Electrovector effect on bone-like apatite crystal growth on Inside pores of polarized porous hydroxyapatite ceramics in simulated body fluid

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Porous hydroxyapatite (HA) disks and blocks with interconnecting pores (pore size $\leq 150 \mu m$, interconnecting window size $\leq 30 \mu m$, and porosity $\approx 75\%$) were polarized and bone-like apatite formations on the pore surfaces were investigated by immersion test using simulated body fluid (SBF) to estimate the electrovector effect in porous bodies. Thermally stimulated depolarization current (TSDC) measurements were used to estimate the stored charges of polarized porous HA disks and blocks as 11 and 7 $\mu C cm^{-2}$, respectively, demonstrating that both the porous disks and blocks were successfully polarized. After immersion in SBF for 14 d, bone-like apatite formations within the pores were observed in the polarized and non-polarized HA disks and blocks. Based on scanning electron microscopic (SEM) images of the pores located around the center of the disks and the blocks (inside-pores), formation of bone-like apatite was accelerated in the polarized HA compared with that in the non-polarized HA. Although the weight of the polarized and non-polarized porous HA increased linearly with immersion time regardless of shape, the rate of weight change of the polarized HA was greater than that of the non-polarized HA. It was demonstrated that the electric charges on the inside-pore surface of porous HA have a positive effect on the formation of bone-like apatite crystals.

Key-words: Hydroxyapatite, Electrical polarization, Simulated body fluid, Porous ceramics, Electrovector effect

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA), a principal bioactive and biocompatible inorganic constituent of human bones and teeth is used in biomedical materials such as bone graft substitutes$^{1,2}$ and dental and orthopedic implants$^{3,4}$.

In particular, porous HA ceramics with interconnecting pores are suitable for bone graft substitutes, because new bone tissue can grow in the pores through the HA substitutes$^{5,7}$. Many studies on the preparation of porous HA, other calcium phosphates, and their scaffolds have been reported$^{5-10}$. Recently, improvements of bone ingrowth have been reported by the addition of bone growth factors such as bone morphogenetic proteins$^{11,12}$. The motivation behind these research efforts was the attempt at early healing of lost tissue; further improvement in the speed of bone ingrowth is expected in clinical practice.

Our previous study has reported that HA ceramics can be polarized in a DC electric field at an elevated temperature$^{13}$. Polarization of HA ceramics occurs due to proton migration, causing an inducement of a surface electric charge$^{14-16}$. In the case of dense HA ceramics, we have confirmed that the polarized surface influences biomedical phenomena$^{17,18}$ and promotes the formation of new bone tissue$^{19-23}$. These consequences have been termed electrovector effects$^{24}$. The potential of the effects will be extended if they can be applied to porous bodies but there has been little research done concerning the relationship between electrovector effects and porous HA ceramics, despite the advantage of the clinical applications$^{25}$. For this reason, the electrovector effects of electrically polarized porous HA ceramics were evaluated in this study by immersion in simulated body fluid (SBF), which is an aqueous solution with an inorganic ion concentration nearly equal to that of human plasma$^{26,27}$. The formation of bone-like apatite crystals within the pores was treated as an index of the electrovector effect on porous bodies. From the perspective of basic research, disk-shaped samples are desirable because their electrical properties can be accurately evaluated within normalized parameters. On the other hand, most clinical applications use porous HA blocks. Considering these circumstances, both types of porous HA ceramics disks and blocks were employed in this study.

2. Experimental procedure

2.1 Materials

Commercial porous HA ceramics for clinical applications consisting of interconnecting pores with an average pore size of $150 \mu m$, an interconnecting window size of $30 \mu m$, and a porosity of approximately 75% were used in this study. Two shapes, disks ($\phi 11 \text{ mm} \times 2 \text{ mm}$) and blocks ($5 \text{ mm} \times 5 \text{ mm} \times 6 \text{ mm}$), were prepared by cutting porous HA ceramics with a low-speed diamond saw (Figs. 1 (a), (b)). The disks and blocks were cleaned with distilled water and ethanol by using an ultrasonic cleaner before the following experiments were performed. Characterization of the porous HA ceramics was done by X-ray diffraction (XRD, Phillips PW1700) measurement and Fourier transform infrared (FT-IR, JASCO FT-230) spectroscopy. From the results of...
the XRD and FT-IR measurements, the porous HA disks and blocks were identified as single-phase hydroxyapatite.

