulo (SP), Brazil*

Abstract

Two types of polymer matrix composites were designed to use as bone replacement: PMMA+HA and PMMA+45S5® Blown Fibers. The materials were tested in vivo for 60 days and compared to two control groups: empty bone defect and porous PMMA. The histology results suggest that both materials provide a suitable scaffold for bone growth with the presence of newly formed bone tissue, blood vessels, osteocytes and osteoblasts cells. For a better understanding of the mechanical properties of scaffolds to bear physiological loads when implanted, compressive tests were carried out. The results showed difference in the compressive behaviour of the two composites, PMMA+HA specimens achieved higher values approaching 9.0 MPa of ultimate strength while PMMA+45S5® had values close to 8.0MPa. Both materials are suitable to bone replacement of small size areas.

Keywords: Bone scaffold; Poly methyl methacrylate; Bio composites; *In vivo* test; Compression test

Introduction

Bone and joints injuries and diseases result in pain and disability in millions of people around the world [1]. Most lesions in bone tissues have potential of suitable spontaneous regeneration, sometimes requiring only the use of conservative therapy or conventional surgical techniques. This is mainly due to the continuous process of bone remodeling that occurs throughout the human life. In cases of large osteochondral defects it is necessary to replace the entire affected site using artificial prostheses that have their limited life. For these reasons, it is extremely important to identify new materials which can repair such lesions permanently avoiding new reconstructive surgeries, especially in young individuals [1]. Polymers and ceramics (in particular hydroxyapatite (HA) and Bioglass®) stand out among the biocompatible materials in the search for new and more durable materials to be used as bone graft. When designing a new material to be used for tissue engineering some important aspects have to be considered such as material biocompatibility and its correlation with the tissue to be replaced, its biostability and integration into host materials and the restoration of normal structure with functional characteristics [2].

Sousa [3] conducted analysis *in vitro* and *in vivo*: Scanning Electron Microscopy (SEM), cytotoxicity, biocompatibility and bone conductivity of a composite consisting of PMMA and HA Carboxy Methyl Cellulose (CMC) as a pore forming agent. According to this study the material does not show toxicity *in vitro* and obtained excellent results of biocompatibility and cell growth *in vivo*. These results are supported by Itokawa [4] research who studied a nonporous PMMA and HA composite and obtained full adhesion of the interface material/bone after 1 year postoperatively without any form of inflammatory reaction. However in both studies this composite has limitations on their employability in bone regions subject to mechanical loading due its frailty.

This proposed study aims to obtain consistent biological and mechanical properties of two kinds of materials into a single composite able to withstand the mechanical loads that act physiologically over a bone region. Using PMMA and 45S5® Blown Fibers this study intended to achieve porous scaffolds with excellent integration and suitable bone strength.

Materials and Methods

Samples preparation

For the manufacture of dense PMMA samples was performed a mixture of PMMA and MMA in a ratio of 1:1. For the porous PMMA samples: 0.029 g CMC; 0.118 g auto-polymerizing PMMA; 0.382 g distilled water and 0.236 g MMA. For the PMMA+HA samples: 1.65 g PMMA; 1.2 g HA; 0.45 g CMC; 5.37 g distilled water and 3.54 g MMA. For the PMMA+45S5® Blown Fibers samples (80% of porous and 4% de microfibers of bioglass): 0.17 g PMMA; 0.10 g 45S5® Blown Fibers (size -10 µm, MO-SCI Health Care); 0.05 g CMC; 0.68 g distilled water and 0.34 g MMA. After insertion of the material into the mould material is submitted to 0.6 MPa for 50 minutes. Immediately after it is boiled in water for 50 minutes and dried at 60°C for 50 minutes.

