

Hydroxyapatite Coating on a Titanium Metal Substrate by a Discharging Method in Modified Artificial Body Fluid

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Purpose: The aim of this study was to evaluate the formation of a hydroxyapatite coating on titanium plate by discharging in typical and modified artificial body fluids. **Materials and Methods:** Japanese Industrial Standard Grade 2 titanium plates were used as specimens. Discharging was performed on the specimens in each solution, and the surface topography of each coating was observed with a scanning electron microscope. The Ca/P atomic ratio and surface characterization of each coating were evaluated by x-ray photoelectron spectroscopy, and crystal phases of each coating were analyzed by Fourier transform infrared spectroscopy and x-ray diffraction. **Results:** Tricalcium phosphate formed on the titanium surface in a 1.5 Ca/P solution with high ion concentrations of calcium (2.5 mmol/L) and phosphorous (1.67 mmol/L). Crystalline HA was formed on the titanium surface in a 1.5 Ca/P solution (Hanks' balanced salt solution without organic molecules) with low ion concentrations of calcium (1.26 mmol/L) and phosphorus (0.83 mmol/L). **Discussion:** A solution containing organic pH buffer was insufficient to form stable coatings on the titanium surface. Coating properties strongly depended on the Ca/P chemical ratio of the applied solution. A Ca/P ratio of 1.5 was appropriate for preparing a calcium phosphate coating on a titanium surface, whereas a Ca/P ratio of 2.5 was excessively high. **Conclusion:** These findings suggest that Hanks' balanced salt solution without organic molecules is the most suitable solution for forming crystalline hydroxyapatite coatings on titanium surfaces by the discharging method. INT J ORAL MAXILLOFAC IMPLANTS 2004;19:66–72

Key words: artificial body fluid, balanced salt solution, hydroxyapatite, titanium

Titanium and its alloys have been used successfully for the replacement of hard tissues. They have been especially useful in dental implant systems. Titanium can spontaneously nucleate on its surface a calcium phosphate layer, a bonelike hydroxyapatite (HA), when it is immersed in an artificial body fluid.^{1,2} The biocompatibility of titanium

strongly depends on this biomineralization process.³

Ban and Maruno⁴ reported that HA was electrochemically deposited on titanium plates in typical simulated body fluids. In the presence of an electrical field, various components suspended in the solution were driven into the titanium surface, forming an HA layer.⁴ However, since these deposits were formed using a relatively low electrical field, additional treatments were necessary to increase their crystallinity and bonding properties.⁵

Deposition of HA on the surface of titanium by electrochemical synthesis using a neutral pH solution has been proposed as another method. Hanawa and Ota reported that an HA-like calcium phosphate precipitation was formed on a pure titanium surface immersed in Hanks' balanced salt solution without organic ions (HBSS⁻).¹ However, the application of HA coatings on titanium surfaces using HBSS⁻ has not been fully investigated.¹

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The authors have proposed that the technique of discharging in an electrolyte is useful for preparing thin calcium phosphate coatings on titanium surfaces. Discharging was generated between the electrolyte and the working electrode (specimen) through a gas layer on the surface of the electrode with large cathodic potential. Calcium phosphate particles suspended in the electrolyte were driven into the specimen surface, and a calcium phosphate layer was formed with a larger electrical field than that used in conventional electrophoretic techniques. This technique is expected to increase the quality of coatings without additional secondary treatments.

Miyazaki and associates reported that a thin anatase titanium dioxide (TiO_2) film could be formed on a titanium surface anodized by discharging in a phosphoric acid/hydrogen peroxide ($\text{H}_3\text{PO}_4/\text{H}_2\text{O}_2$) solution.⁶ Fujimori and associates reported that a thin calcium phosphate film could be precipitated on a titanium surface by discharging in an acidic-mixed calcium phosphate solution.⁷ Kishida demonstrated that a stable HA layer (5 to 10 μm) could be formed through a pretreated anodized layer by discharging in an acidic-mixed calcium phosphate solution.⁸ Because of the large electrical stimulation during the processing, a discharging method has the potential to prepare HA coatings in a single treatment. Ikeda and Igarashi reported that titanium plates anodized by discharging in sodium chloride, sodium fluoride, or potassium iodide solutions could acquire antibacterial properties.⁹ However, the formation of HA coatings by discharging in artificial body fluids has not been reported.

The purpose of the present study was to systematically evaluate the effect of the electrolyte compositions of various artificial body fluids on coatings formed on titanium plates by the discharging method. The surface topography of each coating was observed using a scanning electron microscope (SEM). In addition, surface characterization and the Ca/P ratios of these coatings were determined by x-ray photoelectron spectroscopy (XPS), and the crystalline phases of the coatings investigated by Fourier transform infrared (FTIR) and x-ray diffraction (XRD).