### 2.2 Electrical polarization

The polarization electrodes were attached to the disks and blocks by different layouts. Two platinum electrodes were attached on opposite surfaces of the disks. For the blocks, one electrode was attached around four sides (5 mm × 6 mm) and the other electrode was attached to the remaining two sides (5 mm × 5 mm) for a wide range of polarization of the block surface with electric power at a reasonable level. When the electrodes were attached to the HA blocks by using the same layout as the disks (i.e., opposite sides), a high voltage was required. The HA disks and blocks with the electrodes attached were polarized at 400°C for 1 h and then cooled to room temperature at a DC voltage of 2.0 kV for the disks (Fig. 1(c)) and 2.5 kV for the blocks (Fig. 1(d)). An Atto AE8800 was used to supply the DC power. In the case of the HA disks, negative and positive charges were induced on opposite surfaces. In the case of the HA blocks, negative charges were induced on the four sides of the block and positive charges were induced on the two sides. The negatively and positively charged surfaces on the polarized porous HA were designated the N-surface and P-surface, respectively, while non-polarized surfaces were referred to as 0-surfaces.

After polarization, the stored electrical charges were estimated by thermally stimulated depolarization current (TSDC) measurements from room temperature to 650°C with a heating rate of 5°C min⁻¹ in air by using a Hewlett-Packard 4140B picoammeter. The electrodes were attached to the disks and the blocks for TSDC measurements using the same layout as those used for the polarization.

### 2.3 SBF immersion test

To evaluate the electrovector effect of the polarized porous HA ceramics, polarized and non-polarized disks and blocks were immersed in 30 ml of SBF with pH 7.4 at 37°C for 3–14 d. SBF was prepared using the technique described by Kokubo. After immersion in SBF, the specimens were washed with distilled water and ethanol, and then dried at room temperature in air. The disks, immersed in SBF for 3, 5, 7, and 9 d, were cut into half-rounds by using a low-speed diamond saw. After immersion in SBF for 3, 7, and 14 d, the blocks were cut in half from the original size of 5 mm × 5 mm × 6 mm to 5 mm × 5 mm × 3 mm by using a low-speed diamond saw. The surfaces and the cross-sectional surfaces of the disks and blocks were observed by using a scanning electron microscope (SEM, HITACHI S-2400). For SEM observation, the surfaces of the specimens were sputtered with Pt-Pd using an ion coater (Eiko Engineering IB-2). The weights of the disks and blocks were measured after the immersion in SBF for 3, 5, 7, and 9 and 3, 7, and 14 d, respectively.

### 3. Results and discussion

#### 3.1 TSDC measurement

Figure 2 shows the TSDC curves of the polarized HA disks and blocks. The stored charges (Q) of the polarized HA disks and blocks were estimated according to Eq. (1),

\[
Q = \frac{1}{\beta} \int I(T) \, dT
\]

where \(I(T)\) is the measured current density at temperature \(T\) and \(\beta\) is the rate of heating. The value of \(Q\) for the polarized HA disks was estimated as 11 μC cm⁻² and as 4 μC for the blocks. Here, for the blocks, we could not normalize \(Q\) by the surface area because the area of 0.7 cm² on the N-surface was different from that of 0.5 cm² on the P-surface; the average values of \(Q\) for the polarized HA blocks were estimated as 7 μC cm⁻². It was confirmed that both the porous HA disks and blocks were sufficiently polarized despite the differences in shape and layout of the electrodes.

#### 3.2 Bone-like apatite formation

From the SEM observation of the surface of the porous HA disks and blocks after immersion in SBF for 3 d, the deposition of bone-like apatite crystals was confirmed on all
surfaces: N-surface, P-surface, and 0-surface. There was little difference between the porous HA disks and blocks in terms of the rate of crystal growth of bone-like apatite, probably because the magnitudes of Q of the disks and blocks were equivalent. Figure 3 shows SEM images of the surface pores of porous HA disks after immersion in SBF for 9 d. After immersion in SBF for 9 d, the average thicknesses of the bone-like apatite layers on the surfaces of the porous HA disks were estimated as 3.1±0.2, 2.5±0.1, and 2.6±0.1 μm for the N-, P-, and 0-surfaces, respectively. In contrast, after immersion in SBF for 14 d, the average thicknesses of the layers on the surfaces of the blocks were estimated as 4.8±0.2, 2.4±0.4, and 3.4±0.8 μm for the N-, P-, and 0-surfaces, respectively (Fig. 4). Figure 5 shows the relationship of the thicknesses of the bone-like apatite layers to SBF immersion time for the polarized and non-polarized HA blocks. Based on the SEM observation, it was confirmed that the speed of deposition of the bone-like apatite crystals on the N-surface was faster than that of the 0-surface and P-surface in both the disks and the blocks. These results were caused by the electric charges of the surface pores. In comparison with those of the 0-surface and P-surface, the N-surface attracted a large number of cations such as Ca²⁺, Na⁺, and Mg²⁺. Thus, the N-surface accelerated the rate of crystal nucleation. In contrast, the P-surface was surrounded with anions such as HPO₄²⁻, HCO₃⁻, and Cl⁻, and decelerated the rate of crystal nucleation.¹³

