QUANTITATIVE EVALUATION OF THE HYDROPHILIC PROPERTIES OF POLARIZED
HYDROXYAPATITE

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ABSTRACT

Hydroxyapatite (HAp), a major inorganic component of bone and teeth, has been used as
biomaterials. HAp also possesses the proton conductive property as well as biocompatibility. Due to
proton defects at hydroxyl ion in the process of HAp synthesis, dipole is formed in HAp with hydroxyl
ion and oxide ion from hydroxyl defected proton. By electrical polarization treatment, the electrical
dipoles are reoriented and produce surface electric fields and subsequent surface charges on HAp
surface. This static electrical energy affects the interfaces character and is increased hydrophilicity of
the polarized HAp surface. Hydrophilicity of biomaterials effects on good attachment between the
material surfaces and, biomolecules, cells and tissues. To study the surface change of polarized HAp
with interaction with external environments, surface analysis was performed using X-ray photoelectron
spectroscopy (XPS). The stored charges of the polarized HAp, calculated from thermally stimulated
depolarization currents spectra, were around 5 $\mu C \cdot cm^{-2}$ and the surface potential measured by Kelvin
probe method was $\pm$ 20 V. The results from XPS indicated that the protonation of surface phosphate
ions on the polarized HAp occurred by the adsorption of water in the atmosphere more than on the
non-polarized HAp. It was suggested that there might be changes of chemical bond after interaction
with water molecules in the interfacial layer of the polarized HAp and the changes accelerated the
effect of hydrophilicity as well as the surface energy.

INTRODUCTION

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is well-known as a major inorganic component of
bone and teeth, so such biocompatibility has been used as a bone substitute or a dental implant. In
addition, we have focused that HAp also works as a proton conductor. [1] Because the lattice of
hydroxide ion in HAp is aligned along the c-axis of the crystal structure, protons at the hydroxide can
diffuse along the c-axis via proton defects, which forms during HAp synthesis process. The dipole,

which is formed with hydroxyl ion and oxide ion from hydroxide defected proton, produces polarization ability of HAp. [2,3]

By application of d.c. voltage to HAp at high temperature, the electrical dipoles are reoriented with the proton conduction. The reorientation causes electrical polarization of HAp and this polarization treatment induces surface electric fields and subsequent surface charges on HAp surface. Surface electric charges in polarized HAp have been experimentally demonstrated *in vitro* to affect the interfaces between charged surfaces and ions, proteins, and cultured cells. For example, crystal growth in simulated body fluid and albumin adsorption was changed on the polarized HAp depending on the polarity.[4,5] Osteoblasts, which form bone tissues, could attach and proliferate on the polarized HAp more than non-polarized HAp.[6,7] In porous polarized HAp implanted in rabbit femur, new bone formation, which was stained with Villanueva-Gordner staining, was accelerated compared to non-polarized one after 6 weeks implanted.[8]

A part of these biological changes resulted from increase of wettability of polarized HAp. The wettability of the sample surface can be measured by the static contact angle of water drop and can visualize interfacial energy which sample has potentially. It has been reported that the contact angle for water drop decreased on the polarized HAp compared to non-polarized HAp. [6,7] Because micro structure of HAp, such as grain size or surface roughness, does not change by polarization, it is considered that the hydrophilic conversion by polarization originate in surface potential of the polarized HAp.

In this study, we focused our attention on changes in the surface nano-construction when the polarized HAp interacted with biomolecules. Because water is the most abundant molecule in a living body, the chemical state of water molecules adsorbed onto the polarized HAp surface studied using X-ray photoelectron spectroscopy (XPS), which can evaluate the state of chemical bonds of the interfacial layer of the sample.

MATERIALS AND METHODS

Preparation of the specimens and electric polarization

For preparation HAp pellets, HAp powders were synthesized by the precipitaion method and were pressed into green compacts at 120 MPa. Then the compacts were sintered in water vapor at 1250 °C for 2 h.