MATERIALS AND METHODS

Preparation of Specimens

The material used was Japanese Industrial Standard Grade 2 titanium in plate form ($300 \times 300 \times 1.0$ mm KS-50; Kobe Steel, Kobe, Japan). The plates were divided into specimens of $10 \times 10 \times 1.0$ mm using a wire-type electric discharge machining (W-EDM) device (α -0iA; FANUC, Yamanashi, Japan).

Table 1 Concentration (mmol/L) of Ions in Each Solution

| Ion | SBF | SBF ⁻ | m-SBF ⁻ | HBSS ⁻ |
|--------------------------------|-------|------------------|--------------------|-------------------|
| Na ⁺ | 142.0 | 142.0 | 137.80 | 142.00 |
| K ⁺ | 5.0 | 5.0 | 3.34 | 5.50 |
| Mg ²⁺ | 1.5 | 1.5 | — | 8.00 |
| Ca ²⁺ | 2.5 | 2.5 | 2.50 | 1.26 |
| Cl ⁻ | 148.8 | 148.8 | 142.80 | 140.00 |
| HPO ₄ ²⁻ | 1.0 | 1.0 | 1.67 | 0.83 |
| HCO ₃ ⁻ | 4.2 | 4.2 | — | 4.20 |

Cleaning of Specimens

The prepared specimens were cleaned ultrasonically in acetone for 15 minutes, then in detergent solutions (7 \times ; MP Biomedicals, Aurora, OH) for 15 minutes, and finally in pure distilled water for 15 minutes. The specimens were then dried and stored for 24 hours at 23°C in a desiccator that maintained a humidity of 50%.

Electrolytes

The composition (mol %) of the electrolytes, as prepared in the laboratory and used in this study, is summarized in Table 1. Two of the solutions, simulated body fluid (SBF) and SBF⁻, had a Ca/P ratio of 2.5 mmol/L; the other 2 solutions, m-SBF⁻ and HBSS⁻, had a Ca/P ratio of 1.5 mmol/L. The SBF used here is typical SBF with ion concentrations of 142.0 mmol/L Na⁺, 5.0 mmol/L K⁺, 1.5 mmol/L Mg²⁺, 2.5 mmol/L Ca²⁺, 148.8 mmol/L Cl⁻, 1.0 mmol/L HPO₄²⁻, and 4.2 mmol/L HCO₃⁻. This solution was buffered at a pH of 7.4 with 50 mmol/L tris(hydroxymethyl)aminomethane and adequate HCl.

SBF⁻ was prepared in the same manner as SBF but without buffer. It was prepared to evaluate the efficiency of the organic pH buffers contained in typical SBF. This solution was adjusted to a pH of 7.4 with adequate HCl.

Modified SBF (m · SBF⁻) is made by adding dipotassium hydrophosphate (K_2HPO_4) to an SBF with ion concentrations of 137.8 mmol/L Na⁺, 3.34 mmol/L K⁺, 2.5 mmol/L Ca²⁺, 1.67 mmol/L HPO₄²⁻, and 142.8 mmol/L Cl⁻. To investigate the availability of Ca/P ratio contained in electrolyte, the Ca/P ratio of this solution was prepared to be equivalent to that of HBSS. This solution was adjusted to a pH of 7.4 using HCl.

HBSS⁻ is an HBSS without organic ions and with ion concentrations of 142.0 mmol/L Na⁺, 5.5 mmol/L K⁺, 8 mmol/L Mg²⁺, 1.26 mmol/L Ca²⁺, 140.0 mmol/L Cl⁻, 0.83 mmol/L HPO₄²⁻, and 4.2 mmol/L HCO₃⁻. This solution was adjusted to a pH of 7.4 with HCl.

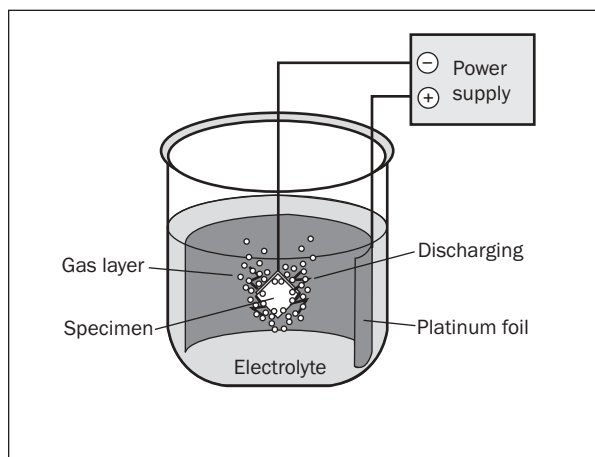


Fig 1 Discharging in an electrolyte.

