THE RELEASE OF ANTIBIOTICS FROM A NOVEL HYDROXYAPATITE WITH BI-MODAL PORE SIZE

+*Hasegawa, M; *Sudo, A; **Barinov, S M; *Uchida; A
+*Department of Orthopaedic Surgery, Mie University Faculty of Medicine, Tsu, Mie, Japan.

Introduction: Porous hydroxyapatite (HA) has been widely used as a bone substitute because of its excellent biocompatibility and osteoconductive ability. Drug delivery systems using porous HA have been developed. For bone ingrowth into the pore of HA, large pore size (more than 100 µm) would be required. On the other hand, for the slow release of drugs, such as antibiotics, growth factors, and anticancer drugs, small pore size would be suitable. We have developed a novel HA with bi-modal pore size distribution, i.e., large and small pores. The purposes of this study were to investigate the slow release of antibiotics from the HA in vitro as well as osteoconduction of the material in vivo.

Materials and Methods: The novel HA was synthesized in a manner described previously [1]. Briefly, HA powder was prepared from CaCl\(_2\), (NH\(_4\))\(_2\)HPO\(_4\), and NH\(_4\)OH by precipitation method. The powder was mixed with 10% gelatin solution. Spherical soft particles containing HA and gelatin were formed by the surface tension forces. Then the samples were compacted by uniaxially pressing at 70 MPa. Porous HA cylinders were prepared by sintering of porous HA microgranules at 1200 °C (HA-A). The mean sizes of crystals and granules were 1 µm and 450 µm, respectively. The size of the cylindrical sample was 4 mm in diameter and 4 mm in height. The bi-modality was due to the intragranular and intergranular pores in the body. The sizes of intragranular pores ranged from 10 nm to 10 µm and the porosity of intragranular pores was approximately 20 vol % (Fig. 1). The size of intergranular pores was 100 µm. Total open pore content was approximately 40 vol %. Commercial porous hydroxyapatite cylinders of the same size were used as controls (Sumitomo Osaka Cement Co, Ltd, Japan) (HA-B). The porosity was approximately 40 vol % and the pore sizes were ranged from 50 µm to 300 µm.

We performed in vitro drug release study using antibiotics impregnated HA. We used 3 antibiotics, including isepamicin sulfate (ISP; Asahi Chemical Industry Co, Ltd, Osaka, Japan), vancomycin hydrochloride (VCM; Eli Lilly Japan KK, Kobe, Japan), and flomoxef sodium (FMOX; Shionogi & Co, Ltd, Osaka, Japan). The minimum inhibitory concentration (MIC) against Staphylococcus aureus was 0.4 µg/ml for ISP and 0.2 µg/ml for FMOX. The MIC against methicillin resistant Staphylococcus aureus was 0.8 µg/ml for VCM. Six samples were prepared for each antibiotics, placing in a bone cement mixer (Mixevac II High Vacuum System, Stryker, Michigan) into which solutions of 100 mg/ml of antibiotics were poured. A vacuum of 500 mmHg was sustained for 10 minutes. The absorption rate was calculated as follows: the change in weight of hydroxyapatite from before soaking to after soaking was divided by its weight before soaking. Each sample was placed in a test tube containing 3 ml of phosphate-buffered saline (PBS) (pH 7.4) and stored in a thermostatic chamber at 37 °C. The PBS was replaced every 2 days for 42 days and after 6 days from HA-B. We could not detect the release from the pulverized samples soaked for 42 days.

The novel HA with bi-modal pore size distribution showed the most rapid release, and it was not detected after 8 days from HA-A and after 6 days from HA-B. We could not detect the release from the pulverized pores into the pores were found. After 3 months the implants showed bone ingrowth into the pores. The material induced no inflammatory responses and no biodegradation occurred.

Discussion: For the slow release of antibiotics, HA is considered to be superior to acrylic bone cement since there is no concern about thermal damage to the antibiotics. In addition, HA has excellent biocompatibility and does not require removal. Any antibiotics including liquid and powder can be soaked into the pores of HA with a simple vacuum system. In this study, HA with nano-level pores might enhance impregnation and slow release of antibiotics. The absorption ratio of the novel HA was higher than the total open pore content. Therefore, a non-uniformity of the packing would exist, probably resulting from the sticking of the granules together due to the gelatin.

Since the novel HA with bi-modal pore size distribution showed the slow release of antibiotics and excellent biocompatibility, it could be useful for the treatment of osteomyelitis.


**Institute for Physical Chemistry of Ceramics RAS, Moscow, Russia.

Fig. 1. Scanning electron micrograph showing small intragranular pores

Fig. 2. Semi-logarithmic scale graphs showing in vitro release of antibiotics from HA-A and HA-B