Development of Porous Ceramics with Bimodal Pore-size Distribution from Single-crystal Apatite Fibers and Its Enhanced Osteoconductivity

ABSTRACT INTRODUCTION:
In orthopedic surgery, bone grafting has been performed to treat diseases and injuries, such as bone tumor and bone fracture. In general, bone implantation is classified into three types: i) auto-grafting, ii) allo-grafting and iii) artificial-bone grafting. Among these bone implantations, auto-grafting is well-known as a common practice and is harvested from the patient. However, there are two serious problems as easily expected, i.e., limitations in supply from the host’s bone and subsequently, the takeout surgery from the normal part of the host. Alternatively, allo-grafting has been performed using donor bones obtained from bone banks; however, it also has problems of supply, immunogenic factors and quality. Thus, for artificial-bone grafting, which would have the least problem in the implantation, porous HAp ceramics, which can be integrated with newly-formed bone of the host, are generally used. Porous HAp ceramics for bone-grafting are required to contain interconnected open pores whose sizes of over 100 µm in diameter, to lead to penetration and proliferation of osteoblasts, vascular ingrowths and integration of newly-formed bone into porous ceramics. Microstructure of porous ceramics with interconnected open pore is also effective for being saturated with a body fluid or as mediums for cell culture. We have developed the porous HAp ceramics with well-controlled porosity and interconnected open pores using single-crystal apatite fibers [1, 2]. In addition, we have successfully prepared the porous HAp ceramics with bimodal pore-size distribution, which has pores of both submicron and over 100 µm in diameter, by firing the apatite-fiber compacts mixed with carbon beads [3]. Our aims of the present investigation were to examine the biocompatibilities of the resulting porous ceramics in vitro using osteoblasts and to clarify the osteoconductivity in vivo using rabbit model.

METHODS:
Fabrication of porous ceramics and their characterization: As previously reported [1], single-crystal apatite fibers were synthesized by a homogeneous precipitation method. Porous HAp ceramics with bimodal pore-size distribution were fabricated from the apatite fibers and carbon beads with 20 or 150 µm in average diameter (Nikabeads; Nihon Carbon Company, Japan) on the basis of the process described in Ref [3]. Hereafter, the porous HAp ceramics using carbon beads of 20 and 150 µm in average diameter are hereafter named “P-HAp(20)” and “P-HAp(150)”, respectively; the porous HAp ceramics fabricated by firing the carbon-free apatite-fiber compacts uniaxially compressed at 30 MPa were named “P-HAp(0)”. In vitro evaluation using osteoblast model: The resulting porous HAp ceramics were biologically evaluated using the osteoblastic cell, MC3T3-E1. About 5.0 x 10^4 cells were seeded on the P-HAp(0), P-HAp(20), P-HAp(150), dense HAp ceramics and control (24-well plate for cell culture). Dense HAp ceramics were fabricated from commercially available HAp powder (HAp-100; Taihei-kagaku company, Japan). We examined the proliferation and morphology of the cells cultured for 1, 3, 5 and 7 days on/in the above-mentioned specimens. As for the cell proliferation, we performed a WST-1 assay using a kit of Premix WST-1 Cell Proliferation Assay System (TAKARA BIO INC, Japan). Cell morphology was observed by a SEM after fixation and freeze-drying. In vivo evaluation using rabbit model: We performed a biocompatibility test using rabbit (Japan white, 16 weeks old, male, weight ~ 3 kg) model. The specimens used in vivo evaluation were P-HAp(150) and APACERAM (control; PENTAX Corporation, Japan). Cylindrical P-HAp(150) with porosity of 70% and APACERAM with porosity of 60% (the size of 4.0 mm in diameter, ~ 8 mm in height) were implanted into tibiae of rabbits. At 4, 8, 12, and 24 weeks after implantation, the rabbits were sacrificed to retrieve the specimens with the surrounding bone tissue, and then undecalcified sections were prepared for the histological evaluation. The sections were stained with hematoxylin and eosin (HE) stain.

RESULTS SECTION:
Porous HAp ceramics with bimodal pore-size distribution: The pure HAp phase was present in a series of the P-HAp(0), (20) and (150). Total porosities of P-HAp(0), (20) and (150) were about 40%, 70% and 70%, respectively. Most of the pores in the P-HAps could be regarded as open pores. This result indicates that the resulting ceramics contain open pores which develop the continuous three-dimensional structure inside the ceramics. The SEM observation indicated that the carbon-free P-HAp(0) contained pores with the sizes of several micrometers originating from interfining of individual fibers. Meanwhile, the P-HAp(20) and P-HAp(150) had two kinds of pores with the sizes of several micrometers derived from interfining of individual fibers and of about 20 µm or 100-200 µm from the carbon beads. Proliferation of MC3T3-E1 on the P-HAps: The results of WST-1 assay showed that the MC3T3-E1 cells cultured on all the specimens showed good proliferation. Morphological observation indicated that the cells attached on the surface and inside macro- pores (100-200 µm in diameter) of the ceramics to proliferate up to nearly confluent. Meanwhile, in the cases of the P-HAp(0) and P-HAp(20) having pores of less than 100 µm in diameter, the MC3T3-E1 cells proliferated only on the surface of the ceramics. Histological evaluation: At 8 weeks after implantation, we performed histological evaluation with HE stain using sections of tibiae containing the implanted P-HAp(150) and a control. The results of the histological observation are shown in Fig. 1. Although newly-formed bone was present in both P-HAp(150) and control, we could observe the much bone formation for P-HAp(150). The P-HAp(150) showed excellent osteoconductivity, as compared with a control. These results indicated that the P-HAp(150) induced an excellent osteointegration with living hard tissues of the host.

DISCUSSION:
We have successfully fabricated porous HAp ceramics with bimodal pore-size distribution using both single-crystal apatite-fibers and carbon beads with 150 µm in diameter. The histological evaluation showed excellent osteoconductivity, as compared with a control. This may be due to the specific pore structure of the P-HAp(150) originating from single-crystal apatite fibers. We conclude that the present P-HAp(150) is effective for a high-performance artificial bone grafting, which will lead enhanced the osteoconductivity with newly-formed bone of the host.

REFERENCES: