

Research Article

## Biological Behavior of MG63 Cells on the Hydroxyapatite Surface

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**Abstract** The response of human bone marrow cell to bone ash-derived hydroxyapatite (HA) and tuna bone-derived HA powders was compared. HA ceramics were prepared from the commercial bone ash and waste of tuna bone. HA powders were prepared by soaking the bone ash and tuna bone in 0.1 M of NaOH solution at 80 °C for 4 hours. Both powders were calcined at 800 °C for 1 hour to completely remove organic and were attritor-milled for 24 hours. The bone ash-derived HA (AHA) and tuna bone-derived HA (THA) ceramics were prepared by cold isostatically pressed and sintered 1200 °C with a dwell time of 1 hour. A human bone cell line MG-63 cells were used to test biocompatibility of AHA and THA ceramics. Cell suspensions in DMEM containing 10% FBS and 1% penicillin-streptomycin were seeded onto 24-well plate containing THA and AHA ceramics. Cell proliferation was evaluated by MTS assay, and cell morphology was observed by SEM.

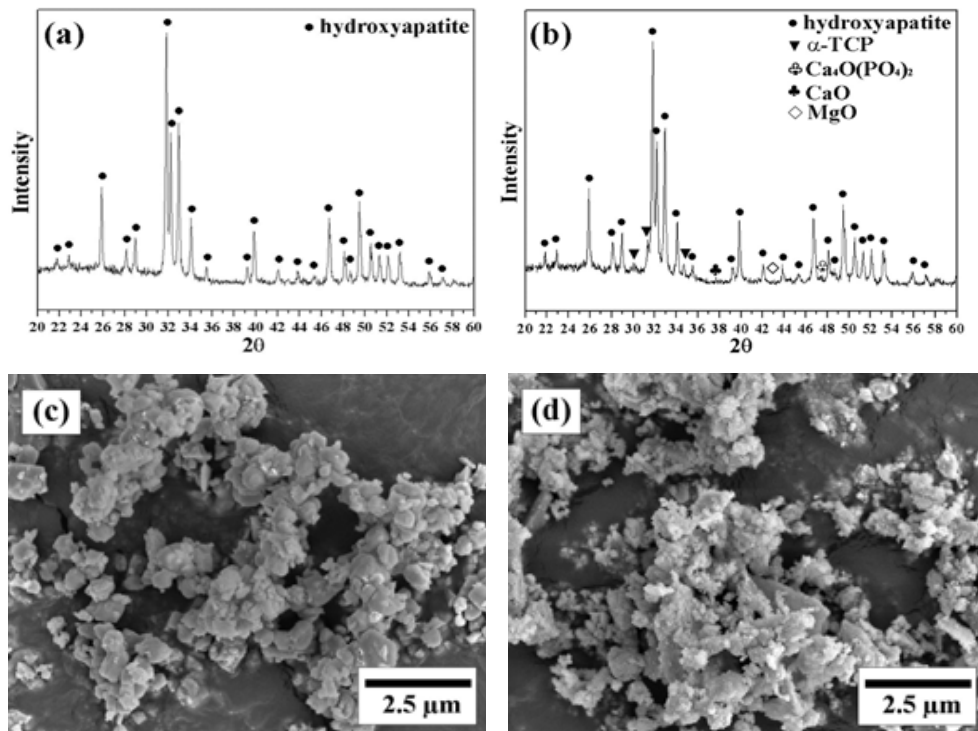
**Keywords** hydroxyapatite; tuna bone; bone ash; cell proliferation

### 1 Introduction

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$ , HA) has achieved the most significant attention because of its compositional similarities to natural human bone and teeth and its excellent biocompatibility [4]. HA is well known to be a biocompatible and bioactive material. When HA is implanted into osteogenic tissue, such as bone defects or medullary cavities, bone formation can occur on its surface, and then the HA becomes directly and strongly bonded to the bone [1]. Possible alternative to suppress degradation of HA is the use of xenogenous bone which is chemically and structurally similar to human bone [2]. We evaluated osteoblast cell response to HA bioceramics recycled from animal bones as a preliminary screen for biocompatibility.

