Transactions of the Materials Research Society of Japan 29[6] 2919-2921 (2004)

Construction of Interconnected Pore Network Using Hydroxyapatite Small Components

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Macro porous materials are attracting much attention mainly because macro pores (>100 μm) are considered as a favorable site for the bone regeneration. Ideally, macro pores should be interconnected, because closed pores cannot allow regenerative activities. Therefore, improvement of the macro pore connectivity has been an urgent matter. We are trying to fabricate an interconnected macro pore network by integrating small components of biomaterials. In this study, ϕ 1 mm spherical hydroxyapatite ceramics with a ϕ 300 μm through-hole (HA beads) was prepared as the small component. By integrating the HA beads, the through-holes were connected each other through gaps among the HA beads. The μ-CT analysis for the integrated HA beads proved that the through-holes and the gaps composed an interconnected network. Bone regeneration in the macro pore network was evaluated by an animal test using two male New Zealand White rabbits at the age of 12 weeks. After 7 days, new bone formation was found in the through-hole of the HA beads that locates on the edge of the implantation site. The new bone was accompanied with cell layers along the outlines. This result showed direct evidence that supports the validity of interconnected macro pores for bone regeneration.
Key words: Bone regeneration, Hydroxyapatite, Porous ceramics, Macro pore, Connectivity

1. INTRODUCTION

Porous calcium phosphate (CP) ceramics are well known as bone regenerative grafts [1-3]. Pores are expected to allow cell migration and humoral transmission to take place in implants, leading to bone regeneration.

For the effective bone regeneration using porous CP ceramics, pore size and distribution are important. Particularly, macro pores larger than ϕ 100 μm are considered as osteoconductive pores [1, 4, 5]. Further, the connectivity of the pores determines the efficiency of porous implants. Ideally, pores should be connected, forming perfectly interconnected macro pore network. However, conventional porous CP ceramics contain many dead pores, resulting in unfavorable performance. Therefore, how to fabricate an interconnected macro pore network using CP ceramics has been attracting great interests.

We are trying to construct a perfectly interconnected macro pore network by integrating tiny biomaterial components with osteoconductive macro structures. In this study, spherical hydroxyapatite ceramics with a through-hole (HA beads) was prepared as a model of the component. Macro pore network was made by gathering the HA beads. The connectivity of the through-holes and inter-bead gaps was evaluated by micro X-ray CT analysis. Osteoconduction in the macro space in the gathered beads was evaluated by a 7-day animal test.

2. METHODS

The HA beads (ϕ 1 mm) with a through-hole (ϕ 300 μm) are fabricated by sintering (1523 K) gelatinous HA spheres made with commercial HA powder (TAIHEI CHEM. IND. CO., LTD., Japan). Each gelatinous HA sphere was provided with a ϕ 500μm through-hole before sintering by a trimming tool. The HA beads were ultrasonic-washed with ultra-pure water for 15 min.

The HA beads were packed in a ϕ 5 x 5 mm plastic tube to make macro pore network. The connectivity of the through-hole and inter-bead gaps was evaluated by image analysis based on a micro X-ray CT data set of the integrated HA beads. Osteoconductivity of the macro spaces in the gathered HA beads was evaluated by an animal test using two male New Zealand white rabbits (12-week-old). One hundred HA beads were filled in a bone defects (ϕ 5 × 5 mm) created on the proximal epiphysis of the tibiae. After 7 days, decalcified tissue section stained with hematoxylin and eosin were prepared, and examined histologically.

3. RESULTS and DISCUSSION

The HA beads were opaque white without any coloring phenomena and major cracks (Fig. 1).

The HA beads were nearly spherical having the average lengths of the major and minor axes of the HA beads were 1.03 mm and 0.87 mm, respectively. Figure 2 shows a scanning electron micrograph (SEM) image of the cross section of the HA beads, showing the HA beads were porous. The HA beads absorbs fluid element such as azo dyes, implicating well connected micro pores in the HA beads. The micro pore networks were considered to be a favorable factor to allow cell migration and humoral transmission in the HA beads.

