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Electrovectorial effect of crystal formation and cell proliferation on polarized ceramics

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Biological behaviors, characterization and crystal growths on the electrically polarized hydroxyapatite (HAp) ceramics were investigated. These specimens were electrically polarized in a dc field. Bone-like crystal growths were studied in a simulated body fluid (SBF) with pH 7.25 at 36.5°C, and in an α-minimum essential medium. Grown crystal layers were observed by scanning electron microscopy, and were analyzed by X-ray diffraction and Infrared spectroscopy. As a result, the bone-like HAp crystals grew rapidly on the negatively polarized surface (N-surface), while the growth was restricted on the positively polarized surface (P-surface) in SBF. It was considered that the "electrovectorial effect" of substrate accelerated the crystal growth on the N-surface, and decelerated on the P-surface. Biological behavior was estimated by the cultivation of osteoblastic and fibroblastic cells on the surface of polarized HAp. Cultured cells were found on the grown bone-like crystal layers for the N-surface, while the cells were directly grown on the specimens for the P-surface. On the polarized HAp ceramics, N-surface had more adhered cultured cells than the P-surface.

Key words: Ceramics, Polarization, Hydroxyapatite, Cell proliferation

1. INTRODUCTION

Studies in the field of implantable calcium phosphate biomaterials have produced impressive results concerning biocompatibility and ability to stimulate tissue formation[1-2]. Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is a main component of bone and tooth minerals. Synthesized HAp is a highly biocompatible ceramic, which has potential for use in a variety of oral, maxillofacial, and orthopedic applications. The knowledge of the behavior and the phenotypic expression of cells concerned by implantation is of great interest. Since HAp ceramics are specifically used in hard tissue implantation, continuing advances of studies on bone-like crystal growth and osteoblastic cell activity are important in the biomaterial field.

The authors described the characterization and cell reaction of calcium phosphates, such as α-TCP ($Ca_3(PO_4)_2$) and HAp[3]. Recently, it has been reported that the accelerations and decelerations of bone-like crystal growth were found on the surfaces of electrically polarized HAp in simulated body fluid by authors[4-8]. In the present study, we investigated the vectorial effects of cultured cells on electrically polarized HAp ceramics.

2. MATERIALS AND METHODS

2.1 Materials

HAp powders were prepared by a precipitation reaction from calcium hydroxide and phosphoric acid. A suspension of calcium hydroxide was stirred and a solution of phosphoric acid was added in drop to product a gelatinous precipitate. The obtained slurry was filtered, dried, and calcined. The resulting powders were finely ground under 200 mesh. HAp ceramic specimens were obtained by sintering of the HAp powders at 1250°C for 2hr. Obtained HAp ceramics were characterized by X-ray diffraction (XRD), infrared spectroscopy (IR), and scanning electron microscopy (SEM).

