Electric poling of cement composites of hydroxyapatite whiskers with chitosan and their chemical properties in simulated body fluid

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Cement composite of calcium phosphate was developed as a novel bioceramic material having biocompatibility, macro-porous structure, resorbability in vivo. The purpose of this study was to prepare and characterize the polarized calcium phosphate-citric acid-chitosan composite and to evaluate the effects of electrical polarization on bioactivity in order to enhance their biocompatibility. The characterization of the cement composite revealed that the surface is consisted with mixed phases of hydroxyapatite and CaHPO4·2H2O, having porous microstructure. The thermal analysis indicated that the appropriate temperature for electrical poling is below 100°C to inhibit the degradation of each component. The electrical measurements revealed that the cement composite has poling ability at 100°C through the proton migration among the lattice sites of OH, HPO4 and H2O molecules. The bioactivity was assessed by immersing the cement composites with or without electrical poling in simulated body fluid and evaluating the deposit and growth of bone-like apatite crystals on each surface. The negatively charged surface was accelerated compared to positively charged and non-poling surfaces.

Key-words : Biomaterials, Hydroxyapatite whisker, Composite cement, Electrical polarization, Electrical property, Simulated body fluid

1. Introduction

In a general understanding, biomedical applications require workability of materials as well as biocompatibility. Also important is the macro-porous structure, which makes feasible cells and tissues to intrude through an artificial implant. Cemented calcium phosphates satisfy the requirements because they have developed consistently for in vivo applications to achieve both safely loose densification and resorbability in vivo.[1–3] In addition to such excellent properties, cemented bodies are superior to sintered bodies in the easiness of addition with other elements such as polymers and proteins, because the addition can be performed through hydration process at room temperature.[4] Considering the advantages, we targeted cemented bodies of calcium phosphates[5] as a novel bioceramic material. For further excellent biocompatibility, we applied electric poling[6–8] to the cemented bodies, because poling treatment of hydroxyapatite (HAp) ceramics enhances biocompatibility both in vitro[9–11] and vivo,[12–14] and regulate the interaction with blood,[15] living cells[9–11] and tissues or defected bones[12,13] and wound skins[14] and blood vessels[16] were effectively healed by poling.

In this study, we employed the method proposed by Toyama for the preparation of cemented calcium phosphate, which uses HAp whiskers with amorphous tricalcium phosphate (ATCP), because the whiskers can be expected to be poled effectively according to the previous studies on ionic conduction properties of HAp whiskers.[17,18] The composites were chosen as the system of HAp/ATCP-citric acid-chitosan.[5] Chitosan is natural biopolymer and has advantages for biocompatibility, biodegradability and osteoconductivity. The cement composites of HAp/ATCP-citric acid-chitosan improves the mechanical property.[5] This report will, first of all, introduce the poling possibility of HAp whiskers, and poling characteristics of cemented calcium phosphate composites. The biomedical availability is conveniently demonstrated using simulated body fluid (SBF).[19]

2. Experimental procedure

2.1 Synthesis and characterization of HA whiskers and powders

Amorphous calcium phosphate (ACP) whiskers were synthesized from the analytical grade reagents of (NH4)2HPO4 and Ca(NO3)2 by the wet method.[4,5] The precipitated ACP powder was washed, dried and ultrasonically dispersed into CH3COOH aqueous solution for 1 min. The prepared ACP suspension was converted into a suspension of HAp whiskers under hydrothermal conditions at 200°C for 2 h in a sealed vessel. The HAp whiskers were washed with hot water and deionized water and acetone. After drying, the HAp whisker was immersed in simulated body fluid (SBF) for 1 h in air. Polarization

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of the HAp whisker compacts was verified by thermally stimulated depolarization current (TSDC) measurement. The TSDC measurements were carried out according to our previous study in air from room temperature to 600°C at a heating rate of 5.0°C/min. The depolarization current was measured with a Hewlett-Packard 4140B pA meter. The values of the polarization charge (Q) were calculated from the TSDC curves using the equation

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

where \( J(T) \) is the measured dissipation current density at temperature \( T \) and \( \beta \) is the heating rate.

2.2 Preparation of calcium phosphate-citric acid-chitosan composite

ATCP powder was mixed with HAp whisker (HAp/ATCP = 0.4:1 in weight ratio) and with CaCO\(_3\) (CaCO\(_3\)/ATCP = 0.3:1 in molar ratio). The citric acid solution (2.0 mol/dm\(^3\)) containing 4.0 mass % chitosan was added to the powder mixture of ATCP-HAp-CaCO\(_3\) (liquid/solid = 1.5:1 in weight ratio) and mixed at room temperature. The mixtures were packed into a mold (φ11 mm, 1 mm in thickness) and hardened for 3 d at room temperature.