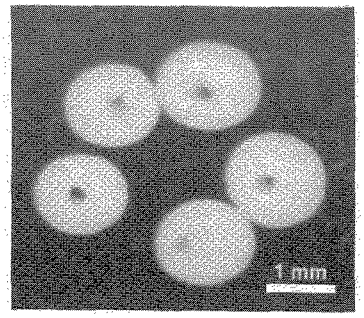

Fig. 1. A Photomicrograph of the HA beads fabricated in this study.

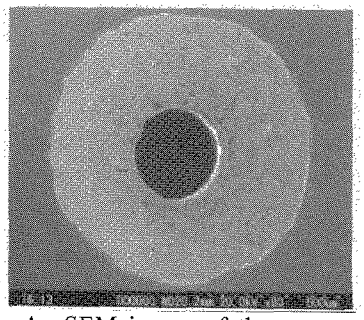

Fig. 2. An SEM image of the cross section of the HA beads, showing the HA beads were porous.

Figure 3 shows a 3-D computer model of the gathered HA beads. The gathered HA beads exhibited the macro porosity of 47.7±1.9%. The micro X-ray CT analysis revealed that the through-holes and inter-bead gaps are composing a single interconnected network.

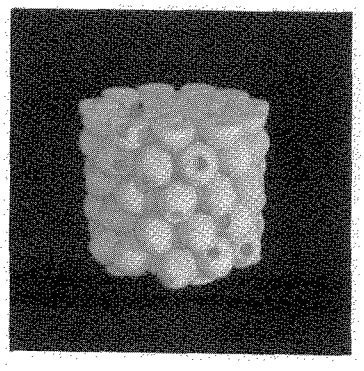

Fig. 3. A 3-D computer model of the gathered HA beads. The gathereded HA beads exhibited the macro porosity of 47.7±1.9%.

The implantation of the HA beads caused no inflammatory responses, or degeneration and necrosis of the surrounding tissue. The HA beads on the tissue sections were infiltrated by cells and fibrous tissue. Remarkable bone formation was found in the through-hole, while fibrous tissue formation was observed allover the implantation site (Fig. 4). The new bone was covered with cell layers along the outlines. The cells directly on the new bone were considered osteoblasts. Erythrocytes were also found beside the new bone, indicating vascularization. Above findings can be considered to lead favorable developments for bone repair. Bone formation in the inter-bead gaps was minimal and localized on the bead surfaces. Mostly, HA beads were surrounded by osteoid layers. Osteoid was considered to be a precursor to new bone. Consequently, bone regeneration in the macro pore network consisted of the through-holes and inter-bead gaps was considered favorable, and the network could be filled with new bone at an early stage.

Bone regeneration in the macro pore has been reported in the previous studies dealing conventional macroporous CP ceramics [6-8]. However, the morphology and size distribution of the macro pores in the CP ceramics used were not simple, spoiling evidences linking bone regeneration to the macropore diameter. On the other hand, the present animal tests provided direct evidence of osteoconduction in the macro pores with a precise diameter.

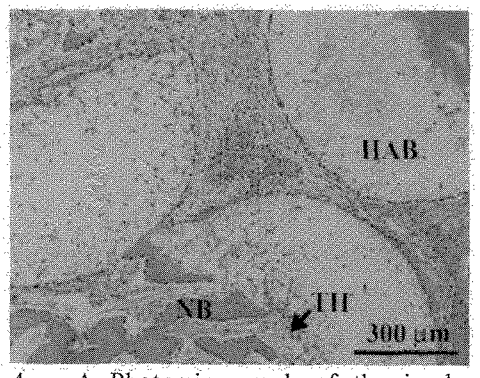

Fig. 4. A Photomicrograph of the implanted HA beads. New bone formation was occurred in the through-hole of the HA beads. HAB: HA bead, TH: Through-hole, NB: New bone.

4. CONCLUSION

This study demonstrated a new method to make a perfectly interconnected macro pore network for bone regeneration by gathering HA beads. The macro pore network consisted of the through-hole of ϕ 300 μm in diameter and inter-bead gaps exhibited the efficiency concerning bone regeneration.

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(Received November 30, 2003; Accepted February 29, 2004)