The polarization performed in a d.c. field of 5 kV/cm at 400 °C for 1 h with a pair of platinum electrodes. The surface faced with cathode side was designated N-surface which has negative charges, and opposite side was referred as P-surface which has positive charges. Non-polarized surface was prepared with heat treatment in the same equipment as a control denoted as 0-surface. To examine the state of water adsorbed into the HAp surfaces in contact with liquid water, samples immersed in

distilled water for 7 d at 37 °C.

Characterization of the specimens

Surface morphology was observed using scanning electron microscopy (SEM, S-3400-NX, Hitachi Ltd.) after acid etching. The surface roughness of the specimens was quantified using a laser profile micrometer with a resolution of 0.01 μm (VF-7500, Keyence). The crystal phases of the product were determined with a powder X-ray diffractometer (XRD) in the 2θ range of 10° to 60° using Cu Kα radiation at 40 kV and 40 mA (D8 ADVANCE, BRUKER). Chemical bonding in HAp was measured using Fourier transform infrared spectrometry (FT-IR, JASCO FT/IR-500 spectrometer) between 4000 and 400 cm^{-1} using KBr pellets.

Assessment of polarization

Evaluation of the polarization was performed using thermally stimulated depolarization currents (TSDC) measurements. To calculate the stored charges of the polarized apatite, the depolarization currents were measured via heating at a constant heating rate 5 °C·min^{-1} from room temperature to 600 °C. Surface potential measurements were performed with a Kelvin Probe (KP) instrument. The potential of the KP tip was calibrated against a commercial 3V button battery in an ambient condition.

Characterization of the surface of the specimens

The chemical compositions of the sample surfaces were analyzed by XPS (JPS-9010MC, JEOL) under ultrahigh vacuum (UHV, 2-4 × 10^{-7} Pa). The chemical species physically adsorbed on the samples would be almost removed in this high vacuum. Mg Kα (1253.6 eV) radiation was used as the X-ray source in the XPS tests. The XPS take-off angle was set to 90°. Charge effects were corrected using the C1s line of adventitious carbon at 285 eV. The XPS spectra were fit using the Casa program to a Gaussian-Lorentzian peak shape with a Shirley baseline to represent the background.

RESULTS AND DISCUSSION

Characterization of the non-polarized and polarized samples

After sintered, relative densities of the samples were more than 95 % of the theoretical density value (3.16 g·cm^{-3}). The morphology of HAp was observed using SEM (Figure 1). The average grain size was 0.5- 2.0 μm. The surface roughness (Ra values) of the HAp was 0.53 ± 0.07 μm.
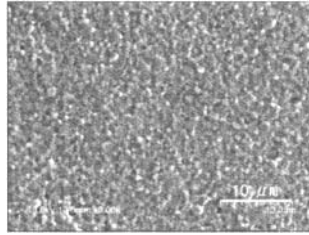
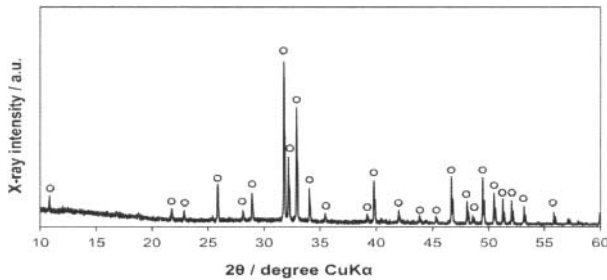Fig. 1. SEM micrographs for HAp pellet.

Figure 2. X-ray diffraction patterns of HAp. Open dots indicated HAp phase.

XRD patterns showed that all samples have single-phase hydroxyapatite structure indicated by open-dots (Figure 2). FT-IR spectra indicted that there were two bands at 3570 and 630 cm^{-1} due to hydroxyl groups (OH) stretching and liberating modes in crystal lattice of HAp, respectively, and two broad peaks around 1200-900 and 600 cm^{-1} assigned to vibration of phosphate ions (PO_4^{3-}) (Figure 3).
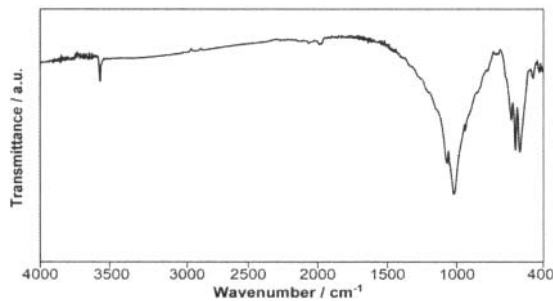

Figure 3. FT-IR spectra of HAp.