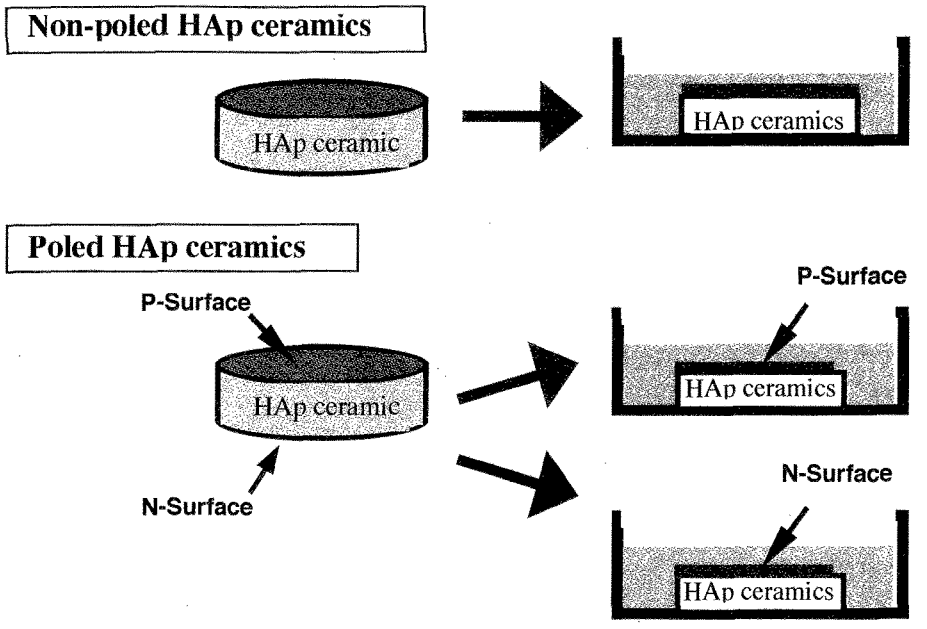


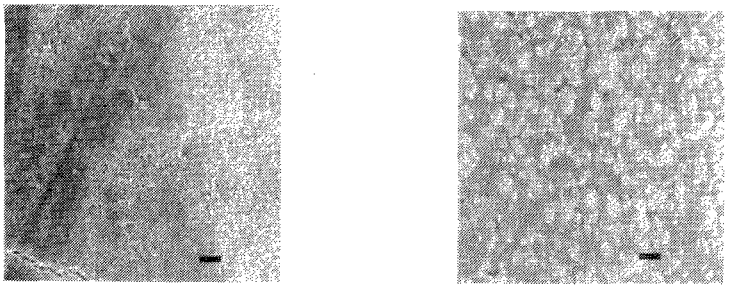
Figure 1. Schematic illustration of sample arrangement for poling and immersion in simulated body fluid.

2.2 Experimental Procedures

The HAp ceramic pellets obtained above were sandwiched between platinum electrode plates, then electrically polarized at 400°C under a dc field for 2 h. The samples were cooled down to room temperature under the electric field of the polarization. For the polarized HAp, negatively polarized surface and positively polarized surface were denoted as N-surface and P-surface, respectively. The surfaces of specimens without poling were referred as O-surface.

For investigation of bone-like crystal growths on polarized HAp surfaces, the electrically polarized specimens were immersed in a 1.5SBF (simulated body fluid)[9], having the 1.5 times inorganic ion concentrations equal to those of human blood plasma, with pH 7.25, at 36.5°C. Several days immersion, grown crystal layers were observed by SEM, and analyzed by XRD and IR.

Biological behavior and vectorial effects on selective cell adhesion of polarized HAp ceramics were estimated by cell cultivation. MC3T3-E1, osteoblast-like cells immortalized by Kodama *et al.*[10], and L929, mouse-derived connective tissue cells, and mouse-derived connective tissue cell, and SK-N-SH, human-derived neuroblastoma were used in this study.



(a) (b)

Figure 2. SEM photographs of the surfaces of electrically polarized HAp ceramics after 12 h immersion in 1.5SBF. (a) : N-surface, and (b) : P-surface.