### 2 Experimental procedure

Two types of HA ceramics were used in this study. One was the commercially bone ash-derived HA (AHA), and the other was the tuna bone-derived HA (THA). Bone ash was commercially obtained and was used as a raw material. Organics in bone ash were removed by soaking in 0.1 M of sodium hydroxide (NaOH) solution at 80 °C for 4 hours. The resulting product was crushed into small pieces and milled in a ball mill pot for 24 hours. The phase identification and particle size of as-prepared powders have been examined via X-ray diffraction techniques; then, two types of powder were calcined at 800 °C. The AHA and THA disks were prepared by uniaxial pressing, cold isostatic pressing and were sintered at 1200 °C for 1 hour under moisture protection. All samples were degreased, ultrasonically cleaned, and sterilized in autoclave at 121 °C. MG-63 cell, a human osteoblast-like cell line, was used to assess the cellular responses to the biomaterials of AHA and THA surface. Cells were cultured in 10 cm dishes in Dulbecco's modified Eagle medium (DMEM, Gibco) containing 10% heat inactivated fetal bovine serum (FBS, Gibco), 1% penicillin-streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5%  $\text{CO}_2$ . The cells were washed with phosphate-buffered saline (PBS, Gibco) and detached with trypsin EDTA solution (0.25% trypsin, Gibco) at 37 °C for 2 minutes. The disks were placed in 24-well plates for MG-63 cells implantation at a set density of  $1 \times 10^4$  cells/cm<sup>2</sup>. The MG-63 cells were further incubated in DMEM solution supplemented for 3 and 5 days. Following incubation of cells with two types of HA for indicated time, cells were washed with PBS; then 35  $\mu\text{m}$  of MTS and 350  $\mu\text{m}$  DMEM of nothing excluded were added per well. After 3 hours of incubation, absorbance in control and treated wells was measured in a plate reader at 490 nm. The samples were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). They were dehydrated in a graded series of ethanol, treated with isoamyl acetate, sputter-coated with



**Figure 1:** XRD patterns of (a) tuna bone-derived HA and (b) bone ash-derived HA powders calcined at 800 °C and FE-SEM micrographs of (c) tuna bone-derived HA and (d) bone ash-derived HA powders.

platinum, and surface morphology was observed by field emission scanning electron microscopy (FE-SEM).

### 3 Results and discussion

Figure 1 shows XRD patterns and microstructures of THA and AHA powders calcined at 800 °C. Figure 1(a) showed that all peaks were identified as HA without expressing any second phases such as tricalcium phosphate and calcium oxide. The THA powder had average particle size of about 0.6–0.7  $\mu\text{m}$  and the powders had irregular shape with agglomeration after ball milling for 24 hours (Figure 1(c)). In case of AHA powder (Figure 1(b)), XRD patterns showed the presence of mostly HA and minimal amounts of  $\alpha$ -tricalcium phosphate ( $\alpha$ - $\text{Ca}_3(\text{PO}_4)_2$ ,  $\alpha$ -TCP), calcium oxide phosphate ( $\text{Ca}_4\text{O}(\text{PO}_4)_2$ ), calcium oxide (CaO), and magnesium oxide (MgO).

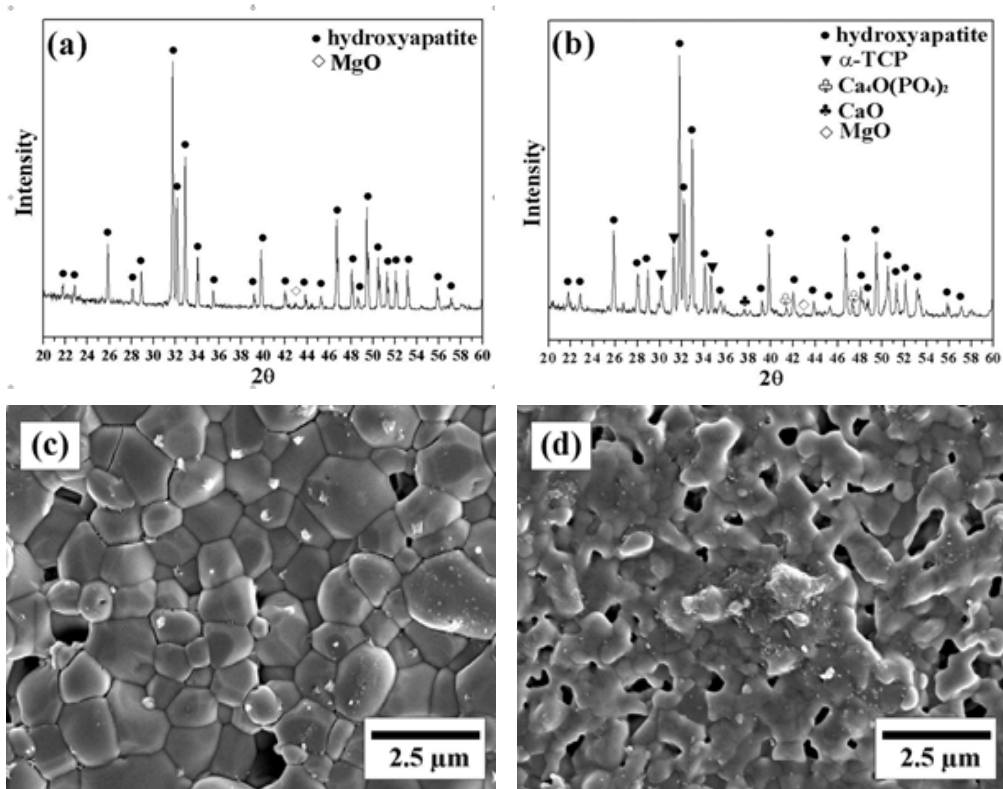
Figure 2 shows XRD patterns and microstructure of THA and AHA ceramics sintered at 1200 °C. XRD patterns (Figures 2(a), 2(b)) were almost similar to the case of the calcined powders, but the XRD signatures exhibited a substantial increase in peak height and a decrease in peak width, thus indicating an increase in crystallinity and crystallite size. A small peak corresponding to magnesium oxide at  $2\theta = 43^\circ$  appeared in the THA powder after sintering. Magnesium is the fourth abundant cation in the human body which is naturally found in bone tissue [3].

