Transactions of the Materials Research Society of Japan 29[6] 2939-2942 (2004)

Osteoconductive Mechanism of Electrically Poled Hydroxyapatite Ceramics

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Biological responses of electrically poled hydroxyapatite (HA) ceramics were investigated by implantation tests using canine tibial and femoral bones. The enhancement mechanism of electrically poled HA ceramics was discussed by histological methods. The HA ceramics were sintered at 1250°C in saturated water vapor. The ceramics were poled in a dc field at 300°C. The electrical surface charge induced by poling was *ca.* 3 $mCcm^{-2}$ calculated from thermally stimulated current measurement data. The flat thick new bone formation with an osteoblast layer was observed on the negatively charged (N) surface. Although no direct-contacting new bone was found on the positively charged (P) surface, osteoids were observed in the vicinity of the ceramics on 7th day after the implantation. The thickness of the new bone contacted to the N-surface on 14th day increased 2-3 times more than that of 7th day after the implantation. The new bone formation developed in the vicinity of the P-surface and directly contacted the matured bone surfaces partly on 14th day. A small volume of the new bone directly contacted the non-poled (0) surface on 14th day. The N-surface of the poled HA promoted the new bone formation, compared to the 0-surface of HA.
Key words: hydroxyapatite, electrical poling, osteoconductivity, Electret, Surface charges

1. INTRODUCTION

We disclosed that hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$; HA) had an electrical polarizability due to proton transport [1, 2] and reported that the surface charges induced by electrical polarization affected interaction to surrounding environment. A pseudo-biological environment using simulated body fluid (SBF) has been widely accepted as an osteobonding estimation of biomaterial surfaces [3]. We have been demonstrated by SBF tests that the negative surface charges induced by an electrical polarization accelerated the overgrowth on the HA ceramic surface but that the positive charge decelerated it [1]. The enhanced osteobonding ability was revealed by *in vivo* implantation tests and corresponded the estimation by the SBF method [4,5].

In the present study, Biological responses of electrically poled hydroxyapatite (HA) ceramics were investigated by implantation tests using canine tibial and femoral bones. The enhancement mechanism of electrically poled HA ceramics was discussed by histological methods.

2. MATERIALS AND METHODS

Powder of HA was prepared by a precipitation synthesis from calcium hydroxide ($Ca(OH)_2$) aqueous suspension and phosphoric acid (H_3PO_4) at room temperature (RT) [6]. Suspension of calcium phosphate was precipitated by adding solution to aqueous suspension (0.5M) continuously at RT with vigorous stir using a propeller mixer. The calcium hydroxide obtained from reagent grade calcium carbonate (calcite, $CaCO_3$) fine powder (Wako, Japan) was dispersed in distilled water. The powder of calcium carbonate was calcined at 1100°C for 3h in air and then hydrolyzed by means of adding distilled water. The amount of phosphoric acid was adjusted so that no other phase than HA was detected in the product heated at 1100°C by XRD analysis. After the adjustment of phosphoric acid amount, the reaction suspension was aged for 4 days with continuous mixing. The obtained slurry was filtrated and then, dried at 60°C for 24 h. The powder calcined at 850°C was uniaxially pressed at 220 MPa. The pellets were sinterd at 1250°C for 2 h in saturated water vapor [7]. The obtained dense HA ceramic disks with the size of 10 mm diameter and 0.7 mm thickness were identified by powder X-ray diffractometry (XRD) and FT-infrared spectroscopy (FT-IR).

The HA ceramic disk cramped with a pair of Pt electrodes was polarized in air at a temperature ranging from 300 to 500°C for 1h in a dc electric field of 1 or 10 $kVcm^{-1}$. The thermally stimulated depolarization current (TSDC) spectra were examined with a handmade measurement cell shielded against stray field and a pico ammeter (Hewlett-Packard 4140B) from RT to 850°C at a heating rate of 5°C/min. The stored electrical charge (Q) was calculated by numerical integration from each of the measured current spectrum [2].

