*Journal of Ceramic Processing Research. Vol. 13, No. 5, pp. 586~589 (2012)*

J O U R N A L O F
**Ceramic Processing Research**

# Fabrication of hydroxyapatite bioceramics from the recycling of pig bone

**Dong Seok Seo[a], Kyu Hong Hwang[b], Seok Young Yoon[c] and Jong Kook Lee[d,*]**

[a]*Jeonnam Technopark, Suncheon, 540-850 Korea*
[b]*School of Nano & Advanced Materials, Gyeongsang Nat'l Univ., Jinju, 660-701 Korea*
[c]*School of Materials Science and Engineering, Pusan Nat'l Univ., Busan, 609-735 Korea*
[d]*Department of Advanced Materials Engineering, Chosun Univ., Gwangju, 501-759 Korea*

**Hydroxyapatite compacts were fabricated by pressureless- and hot-pressing of natural powders recycled from pig bones and their sintered property and biostability investigated. Organics of bone were removed by soaking the bones in a NaOH solution. An organic-free hydroxyapatite powder was obtained by calcination of dried bones and milling by an attritor. Porous hydroxyapatite compacts were fabricated by pressureless-sintering which was carried out at a temperature of 1100 °C for 1 h in a humid atmosphere. Dense hydroxyapatite compacts were prepared by hot-pressing at 1000 °C for 0.5 h under a pressure of 30 MPa in an Ar atmosphere. Almost the detectable peaks in the calcined powder were identical to hydroxyapatite with a small peak of MgO. Sintered hydroxyapatite compacts have a density of 77% by pressureless-sintering and 95% by hot-pressing. An immersion test in buffered water revealed that there was no clear evidence of dissolution for the hot-pressed hydroxyapatite compacts derived from recycled pig bones. By comparison with an extensive dissolution on the surface of an artificial hydroxyapatite compact in buffered water, the hydroxyapatite compacts recycled from pig bones have a superior biostability for in vitro tests.**

**Key words: Hydroxyapatite, Bioceramics, Pig bone, Recycling.**

## Introduction

Hydroxyapatite (HA) bioceramics which have a Ca/P ratio of 1.67 with a chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ have achieved the most significant attention because of their compositional similarities to natural bone and teeth, and their excellent biocompatibility [1, 2]. But HA dissolved during exposure to the in vitro and in vivo environments due to its intrinsically poor mechanical properties especially in wet environments [1]. It is important to predict the possible harmful effects of materials on the surrounding bone tissues [3]. The result of dissolution or particle loosening from implant materials will provoke inflammation or third body friction [4]. Therefore, there is a requirement for fabrication of hydroxyapatite with long time dissolution and degradation resistance. A possible alternative to suppress degradation of HA is the use of natural bone which is chemically and structurally similar to human bone [5]. Natural HA derived from animal bones has the advantage of inheriting a "true" chemical composition and the structure of the raw material. Therefore, it seems to be an alternative solution for products based on synthetic HA.

Pig bone is composed of organic and inorganic components. The organic part contains mainly collagen and proteins, whereas the inorganic component is mainly HA with a small percentage of other elements being incorporated in the structure such as magnesium and sodium [6]. The aim of the present study was to fabricate hydroxyapatite compacts recycled from pig bones. In this study, porous and dense HA compacts from pig bone were fabricated by pressureless-sintering and hot-prssing. Hot-pressing is able to achieve a high densification rate at a lower temperature than that of pressureless sintering [7]. We also tried to investigate the microstructural property of sintered compacts and compared the dissolution of the hydroxyapatite recycled from pig bone with synthetic HA by hot-pressing.

## Experimental procedure

A biologically-derived hydroxyapatite powder in this study was originated from pig bones. The pig bones were irrigated with a brush in running water and dried at room temperature, and organics in each bone were removed by soaking the bones in 0.1% NaOH solution at 80 °C for 4 h. After soaking, the bones were dried overnight. After drying, the bones were calcined at 800 °C for 1 h to obtain organic-free calcium phosphate materials. The calcined bones were manually ground, attritor-milled for 24 h to make the grains smaller, and then dried overnight. Hydroxyapatite powders were uniaxially and cold isostatically pressed into pellets. The pellets were sintered at 1100 °C for 1 h in a humid

*Corresponding author:
Tel : +82-62-230 7202
Fax: +82-62-232 2474
E-mail: jklee@chosun.ac.kr

atmosphere. The dense sintered bodies were prepared by hot-pressing at 1000 °C for 0.5 h under a pressure of 30 MPa in an Ar atmosphere.