Compression tests

To prepare the samples to the compression tests the recommendations of ASTM D695-10 [5] were followed; with specimen dimensions of 12.7 mm diameter and 25.4 mm in length. The following groups were tested:

***Corresponding author:** Lourdes Cristina de Albuquerque Haach, Department of Mechanical Engineering, University of São Paulo, Av. Trabalhador São-Carlense 400, CEP 13566-590 São Carlos (SP), Brazil, Tel:+55 16 3415 3119; E-mail: lourdesa@sc.usp.br

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dense PMMA, porous PMMA, PMMA+HA and PMMA+45S5® Blown Fibers. The tests were performed with a servohydraulic test system Bionix® - MTS® - actuator speed 1.0 mm/min.

In vivo protocol

Were used 14 male rats aged approximately 2 months. The implants used for experimental analysis of bone integration were cylindrical with no hemispherical ends with 2.5 mm in diameter and 1.6 mm in height. Both legs received the scaffold in the side of the upper third tibia. The experimental time was 60 days. The animals were divided in 4 groups as follow: 3 rats bone defect control group (DC), 3 rats porous PMMA control group (MC), 4 rats PMMA+HA group (HA) and 4 rats PMMA+45S5® Blown Fibers (BG). DC group was submitted to a surgery like the others groups but no material was placed into the defect. The bone defect was made with a low speed drill with irrigation and its size was similar to the implants size.

Results and Discussion

Compression tests

The dense specimens behaved very different from the other three materials. The graph of the dense material stress had a progressive increase up to the value of approximately 47.0 MPa followed by a fall of about 10.0 MPa and from there the specimen began to gain strength again (Figure 1). In the other three materials, stress increased from the beginning to the end of the test. The dense specimen showed ultimate strength approximately 10× higher than the other materials at the same level of deformation. Analyzing the average curves of the compression tests, the ultimate strength value of porous PMMA specimen without any aggregate fell by 93.44% in comparison to dense PMMA. Both aggregates (HA and BG specimens) showed higher ultimate strength values when compared to porous PMMA specimens: an increase of 82.54% in HA and 69.83% in BG (Figure 1–inner graphic). In terms of stiffness, dense material showed the highest value as in the case of ultimate strength. The porous PMMA and BG showed the lowest stiffness being very equivalent between the two materials. The HA had greater stiffness than the other two materials but far below of the dense PMMA values.

Histology

After 60 days postsurgical, all DC rats present the surgical site well consolidated. This result can be seen at Figure 2A that shows a new formed bone structure that was capable of close all the defect extension. Some vestige of bone marrow was surrounded by bone at the healing place but it is believed that it would disappear and form regular bone if the experimental time was longer. Porous PMMA without any added biomaterial (MC group) respond as was expected since PMMA is a proven bioinert material. There was no inflammation reaction and the new formed bone was capable of close the defect area. Both material groups (HA and BG) showed good results at bone formation with no important signs of inflammation (Figures 2C and 2D). Some inflammation markers could be seen but it is consider normal since the defect was deep and the scaffold was in contact with the bone marrow. Some vestige of HA scaffold can be seen (Figure 3A) and present an oblong shape. HA and BG new formed bone contains osteocytes, blood vessel (Figures 3 and 4) and biomaterial vestiges well integrated with the surround bone tissue.

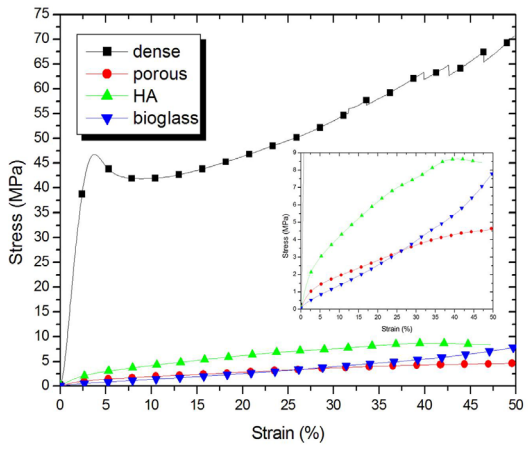

Figure 1: Stress (MPa) x Strain (%) average curves for materials tested. The results with lower stress values can be seen with details at the inner graphic.