The polarized samples were implanted in the femoral and tibial diaphyses fully described in a previous report [4]. The experiments were carefully completed by veterinarians in accordance with the Guideline for Animal Experimentation (Tokyo Medical & Dental University) as well as the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Pub. No. 85-23, Rev. 1985). Rectangular holes of 1.4×5.0 mm^2 at intervals of more than 15 mm were obtained on the bones of beagle dogs with a 0.7-mm

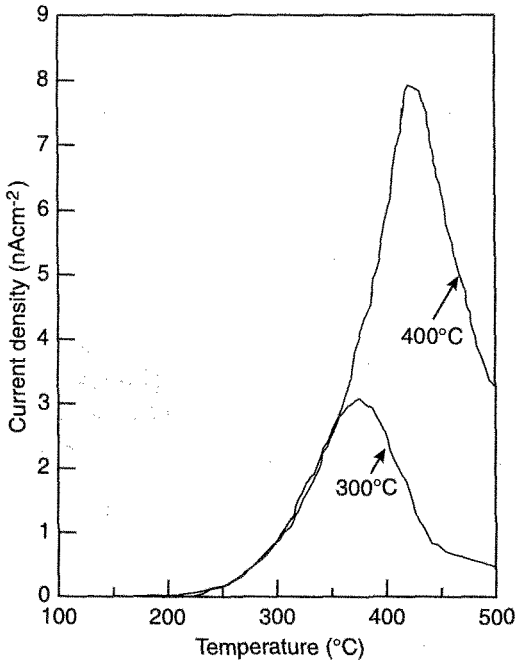

Fig.1 TSDC spectra of electrically polarized HA ceramics.

dental fisher bur. The gaps between the observational faces of the samples and the cut cortical bone faces were fixed at 0.2 – 0.3 mm. The samples were rigidly held by the friction between the lateral faces of the samples and the bone faces. The bones containing the samples were harvested at 7 and, 14 days after the implantation. The extracted tissues were decalcificated with Plank-Pychlo solution for 1-2 weeks. The morphological evaluations of the tissue reaction in the cavity between the ceramics and cortical bones were examined using transversal sections stained with hematoxylin eosin (H-E).

RESULTS AND DISCUSSION

The obtained HA ceramic samples had a relative density of 98%. The samples were recognized to be a single phase of hexagonal HA containing the OH^- group, based on their XRD patterns and IR spectra.

The TDSC spectra (Fig.1) of the HA ceramics polarized in a dc field of 1.0 $kVcm^{-1}$ at 300 and 400°C for 1h. The curve increased at *ca.* 180°C and reached maximum at 375°C for the sample polarized at 300°C and 420°C for the sample done at 400°C, and then decreased. The calculated charge storage (Q) was 3.92 and 15.1 $mCcm^{-2}$, respectively [8]. The maximum current density of 3.21 $nAcm^{-2}$ was much lower than those of the ferroelectric-paraelectric transition ceramics, such as a $BaTiO_3$. However, the average stored charge (3.9 μCcm^{-2}) was well corresponded to the published result [2, 5, 6] and was almost half of the $BaTiO_3$ [2]. Therefore, it was confirmed that the large electrical charges were preserved in the polarized HA ceramics. We abbreviated the negative charge-induced surface, the positive charge-induced surface, and the surface of the non-polarized HA as the N-surface, P-surface and 0-surface, respectively.

The TSDC spectrum showed that the polarized HA ceramics stored large electrical charges and that the decay process of depolarization was very slow. The electrical properties suggested that the electrically polarized HA was an electret [2, 8].