The TSDC analysis is a widely used experimental technique for the study of the dielectric relaxation. Representative TSDC spectra of the polarized HAp samples are shown in Figure 3A. The TSDC curve begins to increase at ca. 200-250°C, reaches its maximum at ca. 450-550°C, and then gradually decreases. The average stored charges of the polarized samples calculated from the TSDC curves were around 5 $\mu C \cdot cm^{-2}$. Because the sample after TSDC assessment was depolarized and could not use other studies, we determined the surface electronic property of the samples by surface potential using Kelvin probe method.[9, 10] The method is a non-contact and non-destructive and can measure the static electricity difference between the sample and a vibrating capacitance metal probe in an ambient condition. The polarized HAp used in XPS test was measured ± 20 V, compared to non-polarized was 0 V.
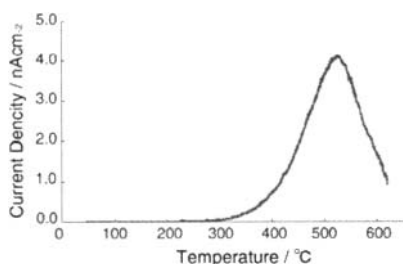

Figure 3A. TSDC spectrum obtained from polarized HAp.

Water molecules adsorbed on HAp surfaces

XPS surveys of HAp before electric polarization were showed in Figure 4. The elements with the highest detected concentrations were oxide (O), calcium (Ca), phosphorus (P), and carbon (C). The survey spectra showed little change after polarization treatment.
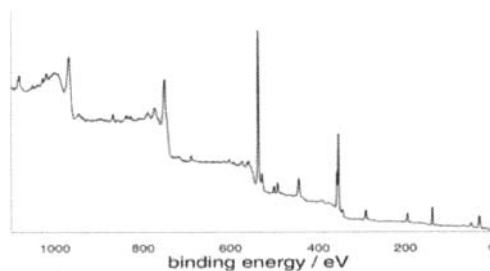

Figure. 4. The survey XPS spectra of the non-polarized HAp.

The relative concentration of elements on the non-polarized and polarized HAp surface after aged water was summarized in Table I. The relative concentration of elements was calculated using the peak intensity of O 1s, P 2p, Ca 2p, and C 1s. Aromatic carbon was removed as a carbon contamination. The atomic concentration (at %) of each element in HAp was almost stable under polarization.

Table I. The relative concentration of elements on HAp surface using XPS (at %).

element	0-surface	N-surface	P-surface
O	59.6	61.1	60.2
Ca	19.1	20.2	19.4
P	13.8	14.2	13.9
C	4.9	3	4.8

The spectrum of P 2p bond was detected at 133.1–133.2 eV, and the binding energy (BE) value in the polarized HAp shifted to higher value than the value in the non-polarized HAp (Figure 5(a)). The result indicated that the chemical state of phosphate (PO_4^{3-}) in all samples resulted from HAp and the protonation of surface PO_4^{3-} and forming acidic phosphate ions (HPO_4^{2-} and $H_2PO_4^-$) occurred by the adsorption of water in the atmosphere more on the polarized HAp than on the non-polarized HAp.[11] The XPS spectra of the BE regions of Ca 2p electrons obtained from the non-polarized and polarized HAp were shown in Figure 5(b). Ca 2p core level presented the double bonds at a BE of 347.1-2 and 350.6-7 eV, which assigned to Ca $2p_{3/2}$ and Ca $2p_{1/2}$ of Ca 2p orbit. The BE value of the N-surface shifted to higher value than the value of other surfaces. The results indicated that the chemical state of Ca in all samples resulted from HAp and the polarized HAp contained more $CaHPO_4$ or $Ca(H_2PO_4)_2$ than the non-polarized HAp.
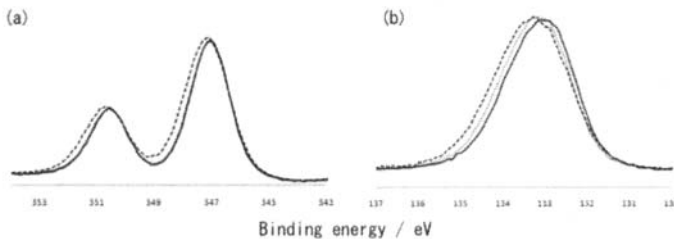