Conclusions

The composites proposed in this paper to be used as bone scaffolds

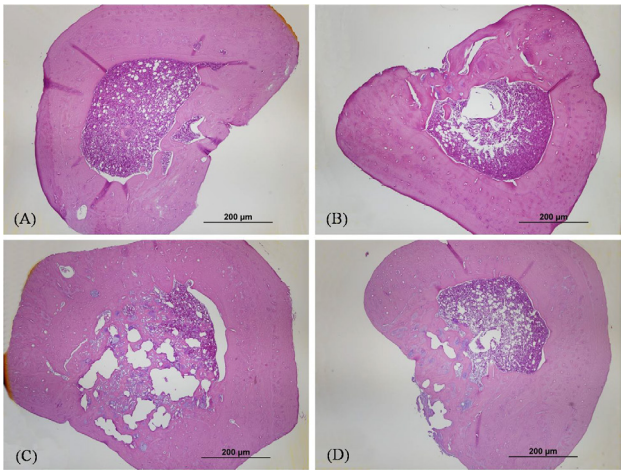

Figure 2: Scaffolds sites in all groups after 60 days of implantation (4x). (A) defect control, (B) material control, (C) hydroxyapatite and (D) 45S5® Blown Fibers.

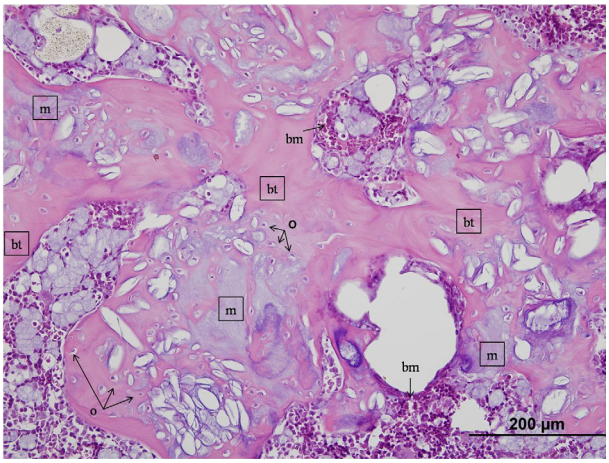

Figure 3: Histological image of hydroxyapatite scaffold after 60days of bone implantation (20x). Osteocytes (o), bone marrow (bm), material (m) and new formed bone tissue (bt) are present.

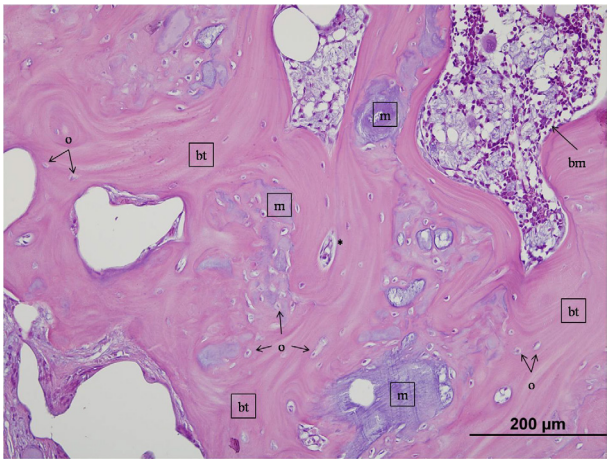

Figure 4: Histological image of 45S5® blown fibers scaffold after 60days of bone implantation (20x). Osteocytes (o), bone marrow (bm), material (m), blood vessels (*) and new formed bone tissue (bt) are present.

showed good biological results, bone growth at the site without the presence of significant inflammatory reaction. The results of the mechanical tests showed that both materials are not as resistant to compression as normal bone tissue, thus indicating the use in small areas of bone replacement or removal of load on the member during bone growth and healing in the implant.

Acknowledgments

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