Figures 2(c) and 2(d) present FE-SEM micrographs of the surfaces of THA and AHA ceramics sintered at 1200 °C. THA had denser microstructure than AHA ceramics, but the grain size of the THA was larger than that of AHA.

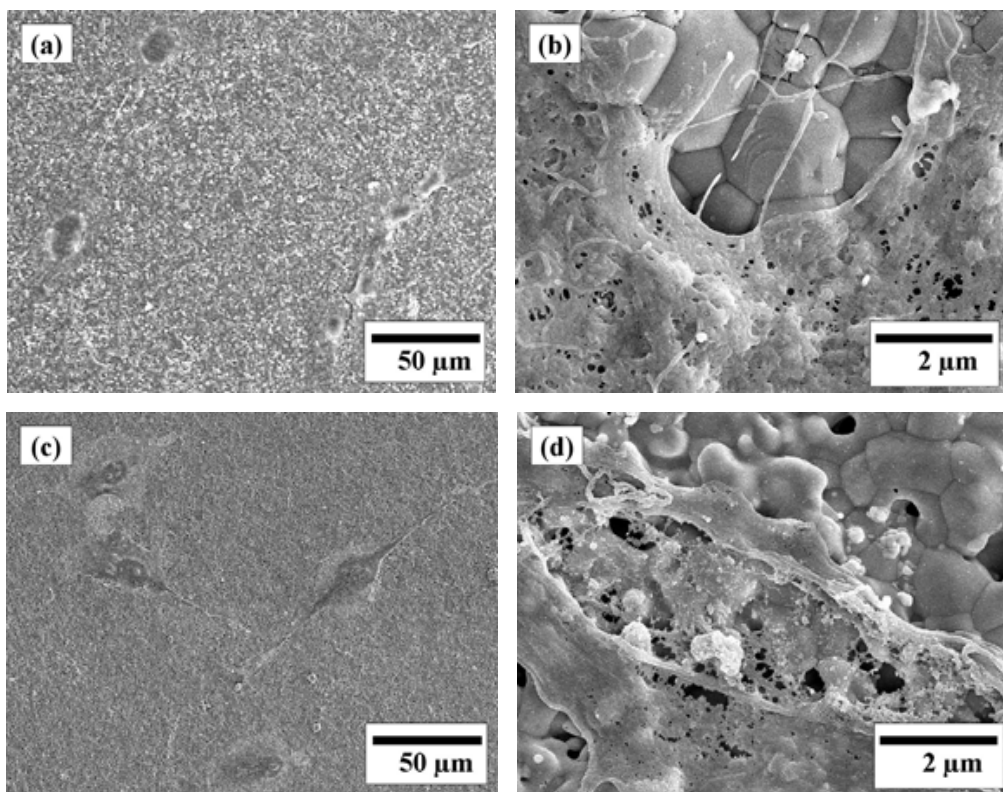
Using Cell proliferation and morphology on the surface of HA surfaces after 3 and 5 days of culture were shown in Figure 3 and Figure 4. MG-63 cell spread and flattened on the HA ceramics as shown in Figures 3(a) and 3(c). They were of polygonal shape with filopodial extensions, which are indicative of very thin extensions of cell spreading. The MG-63 cell showed a lower rate of adherence on the THA surface than on the AHA surface. Cells formed bridges across the undulations and spread over them Figures 3(b) and 3(d). On the AHA surface, phagocytosis, apparent degradation of HA substrate, and small mineralized nodules on cell surface were observed.

