行政院國家科學委員會補助專題研究計畫 ☑ 成 果 報 告
□期中進度報告

應用電氣極化技術改變氫氧磷灰石之表面電位對骨內植入生醫材料表面改質之研究

計畫類別：☑ 個別型計畫　□ 整合型計畫
計畫編號：NSC　93－2314－B －038－022　－
執行期間：民國 93 年 08 月 01 日起至民國 94 年 07 月 31 日

計畫主持人：鄧乃嘉
共同主持人：黃文成、林哲堂
計畫參與人員： 黃濬賢

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

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中　華　民　國　94 年　8 月　21 日

行政院國家科學委員會專題研究計畫成果報告

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Introduction:

The manipulation of cells by inert or biomaterials has therefore long been a focal theme of biomaterial science, microbiology, and biochemistry because cells and bacteria are known to variably adhere to solids, depending on their surface charge. Tissue engineering has rapidly developed with the manipulation of cells in vitro using scaffolds from biomaterials. However, the dependence of cell adhesion on the electrical charge of these materials is not yet generally accepted because of a lack of evidence. We recently demonstrated changes in the rate of bone-like crystal overgrowth on electrically polarized ceramics of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAp) in a simulated body fluid. The effect of polarization on the acceleration and deceleration of grown hydroxyapatite layers was reported by Yamashita et al. This inductive effect by the polarized ceramics could potentially be useful to manipulate biomineralization.

Objectives:

We proposed that nucleation and crystal growth took place because of the interaction of the supersaturated ionic groups in simulated body fluid with the accumulated charges on the polarized HAp surface. On the basis of these findings, we examined the effect of electrically charged HAp ceramics on selective cell adhesion.

Materials and Methods:

Preparation of polarized HA samples

HA powder synthesized by the wet method was calcined at 850˚C and pressed into a mold at 200 MPa. The HA ceramic compacts were sintered in a saturated water vapor atmosphere at 1250˚C for 2 h. The highly crystalline HA samples, having a density of more than 98%, were cut into pieces 4 mm＊2 mm＊0.5 mm (thick). The HA samples were electrically polarized in a d.c. field of 1.0 $kV \cdot cm^{-1}$, 2.0 $kV \cdot cm^{-1}$, with a pair of platinum electrodes in air at 300˚C for 1 h. After sterilization with ethylene oxide gases, polarization of the HA samples was verified by thermally stimulated depolarization current (TSDC) measurement.

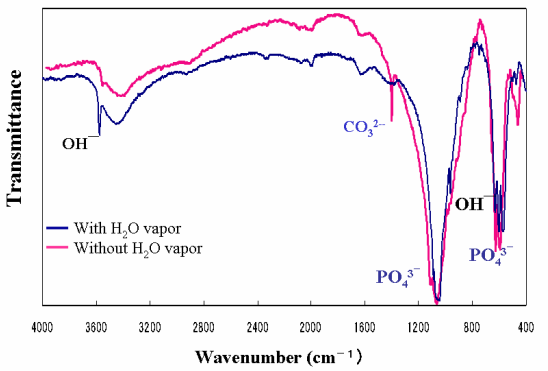
Cell cultivation on HAp ceramics

Scanning electron micrography (SEM) was used to further investigate both cell proliferation and colony morphology with different cell types on HAp ceramics. The HAp ceramics were made by sintering compressed HAp powders at 1250°C for 2 h, which were then electrically polarized as described. The HAp ceramics without surface polarization, used as a control, will be referred to as the O-surface. Mouse-derived osteoblast-like cells (MC3T3-E1), mousederived connective tissue cells (L929), and human-derived neuroblastoma cells (SK-N-SH) were used to test selective adhesion on these polarized HAp ceramics. These cells were seeded in a culture flask at a concentration ensuring exponential growth for 5–7 days. L929cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), whereas both MC3T3-E1 and SK-N-SH cells were cultivated in a-MEM supplemented with 10% FBS. Two milliliters of a cell suspension (6.7 × 104 cells/ml for L929, 5.5 × 104cells/ml for MC3T3-E1, and 1.7 × 105 cells/ml for SK-N-SH cells) was then divided into tissue culture multiwell plates and incubated in an atmosphere containing 5% CO2