After sintering, the compacts were polished with 1 μm diamond paste. The crystal phases of the powders and the compacts were analyzed by X-ray diffraction (XRD). Elemental analysis of the hot-pressed HA was carried out by energy dispersive X-ray spectrometry. The microstructures of the compacts were examined using a scanning electron microscope (FE-SEM). The polished disks were immersed in 40 ml of pH 7.4 distilled water buffered using 0.05 M tris at 37 °C for 3 and 7 days. At the end of immersion, all samples were washed with ethanol and their dissolution behavior examined using a scanning electron microscope.

## Results and discussion

Fig. 1 shows the microstructure and XRD pattern of pig bone powder before calcination. Small pieces of pig bone had a size of 40-150 μm with large agglomeration and a color of ochre. Most of the animal bone is a complex composite material consisting of approximately 10% water, 30% organic phase (mainly collagen fibrils) and 60% inorganic material (predominantly carbonated hydroxyapatite). All of the peaks in XRD patterns of pig bone are only attributed to hydroxyapatite with a few broad peaks due to the low crystallinity and small crystallite size. It is well known that natural hydroxyapatite particles in the bone are embedded, well-arrayed in the collagen fibrils with a nanocrystalline size (25-50 nm) and rod-like shape.

The color of the NaOH treated pig bone was yellowish white, but after calcination, it turned to white, suggesting complete removal of organic substances. Organic-free pig bone pieces were easily milled to hydroxyapatite powder by an attritor. An XRD pattern and microstructure of hydroxyapatite powder from pig bones are shown in Fig. 2. The HA powder derived from pig bone had an average particle size of about 1.0-1.3 μm and the particles had an equiaxed shape with weak agglomeration after ball milling for 24 h. All of the peaks were only attributed to hydroxyapatite without tricalcium phosphate (TCP) and calcium oxide. However, a small peak corresponding to magnesium oxide ($2\theta = 43$ °) appeared in the pattern. Magnesium is the fourth abundant cation in the human body and is naturally found in bone tissue [8]. HA powders recycled from pig bones had an average grain size of 1 ìm, whose particles showed a narrow particle size distribution.

Fig. 3 shows infrared absorption spectra of HA powder after calcination. Vibrations of P-O bonds are assigned to the absorption bands within wave number ranges of 471-603 $cm^{-1}$ and 992-1091 $cm^{-1}$. Absorption bands of 2860 and 2929 $cm^{-1}$ by stretching vibrations of C-H bonds are not assigned in this HA powder, which indicates the complete removal of organics by calcination at 800 °C for 1 h. The remaining bands in the high wave number range result from vibrations of O- H bonds.

In the case of XRD patterns of pressureless-sintered and hot-pressed compacts, all detectable peaks in both compacts are identical to HA lattice planes which were almost similar to the case of the calcined powders, as shown on Fig. 4. But the XRD signatures exhibited a substantial increase in peak height and a decrease in peak width, thus indicating an increment of crystallinity and crystallite size. SEM micrographs of polished surfaces in pressureless-sintered and hot-pressed HA are shown in Fig. 5. The relative density of pressureless-sintered HA is about 77% and an average grain size of this specimen is about 2-2.5 μm. Compared with an artificial HA specimen, the density of HA derived from pig bone is very low because the