Figure 4 shows cell grown on surfaces after 5 days of culture. Figure 4(d) shows a representative HA crystals that were found at all time points. In this figure, it can be seen that each of the spherical particles is composed of smaller plate-like pieces of HA.

Higher adhesion on the surface does not necessarily suggest that the cells are viable and functional. Thus, the cell viability was assessed using the MTS assay. Figure 5 shows the absorbance values obtained for cells adhered to surfaces for 3 and 5 days of culture. The results indicate that the cells are more viable on AHA surface than on THA surface.

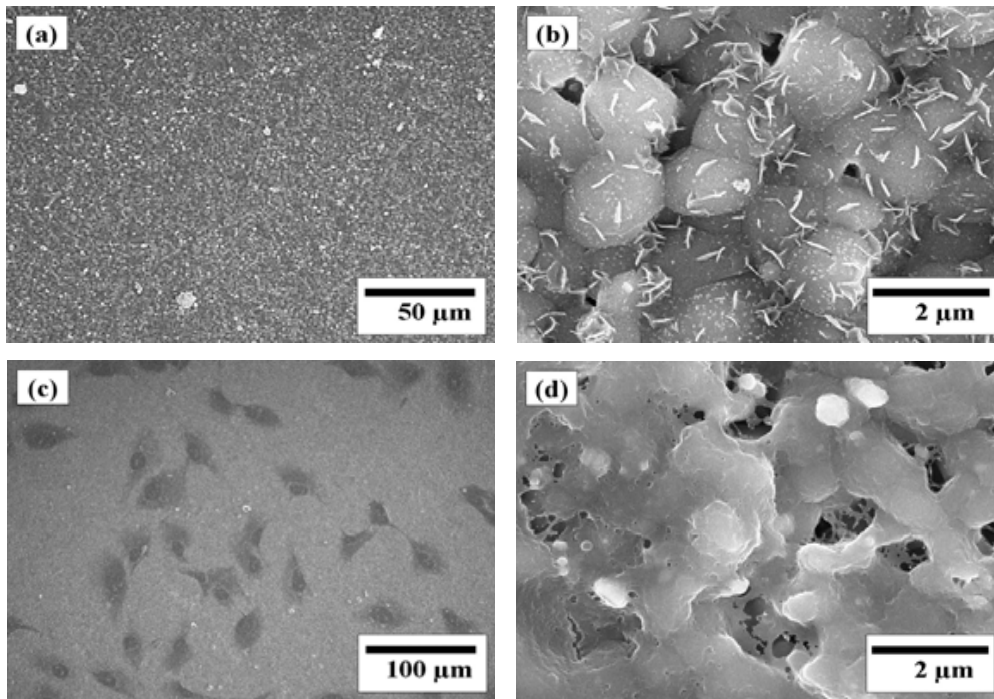


**Figure 2:** XRD patterns of (a) tuna bone-derived HA and (b) bone ash-derived HA powders sintered at 1200 °C and SEM micrographs of (c) tuna bone-derived HA and (d) bone ash-derived HA sintered at 1200 °C.

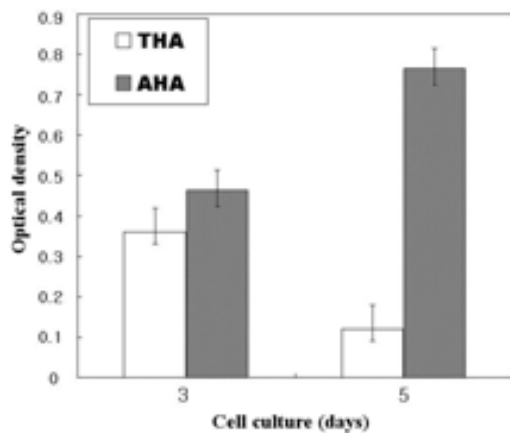


**Figure 3:** FE-SEM micrographs of MG-63 cells after 3 days of culture on THA (a), (b) and AHA (c), (d).





**Figure 4:** FE-SEM micrographs of MG-63 cells after 5 days of culture on THA (a), (b) and AHA (c), (d).



**Figure 5:** Cell viability measured as absorbance using MTS assay after 3 and 5 days for cell culture on THA and AHA.

#### 4 Conclusion

Hydroxyapatite (HA) derived from bone ash and tuna bone was prepared by pressureless sintering, and its MG-63 cell behavior was compared. MG-63 cell spread and flattened on the HA surface. They were of polygonal shape with filopodial extensions, which are indicative of very thin extensions of cell spreading. The MG-63 cells showed a lower rate of adherence on the THA surface than on the AHA surface. However, the *in vitro* results indicate that both THA and AHA ceramics recycled from animal bone wastes can be used as a biomedical substance.

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