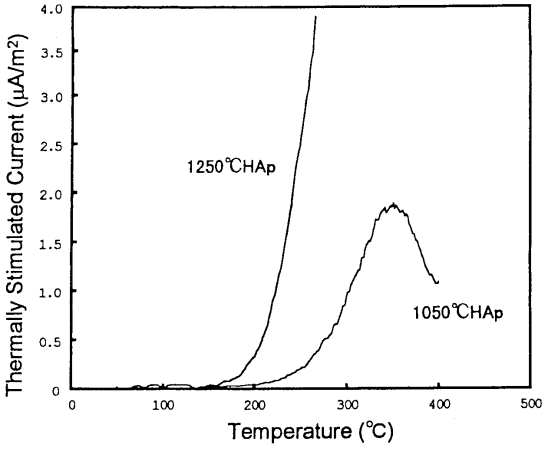
at 37°C. Cells on the tissue culture multiwell plate without HAp ceramic sample were used as the cultivation control

Results:

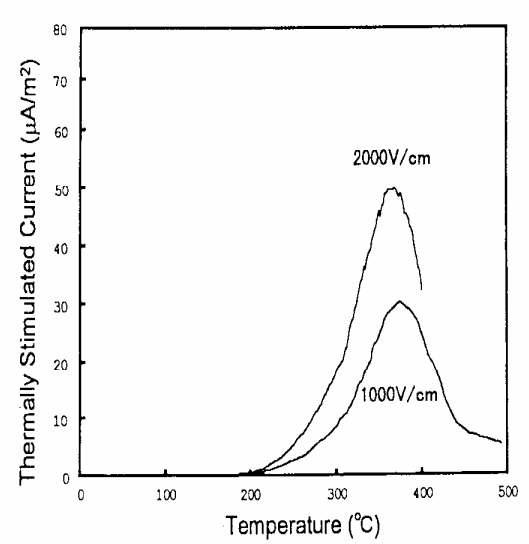
Polarization of the HA samples after the sterilization was verified by TSDC measurement. The curve increased from *150 to 350˚C and then gradually decreased. The stored charges were calculated at 0.05 mC cm^{-2}. It was confirmed that the charges of the polarized.HA samples were retained after sterilization.



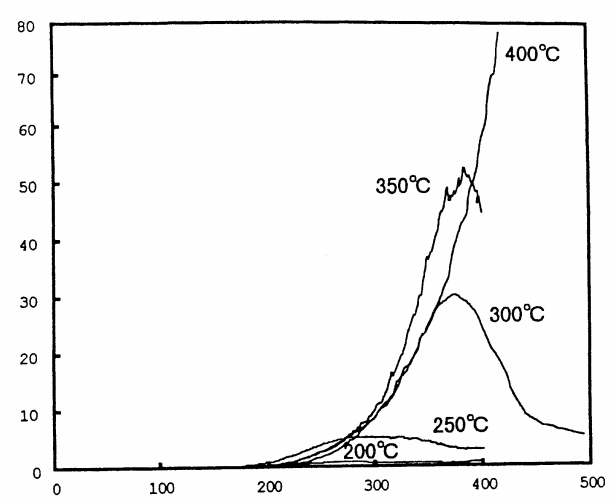
The HA sample sintered under 1250˚C and 1050˚C and polarized under 1kV/cm at 300˚C was verified with TSDC.