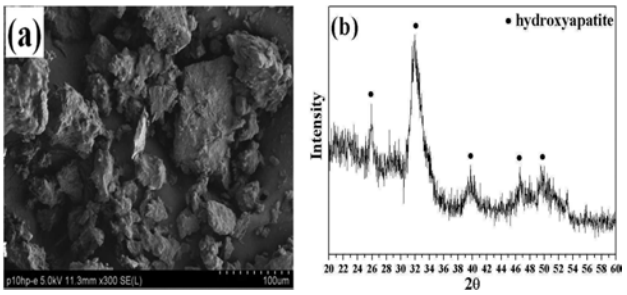


**Fig. 1.** Microstructure and XRD pattern of pig bone pieces.

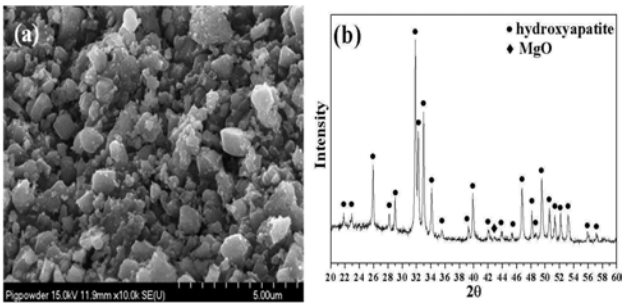


**Fig. 2.** Microstructure and XRD pattern of HA powder recycled from pig bones after a calcination at 800 °C for 1 h and ball milling.

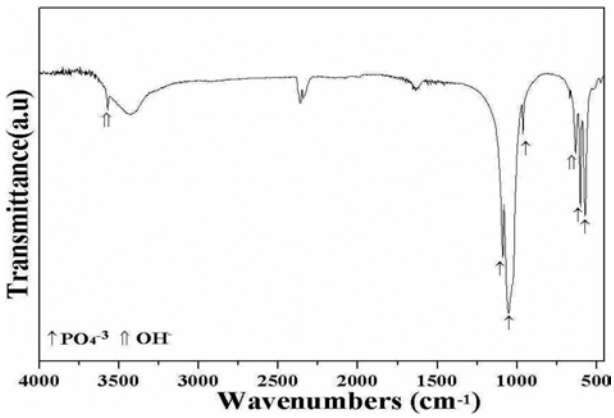


**Fig. 3.** FT-IR spectra of the HA powder recycled from pig bones after a calcination at 800 °C for 1 h and ball milling.