Figure 5. XPS of Ca 2p (a) and P 2p (b) core level spectra of non-polarized HAp. The line shows 0-surface HAp, the short-dashed line is N-surface, and dotted line is P-surface.

The XPS spectrum of the O 1s BE consists of three fitting peaks at 531.0-1, 532.1-3, and 533.3-4 eV (Figure 6(a)). The peaks of 531.0-1 attributed to hydroxyl groups (OH^-) and P-O of PO_4^{3-} groups and 533.3-4 eV attributed to physically adsorbed water (H_2O) or C=O, respectively.[12] Because the peak at 532.1-3 eV was detected between at the higher BE values shifted from P-O and O-H, and at the lower BE from H_2O, it may be assigned to the O 1s BE of the surface P-OH groups in HAp resulted from the protonation of surface PO_4^{3-}.[13,14] The O 1s BE of the P-OH groups in the polarized HAp increased compared to the non- polarized HAp (Figure 6(b)).
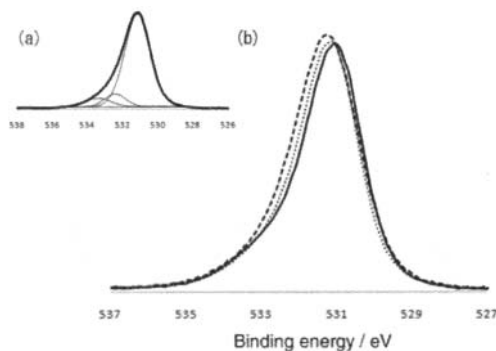

Figure 6. XPS of O 1s core level spectra of non-polarized HAp with fitting curves (a). The line shows 0-surface HAp, the short-dashed line, N-surface, and dotted line, P-surface (b).

The lowest BE peak, contained a large amount of OH^- and PO_4^{3-}, was compared with two other peaks (Table 2). There was no clearly difference of [H_2O]/[O-H, P-O] ratio of all surfaces. In contrast, [P-OH]/[O-H, P-O] ratio was increased due to the polarization treatment. The XPS assessment suggested that PO_4^{3-} on the polarized HAp surface was more likely affected upon protonation by the adsorption of water molecules than on the non-polarized HAp surface. Because the surface P-OH groups have been identified as an absorption site for water and CO_2, it leads us to expect that the polarized surface, especially N-surface, serve as the predominant adsorption site for water.[15]

Table II. Percentage (%) of the deconvoluted peaks ratio in the O 1s XPS spectra.

	0- surface	N- surface	P- surface
P-OH /O-H, P-O	19.7	24.4	18.1
H_2O/O-H, P-O	7.7	8.9	7.3

CONCLUSION

We demonstrated that nano-construction of polarized HAp surface changed in an ambient condition by using XPS in this study. The XPS result, surface P-OH increased on the polarized HAp, suggested the possibility that the construction change of the surface structure will be involved in the hydrophilic conversion of the polarized HAp surface as well as the surface energy. The current work offers one of the possible mechanisms of hydrophilic conversion process for practical applications by the polarized treatment, however, contact angle measured in an ambient condition and XPS results in UHV in this study did not correlate closely. In addition, it is required further investigation whether the state of water molecule adsorption is reversible or irreversible phenomenon.

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