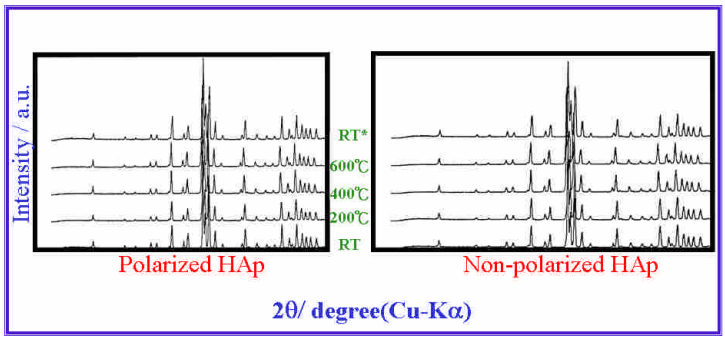
The HA samples polarized under 1.0kV/cm and 2.0 kV/cm, at 300˚C was verified with TSDC.



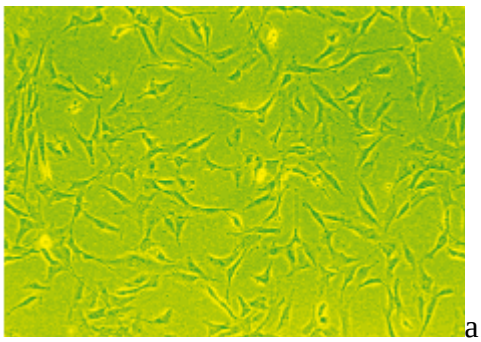
The HA samples was polarized under 1.0kV/cm at different Temp.

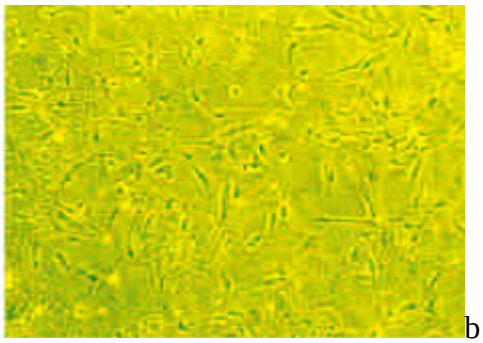


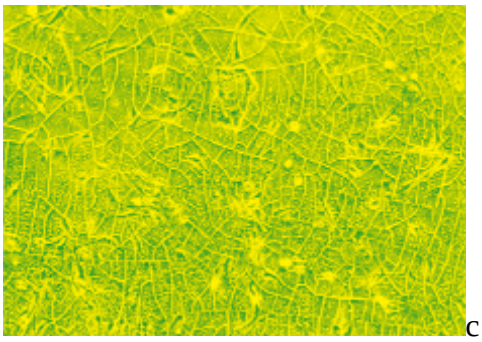
X-ray diffraction of the HA sample before polarization and after polarization. No significant different pattern before and after



Phase contrast micrographs of MC3T3-E1 cells on electrically polarized HAp films sputtered on glass plates at 23, 42, and 73 h after cultivation. The cells were adhered and grown on glass (a1), on negatively (b1) and positively (c1) polarized HAp films after 23 h cultivation.

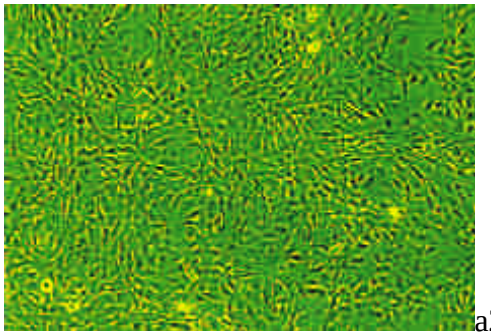

a1

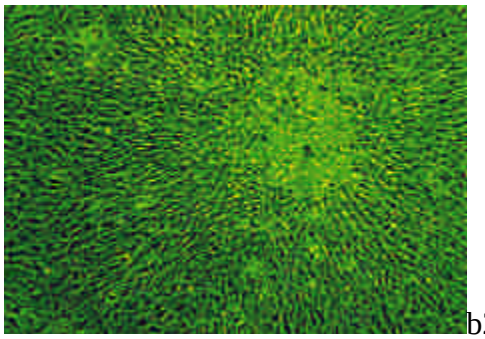

b1

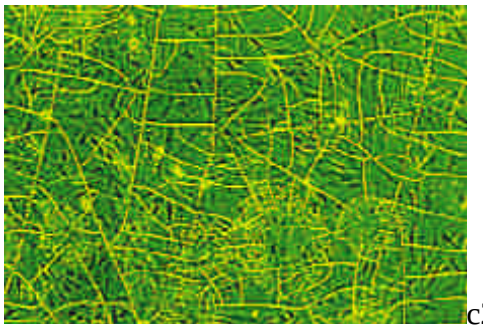

c1

The photographs of(a2), (b2), and (c2) show adhered cells on glass, negatively and positively