The cement composite was characterized using X-ray diffraction (XRD, Phillips PW1830), Fourier transform-infrared spectroscopy (FT-IR, JASCO FTIR4100) and Thermo Gravimeter and Differential Thermal Analysis (TG–DTA, Mac Science TG-DTA2000), especially TG–DTA with XRD was performed to determine the poling temperature without dehydration.\(^{20}\) Impedance measurements were carried out in air using an impedance analyzer (16048A Hewlett Packard) over a frequency range of 100 Hz–1 MHz. A four-probe alternate-current (ac) method with platinum ion blocking electrodes and attached platinum wires was used to measure the complex conductivity. The resistance (\( R \)) of the cement composites was determined from the obtained Cole–Cole plots. For a given geometry, the ion conductivity (\( \sigma \)) at each temperature and the Arrhenius plots for the cement composites were calculated.

2.3 Electrical polarization of cement composites

The cement composites were electrically polarized with a pair of platinum electrodes at 100°C in direct-current (dc) electric fields of 5 kV/cm for 1 h in air. The negatively charged

Fig. 1. XRD patterns of the cement composites of HAp/ATCP-citric acid-chitosan after 3 d hardening with (a) or without (b) poling treatment consist of mixed phases of HAp (open circles) with CaHPO\(_4\)·2H\(_2\)O (closed circles).

Fig. 2. FT-IR spectrum of the cement composite after 3 d hardening.

Fig. 3. TG–DTA curves of chitosan (a), the mixture of chitosan with citric acid solution (b), ATCP (c) and the cement composites after 3 d hardening (d).
and positively charged surfaces of the cement composites are designed N-surface and P-surface, respectively. The non-poling surface is the 0-surface. Polarization of the cement composites was verified by TSDC measurement. The TSDC measurements were carried out in air from room temperature to 600°C at a heating rate of 5.0°C/min as described above.

2.4 Simulated body fluid evaluation

The electrically polarized cement composites were immersed in simulated body fluid (SBF) with pH 7.4 at 37°C for 1, 3 and 7 d. SBF solution was prepared using the technique described by Kokubo et al.19) After immersion in SBF, the composite specimens were washed with deionized water and ethanol, and then dried at room temperature in air. The surfaces were sputtered with Pt-Pd using an ion coater (Eiko Engineering IB-2). The sputtered surfaces were observed using a scanning electron microscopy (SEM, Hitachi S-2400).

3. Results and discussion

3.1 Characterization of cemented composites

The XRD analyses demonstrated that the cement composites of HAp/ATCP-citric acid-chitosan after 3 d hardening and with or without poling treatment [Figs. 1(a) and 1(b), respectively] consist of mixed phases of HAp with CaHPO₄·2H₂O. The cement

![Fig. 4. TSDC curves of the cement composites polarized at 100°C in dc electric fields of 5 kV/cm for 1 h in air (a) and the non-poling cement composites (b).](image)

![Fig. 5. TSDC curves of the HAp whiskers polarized at 100°C in dc electric fields of 5 kV/cm for 1 h in air (a) and the non-poling HAp whiskers (b).](image)
The cement composite exhibited stretching vibrations of the PO$_4^{3-}$ at 1035 and 568 cm$^{-1}$, showing typical HA patterns (Fig. 2). The FT-IR spectrum for the cement composite shows peaks assigned to carboxyl group absorption at 1270, 1300, 1390, 1550 and 1650 cm$^{-1}$ and to amide group absorption peaks observed at 1200 cm$^{-1}$. The absorption peaks observed at 1450 and 1470 are COO-Ca, indicating the composition of calcium phosphate, citric acid and chitosan.

The TG–DTA curve for chitosan shows exothermic peaks at 315, 330 and 540°C (Fig. 3(a), indicated by arrows). The TG–DTA curve for the mixture of chitosan with citric acid solution shows exothermic peaks at 210, 315, 530 and 560°C, indicating the degradation of chitosan (Fig. 3(b), indicated by arrows). The TG–DTA curve for ATCP shows exothermic peaks at 640°C (Fig. 3(c)). The exothermic peaks for the mixture of calcium phosphate and organic materials generally shift to higher temperature compared with that for organic materials. The cement composite exhibited exothermic peaks of the mixture of chitosan with citric acid at 280 and 350°C and ATCP at 640°C (Fig. 3(d), indicated by arrows). TG–DTA curves indicated that the appropriate temperature for the electrical poling is below 100°C to inhibit the degradation of each component.