The number of the migrated protons, which the electrical polarization was attributed to, was estimated as less than 0.5% of the total number in the HA ceramic substrate [2]. Moreover, no significant difference in XRD patterns and FT-IR spectra was found before and after polarization under any condition. Therefore, the

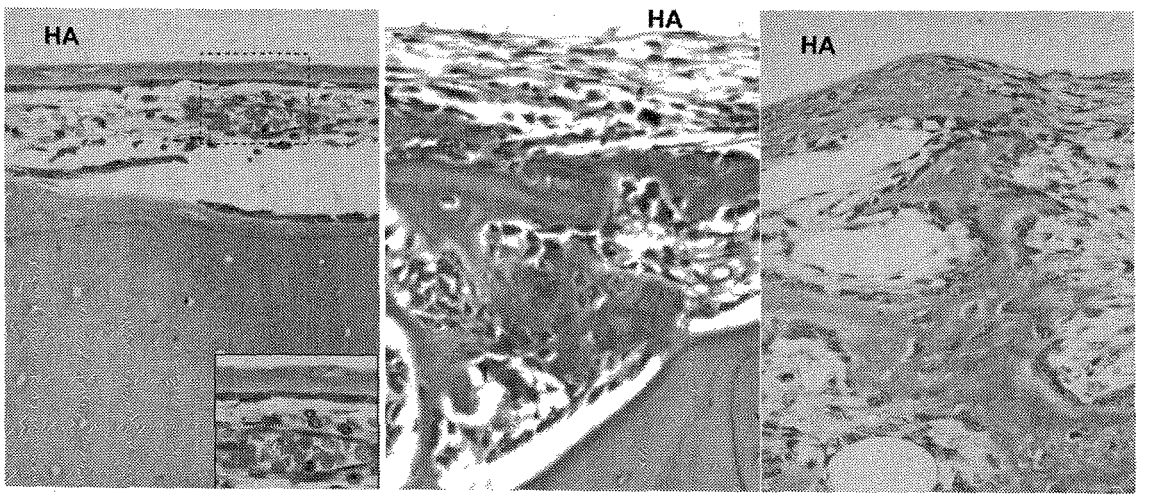

Fig. 3 Histological sections of the vicinities of N-surface (left), 0-surface (center), and P-surface (right) 7 days after implantaion.

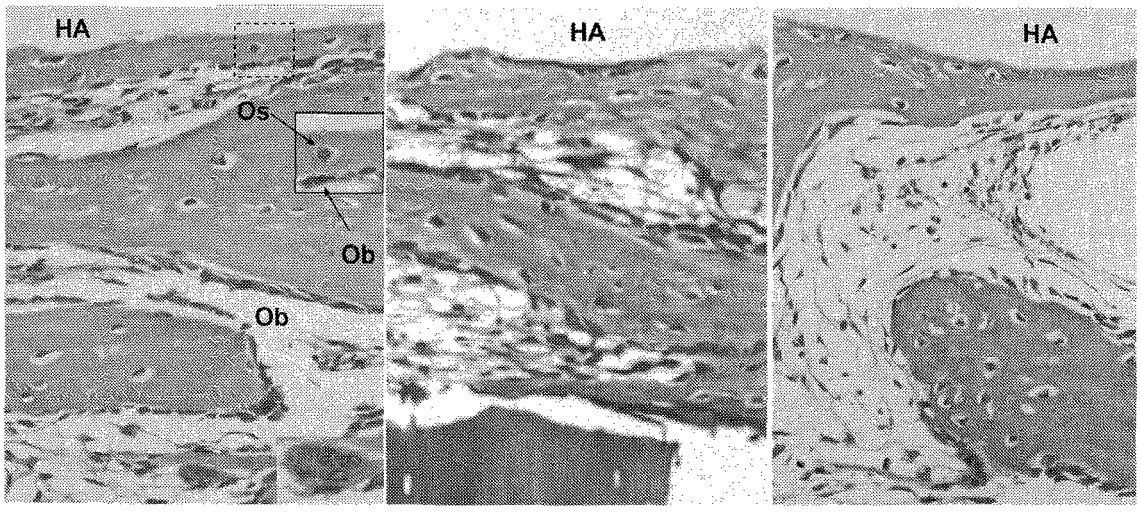

Fig. 4 Histological sections of the vicinities of N-surface (left), 0-surface (center), and P-surface (right) 14 days after implantaion.

chemical changes in the surface properties were possible to neglect.