All solutions were stored in polypropylene bottles before the studies were undertaken.

HA Coating Protocol

The specimen was connected to the cathode of the coating device developed in the laboratory. The specimen was then immersed in each electrolyte. A $50 \times 100 \times 0.1$ -mm platinum plate was used as a counter-electrode. Discharging was generated between the electrolyte and the working electrode through a gas layer on the surface of the electrode with increasing cathodic potential. Discharging was maintained at 1 A and 10 V (416 mA/cm^2) for 540 seconds (Fig 1). This condition was determined by a preliminary investigation. After this process, specimens were washed in pure distilled water and stored for 24 hours at 23°C in a desiccator that maintained a humidity of 50%.

Surface Observation

The specimens were prepared by gold vaporizing with a vacuum evaporation device (IB-2; Eiko Engineering, Ibaraki, Japan). Then the surface topographies of the coatings on the specimens were observed by SEM (S-2360N; Hitachi, Tokyo, Japan).

Surface Characterization

The surfaces of the coated specimens were analyzed by XPS (ESCA-3400; Shimadzu, Kyoto, Japan). High-resolution spectra of Ti2p, O1s, C1s, Na1s, P2p, Ca2p, Cl2p, and K2p were analyzed using MgK α radiation. An emission current of 20 mA and an accelerated voltage of 8 kV were applied in the analysis. The binding energies for each spectrum were calibrated using a C1s spectrum of 285.0 eV. The Ca/P ratio of each coating was calculated using the relative concentrations of calcium and phosphorus. Results of the test were expressed as the mean \pm

standard deviation (SD) of 6 specimens. Findings were analyzed statistically using an analysis of variance (ANOVA). Significant differences were considered to exist when $P < .01$.

Crystal Phases

Each coating was ground into particles and prepared as potassium bromide (KBr) pellets that were analyzed by an FTIR analyzer (WS/IR-7300; JUSCO, Tokyo, Japan) with a spectra resolution of 4 cm^{-1} to characterize the various functional groups on the coating surface. The crystal phases of each surface of the coated specimens were identified by thin-film XRD (XRD-6100; Shimadzu) with CuK α radiation. The XRD was operated at 40 kV (40 mA) with a scanning speed of 0.005 deg/second and a scanning range of 20 to 50 degrees. All data were confirmed at least 6 times in repeated investigations.

RESULTS

Surface Observation

SEM of specimens in each electrolyte are shown in Fig 2. The amounts deposited on the SBF specimens were extremely small compared to the other specimens. The amounts of coatings observed to be on specimens with SBF $^-$ were also small. On the other hand, uniform and granular coating particles were observed on the specimens with m-SBF $^-$ and HBSS $^-$.

Surface Characterization

Ca/P ratios of surface deposits with SBF and SBF $^-$ were remarkably high ($P < .01$), 2.25 and 2.69, respectively. In addition, the relative concentration of carbon on the specimens with SBF (mean \pm standard deviation) was $24.47\% \pm 0.4\%$, which was much larger than those of the other specimens ($12.7\% \pm 0.7\%$ on the specimens with SBF, $10.5\% \pm 0.6\%$ on the specimens with m-SBF $^-$, and $12.0\% \pm 0.3\%$ on the specimens with HBSS $^-$; $P < .01$).

The Ca/P ratios of the coatings on the specimens with m-SBF $^-$ and HBSS $^-$ were approximately 1.59 and 1.71, respectively. The 1.71 Ca/P ratio of HBSS $^-$ was consistent with that of human bone.¹⁰ A peak of Na1s was detected only with HBSS $^-$ (Fig 3).

Crystal Phases

Figure 4 shows the FTIR spectra of a typical specimen. Spectra attributable to HA were not detected on specimens with SBF and SBF $^-$.

PO $_4^{3-}$ adsorption peaks around $1,000 \text{ cm}^{-1}$ and 600 cm^{-1} were observed on specimens with m-SBF $^-$.¹¹ However, since the peak of $3,570 \text{ cm}^{-1}$