### 3.2 Poling and electrical properties of cement composites and HAp whiskers

The result of TSDC curve confirmed poled state of the cement composite through the observation of thermally released current, which was observed at measuring temperature range up to 200°C.
and the maximum value of which was 1 nA/cm² [Fig. 4(a)]. The integration of the observed current gives 3–4 µC/cm² as stored charges in the cemented body, as high as HAp ceramics.7) The curve, on the other hand, of non-poled body showed no peak in the measurement, suggesting no charge storage in the body [Fig. 4(b)].

To investigate the poling mechanism, poling of HAp whiskers was carried out with use of compressed powders of the whiskers. The observed curve shows the maximum peak of 1.2 nA/cm² and the current was released over the temperature range up to 300°C [Fig. 5(a)]. The calculated charge storage was 1–2 µC/cm², almost the same magnitude of that of the cemented composite. The comparison of the two curves of TSDC suggests the same poling mechanism as each other, which can take place due to proton movement by applied voltage, because the HAp whiskers contain a lot of lattice hydrogen from a deduced composition17,18) of Ca₉.₈(HPO₄)₀.₈₂(PO₄)₅.₁₈(OH)₁.₁₈·nH₂O for HAp whiskers. The TSDC curve of non-poling HAp whiskers did not increase [Fig. 5(b)].

To characterize the ionic conduction in the cemented body, complex impedance measurements were done at the temperatures of 50 to 170°C in the air. Though the impedance was too high for the conductivity to be calculated at lower temperatures [Figs. 6(a) and 6(b)], a pair of semi-circles was measured at higher temperatures [Figs. 6(c) and 6(d)]. Using an intercepted point on the vertical line of real part of impedance at lower measuring frequency, the conductivity was calculated as 5–6 × 10⁻⁸ S/cm at around poling temperature. Based on these values, the Arrhenius plot was constructed and the activation energy for conduction was obtained 0.2 eV [Fig. 6(e)], rather lower than that for HAp ceramics.20) The result may imply that protons move among the lattice sites of OH, HPO₄ and H₂O molecules due to poling.

3.3 SBF immersion
The SEM photographs of cemented bodies exhibit porous microstructure with pores of ca. 100 µm in diameter and plate-like microcrystals with a few µm in width of plates [Figs. 7(a) and 7(b)]. The pores appeared on the surface of the cement composites were considered to be caused by the dissolution of citric acid. The surfaces of the cement composite with or without electrical poling after SBF immersion for 1, 3 and 7 d are shown.
in Fig. 7. The deposits of bone-like crystals had formed and grown to ca. 1µm in diameter on the N-surface after the immersion for 1 day [Fig. 7(d)], whereas there were few deposits on the 0-surface and P-surface [Figs. 7(e) and 7(f)]. After the immersion for 3 days, the deposits of bone-like crystals were also observed on the 0-surface and P-surface [Figs. 7(f) and 7(h)]. The amount of the deposits of bone-like crystals was the largest on the N-surface of the polarized HAp bulk, the ion concentrations were different from that of the 0-surface.21) The differences were thought to be caused by the attraction of calcium ions to the N-surface. The attracted calcium ions were supposed to play and important role in the deposition of bone-like crystals through the higher supersaturation against apatite. Therefore, the possible explanation for the acceleration of the formation and growth of bone-like crystals against apatite. Therefore, the possible explanation for the acceleration of the formation and growth of bone-like crystals between the employed composite surfaces.

Near the N- and P-surfaces of the polarized HAp bulk, the ion concentrations were different from that of the 0-surface.21) The differences were thought to be caused by the attraction of calcium ions to the N-surface. The attracted calcium ions were supposed to play and important role in the deposition of bone-like crystals through the higher supersaturation against apatite. Therefore, the possible explanation for the acceleration of the formation and growth of bone-like crystals is the attraction of calcium ions to the N-surface of the cemented composite in SBF solution.

4. Conclusions

In this study, the calcium phosphate-citric acid-chitosan composite was prepared and characterized. The characterization of the cement composite revealed that the surface is consisted with mixed phases of HAp and CaHPO4·2H2O, having porous microstructure. The thermal analysis indicated that the appropriate temperature for electrical poling is below 100°C to inhibit the degradation of each component. The electrical measurements revealed that the cement composite has poling ability at 100°C through the proton migration among the lattice sites of OH, HPO4, and H2O molecules. The bioactivity assessment by immersing the cement composites with or without electrical poling in simulated body fluid indicated that the deposit and growth of bone-like apatite crystals on the N-surface was accelerated compared to P- and 0-surfaces.

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