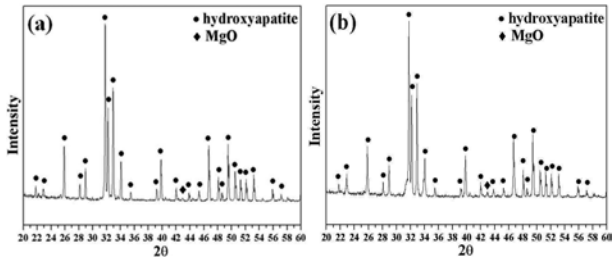


**Fig. 4.** XRD patterns of (a) pressureless-sintered at 1100 °C for 1h and (b) hot-pressed at 1000 °C for 0.5 h.

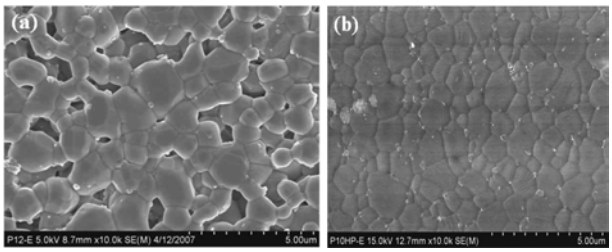


**Fig. 5.** Microstructure of (a) pressureless-sintered at 1100 °C for 1h and (b) hot-pressed at 1000 °C for 0.5 h.

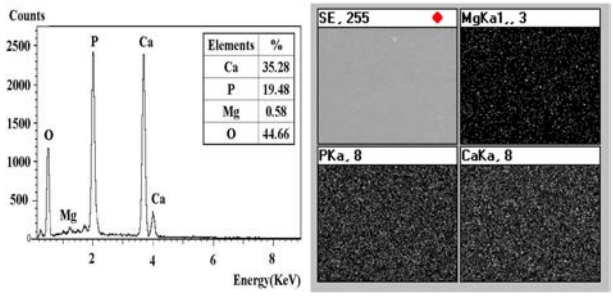


**Fig. 6.** EDS analysis of the hot-pressed HA.

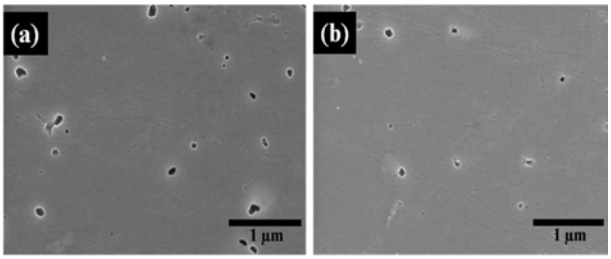


**Fig. 7.** Microstructural evolution of hot-pressed HA aftere an immersion in buffered water for (a) 3 and (b) 7 days.

average particle size of the starting powder from pig bone is larger than that of artificial HA powder. Porous HA exhibits strong bonding to the bone. The pores provide a mechanical interlock leading to a firm fixation of the material. However, despite biocompatibility, mechanical performance of porous HA is very poor compared to bone.

To improve the HA density, it is effective to use a pressure-sintering technique. In this experiment, hot-pressed HA derived from pig bone has a sintered density about 95% with coarse grains of 1-2 μm, which could enhance the mechanical strength and fracture toughness of HA ceramics.

Fig. 6 presents an EDS analysis of the hot-pressed HA. The HA was composed of calcium, phosphate and magnesium ions. EDS mapping of Mg showed that the HA was composed of Mg-rich HA. Magnesium has been known as one of the cationic substitutes for calcium in the HA lattice [9, 10]. The concentration of Mg ions in natural bone may be as high as 1 wt% [11]. The Ca/P ratio of the hot pressed hydroxyapatite from pig bones was higher than that in the stoichiometric (synthetic) material.

Fig. 7 shows microstructures of the HA during immersion in buffered water. After the immersion periods at 3 and 7 days, there was no clear evidence of dissolution of the HA. From our previous report [12], synthetic HA was soluble in a liquid environment although it was phase-pure with a high sintered density. During the immersion, the surface dissolution initiated at the grain boundaries of the synthetic HA and the grain boundary dissolution extended to decohesion of grains. However, hot-pressed HA from pig bones was less dissolved than hot-pressed synthetic HA.

## Conclusions

Porous and dense hydroxyapatites derived from pig bones were prepared. The pig bone derived-HA consisted mainly of HA and a small amount of MgO. The density and average grain size of pressureless-sintered HA were about 77% and 1.5 μm. In the case of hot-pressed HA, sintered density was about 95% with coarse grains of 1-2 μm.There was no clear evidence of dissolution on the surface of the HA. As Mg in HA may suppress surface dissolution, it is believed that pig bone derived HA can be a good substance for medical applications, where biological stability of HA is required.

## Acknowledgments

This study was supported by research funds from Chosun University 2009.

## References

1. J.T. Edwards, J.B. Brunski, H.W. Higuchi, J. Biomed. Mater. Res. 36 (1997) 454-469.
2. H. Oonishi, Biomaterials 12 (1991) 171-178.
3. H.K. Koerten, J. Van der Meulen, J. Biomed. Mater. Res. 44 (1999) 78-86.
4. M. Rokkum, M. Brandt, K. Bye, S. Waage, A. Reigstad, J. Bone Joint Surg. 81 (1999) 582-589.
5. C.Y. Ooi, M. Hamdi, S. Ramesh, Ceramic International 33 (2007) 1171-1177.
6. S.R. Krishna, C.K. Chaitanya, S.K. Seshadri, T.S.S. Kumar, Trends Biomater. Artif. Organs 16 (2002) 15-17.
7. G. Muralithran, S. Ramesh, Ceramics International 26 (2000) 221-230.
8. M.P. Staiger, A.M. Pietak, J. Huadmai, G. Dias, Biomater.

27 (2006) 1728-1734.
9. W.L. Suchanek, K. Byrappa, P. Shuk, R.E. Riman, V.F. Janas, K.S. TenHuisen, Biomater. 25 (2004) 4647-4657.
10. N.A.M. Barakat, M.S. Khil, A.M. Omrand, F.A. Sheikhd, H.Y. Kim, J. Mater. Process. Tech. 209 (2009) 3408-3415.
11. P.K. Wlodarski, K. Haberko, M. Haberko, A. Pyda, K.H. Wlodarski, Folia Biolog. 53 (2005) 183-187.
12. Y.G. Kim, D.S. Seo, J.K. Lee, J. Phys. Chem. Sol. 69(2008) 1556-1559.