Figure 6 shows SEM images of a pore 0.5 mm from the 0-surface, N-surface, and P-surface of HA disks after immersion in SBF for 5 d. It was found that bone-like apatite crystals were observed only on the polarized disks and not observed on the non-polarized disks. Figure 7 shows SEM images of inside center pores’ cross-sectional surface of non-polarized and polarized disks after immersion in SBF for 7 d. Bone-like apatite crystals were not observed on the non-polarized disks, while the inside pores of the polarized disks were covered with deposited crystals of bone-like apatite. The enhanced deposition suggests that the induced charges inside the pores can affect the formation of bone-like apatite crystals. In the pores inside the HA ceramics, the differences of the N-surface and P-surface were not visible as were the surface pores. The reason is the complex structure

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**Fig. 3.** SEM images of surface pores of (a) 0-surface, (b) N-surface and (c) P-surface porous HA disks after immersion in SBF for 9 d. G: grown apatite layer H: HA ceramics.

**Fig. 4.** SEM images of surface pores of (a) 0-surface, (b) N-surface and (c) P-surface porous HA blocks after immersion in SBF for 14 d. G: grown apatite layer H: HA ceramics.

**Fig. 5.** Relationship of thickness of deposited bone-like apatite layer on the polarized and non-polarized porous HA blocks and SBF immersion time at 37°C.
Fig. 6. SEM images of the pore 0.5 mm from (a) 0-surface, (b) N-surface and (c) P-surface of HA disks after immersion in SBF 5 d.

Fig. 7. SEM images of inside center pores cross-sectional surface of (a) non-polarized and (b) polarized porous HA disk after immersion in SBF for 7 d.

Fig. 8. Weight changes of the polarized and non-polarized porous HA blocks after immersion in SBF at 37°C for 3 to 14 d.

of the inside pores. Because of the complex structure, the N-surface and P-surface appeared inside the ceramics at random.

The weights of the non-polarized and polarized disks and blocks increased over time. After immersion in SBF for 9 d, the increments of the non-polarized and polarized disks reached values of 2.4 ± 0.4 and 3.8 ± 0.2 mass%, respectively. For the blocks, the relationship of weight changes to SBF immersion time is shown in Fig. 8. It was found that the weights of non-polarized and polarized blocks increase almost linearly over time. After immersion in SBF for 14 d, the increments of the non-polarized and polarized blocks reached values of 5.6 ± 0.5 and 7.3 ± 0.5 mass%, respectively. Therefore, it is concluded that the depositions of bone-like apatite on the polarized disks and blocks were accelerat-
ed compared with those of the non-polarized disks and blocks. The enhancement of the deposition strongly suggests that the electric charges had a very positive effect on the formation of the bone-like apatite crystals.

4. Conclusion

In this study, electrovector effects were confirmed on commercial porous HA ceramics, which consisted of interconnecting pores. Electrically polarized porous HA ceramics were compared with non-polarized ceramics in terms of their ability to form bone-like apatite crystals in SBF. In SBF immersion tests, bone-like apatite crystal formation was observed on the non-polarized and polarized porous HA ceramics. The average thickness of the bone-like apatite crystal on the polarized porous HA ceramics was thicker than that on the non-polarized ceramics. In addition, the weight increments of the polarized porous HA ceramics after immersion in SBF was larger than were those of the non-polarized ceramics. This indicated that the deposition speed of bone-like apatite crystal on the polarized porous HA ceramics was faster than that on the non-polarized ceramics, and that polarized porous HA promotes new bone ingrowth.

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