polarized HAp films afterafter 72 h cultivation,


a2


b2


c2

we observed multiple layers of cells and enlarged colonies of osteoblast-like cells on the negatively polarized surface. The morphology of this colonization, comprised of multilayers of cells, suggests the possible occurrence of differentiation to osteoblastic cells in the initial stages of ossification. The number of adhered cells on a positively charged surface, however, was less than that on the control surface; thus, the positively charged surface inhibited proliferation of cells. Thus, the effects of surface charges are consistent in that opposite charges cause opposite effects on cell adhesion. In general, two kinds of adhesion of cells are considered: one is a physical bond of the cells, cell-cell adhesion, which is formed among adjacent cells and the other is the attachment to the matrix, cell-matrix adhesion, in which the

adhesive proteins of extracellular matrices participate. The cell-matrix adhesion is important for construction of the multicellular organism body, such as during the development of bone.

Discussion:

The interaction between surface charges and surrounding substances contributing to cell adhesion is considered that the chemical substances in the medium surrounding the HAp ceramic, such as inorganic and organic ions, ionic groups, amino acids, and proteins, have the same opportunity to contact and anchor on the surface of the matrix. However, the charged groups and vesicles around the matrix in a medium can be repulsed or attracted by electrical charges on the matrix surface. Both electrical repulsion and attraction depend on the surface energy of the matrix surface. The group's anchoring state on the matrix surface can be regarded as a competitive reaction. In the competitive reactions of ion anchoring and cell adherence, a Ca2+ ion has two significant roles: one is the effect on crystal nucleation, the other on adhesion of a protein. The surface charges built by polarization have the role of promoting this competitive reaction, especially the Ca2+ ion behavior. Bone-like apatitic layers were formed on the Nsurface on the crystalline base simultaneously with Ca2+ anchoring. For nucleation of an accumulated layer, Ca2+ ions are predominantly adsorbed on the N-surface because of their superior binding affinity relative to the other cations in the medium, such as Na+, K+, and Mg2+.16 Besides Ca2+ adsorption on the N-surface, Ca2+attracts cell adhesion proteins, such as integrins, fibronectin, and osteonectin, which show divalent cation-dependent ligand binding.,the role of divalent cations has been clarified through protein crystallography. The adhesive protein detects the Ca^{2+}ion and anchors it and then the protein is tightly joined to the cell. The bone-like layers formed afford many more cell-adhesive protein contacts; the adhered and proliferated cells on the matrix grow into a bone-like layer. Thus, cell anchoring and growth were synergistic in a concerted ionic reaction on the polarized surface. On the P-surface, the positive charges attract anionic groups. In this case, the anionic groups such as $HPO4^{2-}$ and/or $HCO3^{2-}$ act as antiadhesive molecules. The anion groups do not induce bone-like apatite layers, and thus, cell adhesion is limited on the P-surface.

The improved cell adhesive properties on the Nsurface of HAp encourages further research at this interface of physics, engineering, chemistry, and cell biology. The behavior and the phenotypic expression of cells on polarized HAp demonstrated in this work could have significant ramifications for a variety of biomaterials for oral and orthopedic applications, long sought in bone healing and osteoblast response to biomaterials.

Reference:

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可供推廣之研發成果資料表

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國科會補助計畫	計畫名稱： 計畫主持人： 計畫編號：　　學門領域：
技術/創作名稱	
發明人/創作人	
技術說明	中文： （100~500 字）
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