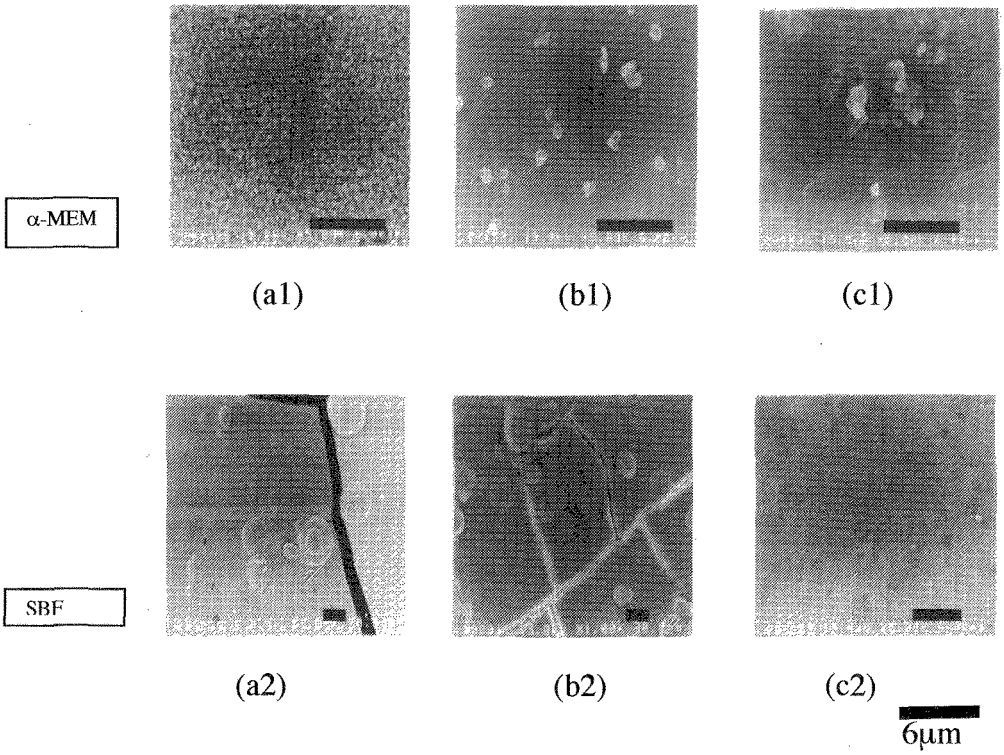


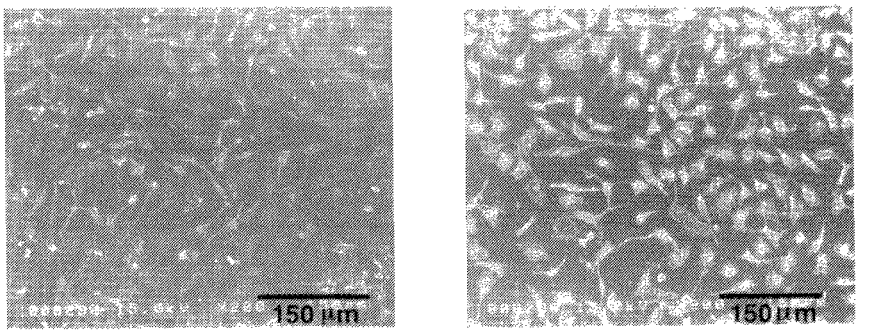
Figure 3. SEM photographs of N- (a), O- (b), and P-surface (c) of polarized HAp ceramic after 7 days immersion in α-MEM (a1,b1,c1), and in SBF(a2,b2,c2).

Cells were seeded in culture flask at a concentration ensuring exponential growth for 5-7 days. L929 cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and MC3T3-E1, and SK-N-SH cells were in α-MEM supplemented with 10% FBS. Every 2ml of suspension of cells ($6.7x10^4$ cells/ml for L929, $5.5x10^4$ cells/ml for MC3T3-E1 cells, and $1.7x10^5$cells/ml for SK-N-SH cell) were divided into culture multiplates and incubated in an atmosphere containing 5% CO_2 at 37 ^{o}C. Cell behaviors were evaluated by cell proliferation tests and morphological observation.

3. RESULTS AND DISCUSSION

The HAp ceramics used in this study were identified as pure HAp by XRD and IR. Result of SEM observation on polarized HAp surfaces revealed that bone-like crystal growth was accelerated on the N-surface, whereas crystal growth was less observed on the P-surface. Grown crystal on the P-surface was less than that on the O-surface, thus, the crystal growth was restrained on the P-surface. These results agree with those of HAp films mentioned in previous works[11-13]. It was considered that the N-surface attracts cations, calcium ions in this case, and the rate of local HAp nucleationis. It is supposed that the aligned dipoles of polarized HAp ceramics accelerate the bone-like crystal growth on the N-surface, and decelerated on the P surface, caused from reorientation of the dipole moments in the HAp structure.

The morphological evaluation by SEM indicated that L929, MC3T3-E1, and SK-N-SH cells were proliferated well in every cultured plate of non-polarized and polarized HAp surfaces. The L929 cells on N-surface of HAp were more adhered than those on O-surface. SEM photographs of L929 cells after 1 day cultivation on O- and N-surface of HAp were shown in Fig.3. L929 cell growth on P-surface was, on the other hand, restricted, and less adhered cells existed on the P-surface. The relative growth rates from 1 day to 4 days after incubation were 98% for the N-surface and 70% for the P-surface.



(a) (b)

Figure 4. SEM photographs of L929 cells on O-surface: (a) and N-surface :(b) on HAp ceramic surface after 1 day cultivation.

In case of MC3T3-E1 cell, cell behaviors on polarized HAp were similar to those of L929 cells. But, the L929, MC3T3-E1 and SK-N-SH cells had the different growth rates one another on the polarized HAp ceramics, and the differences of growth rates of these cells on polarized HAp ceramics explained the distinction among the vectorial effects to selective cell adhesion, which effects were ascribed to electrically poling. Electrical poling induces an additional vectorial effect on cell adhesion, stimulate and regulate the cell growth.

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