Seven days after the operation, the newly formed bone layers of 0.02 mm average thickness were in direct contact with the N-surfaces (Fig. 3a). On the other side of the newly formed bone layer, the small flat-shaped osteoblastic cells were structured in a monolayer. The maturing osseous cells were already included in the newly formed bone. The monolayered flatten small osteoblastic cells were also observed on the surface of the cut cortical bone tissues facing the N-surface and partly lengthened toward the N-surface. In the area of the newly formed bone tissues, a large amount of blood capillaries, fibroblasts and myeloid cells were observed. The newly formed bones were isolated from the 0-surface and surrounded with fibrin layers (Fig. 3b). In the vicinity of the 0-surfaces, the fibrin layers with blood capillaries including fibroblastic cells with fusioform nuclei were observed. Osteoblastic cells were found on the partial surfaces of the immature bone tissues.

Seven days after the implantation, no hemorrhage reaction was found in the vicinity of the P-surface. Many osteoid-like tissues were surrounded by large square-shaped osteoblastic cells (Fig. 3c). Some parts of the osteoid tissues showed light stainability. Consequently, the H-E stainablity of the osteoid tissues varied widely. Considering that the decalcification in the proximity of the P-surfaces in the section preparation was achieved much earlier than on the other side in the vicinity of the N-surfaces, the calcification in the P-surface vicinity progressed more slowly. Although parts of the newly formed bone surfaces appeared to be directly contacted to the P-surfaces, at almost all the interfaces, fibrous tissues were found in higher magnification observation. The newly formed bones directly contacted to the P-surface were osteoid tissues lengthened from the cut cortical bone surfaces toward the ceramic surfaces. The bone formation with layers of the large osteoblastic cells was also observed on the cortical bone surfaces facing the P-surface with a clear cement line indicating a boundary between newly formed and old bones. A large amount of blood vessels observed as vacuoles occupied the space among the osteoid tissues.

On postoperative day 14, the thickness of the newly formed bones bonding to the N-surface significantly increased compared with that on day 7 (Fig. 4a). On the cortical bone surface toward the N-surface, there were many newly formed multi-layered bone tissues. The entire newly formed bone tissues were structured in networks and included the osseous cells and blood capillaries as well as being surrounded by layers of small flatten osteoblastic cells. Although a large amount of newly formed bones were observed with osteoids in the vicinity of the 0-surface surrounded with osteoblastic cell layers and many capillaries, monolayers of flatten cells existed at the interface between the bones and the 0-surface (Fig. 4b). At 14 days, although the newly formed bones were directly contacted to the P-surface, all of the directly contacting bones derived from the osteoids were lengthened from the direction of the cut cortical bone surfaces (Fig. 4c). The directly contacting bone tissues were accompanied by osteoblastic cell layers. While the area of the newly formed bones in the gap looked almost the same as on day 7, each osteoid was noticeably enlarged. Moreover, the osteoids were significantly stained dark uniformly. Many large vacuoles recognized as blood vessels were found among the osteoids.

CONCLUSION

Based on the TSDC studies, it was demonstrated that the HA ceramics were polarized by the transportation of the protons in the OH^- columnar structure. The large polarization charge was long enough to enhance the biological reactivity of the HA ceramics for biomedical use. The charged surfaces of the HA ceramics

promoted bone reconstruction in wide defects, whereas the processes varied according to the charge polarity.

ACKNOWLEDGEMENTS

This work was partly supported by a Grants-in-Aid from the Japan Society for the Promotion of Science (#15360338), the Mitsubishi Foundation, the Murata Scientific Foundation, a grant for Development of Advanced Medical Technology from the Ministry of Education, Science, Sports and Culture of Japan.

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(Received November 30, 2003; Accepted February 29, 2004)