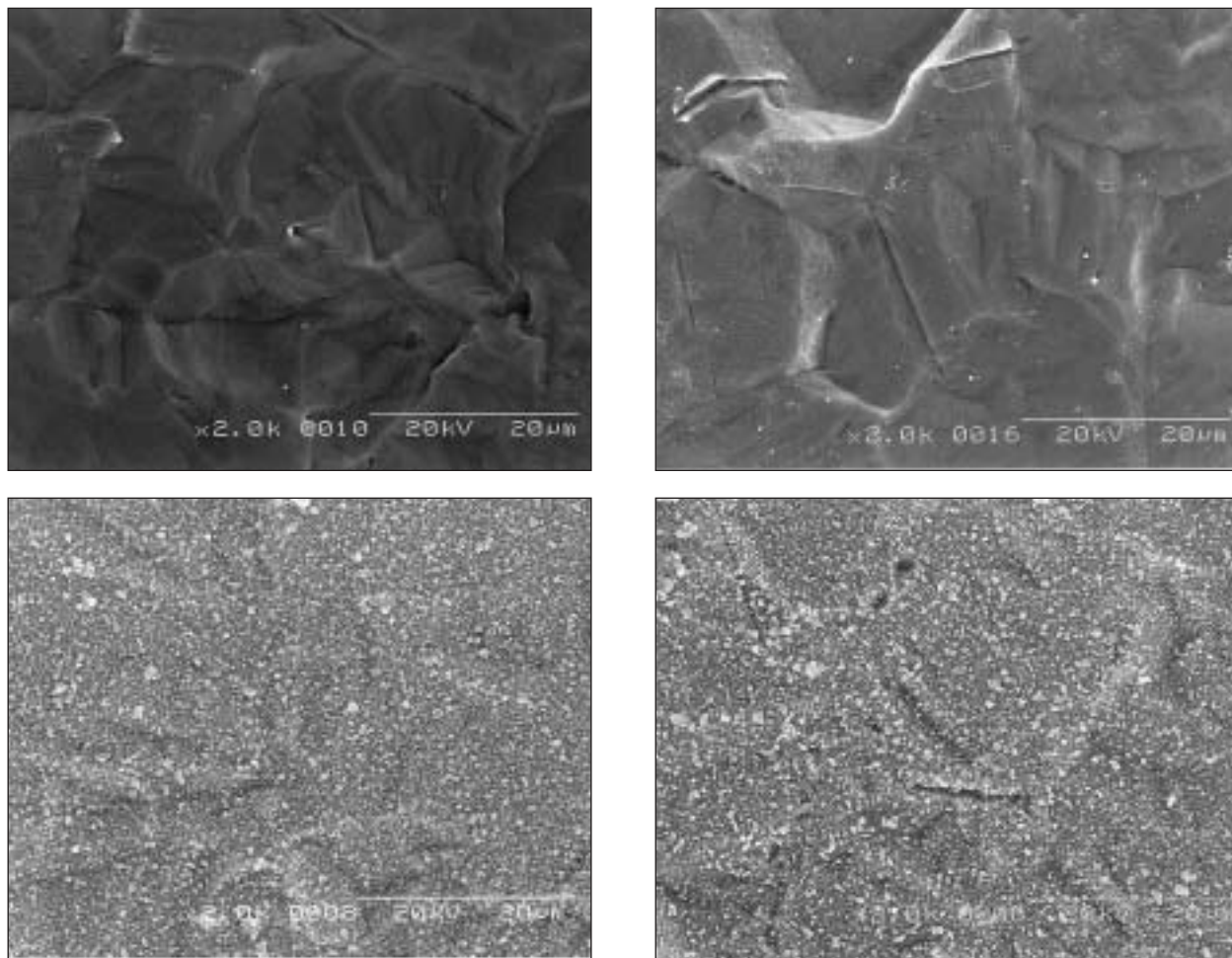


Fig 2 SEM micrographs of specimens with each electrolyte (magnification $\times 2000$). (Above left) SBF; (above right) SBF⁻; (below left) m-SBF⁻; (below right) HBSS⁻.

attributed to hydroxide (OH⁻) groups¹² was not detected on the specimens with m-SBF⁻, the spectra on specimens with m-SBF⁻ consisted of those of tricalcium phosphate (TCP).

PO₄³⁻ adsorption peaks around 1,000 cm⁻¹, 600 cm⁻¹, and 580 cm⁻¹ were detected on specimens with HBSS⁻. Since the peak of 3,570 cm⁻¹ attributed to hydroxide groups was detected on these specimens, HA could have been coated on their surfaces. In addition, the CO₃⁻ peaks around 1,500 cm⁻¹ indicated that the phosphate groups of the HA coatings were partially replaced with carbonate groups as well as the mineral phase of bone.¹³ Because the PO₄³⁻ peaks of the coatings were split around 600 cm⁻¹ and 580 cm⁻¹, the crystallinity of these coatings was relatively high.¹¹ The XRD patterns of a typical specimen are shown in Fig 5. Although no specific patterns related to HA were observed on the other specimens, HA patterns were clearly observed on specimens with HBSS⁻. In addition, a peak of CaO was observed on specimens with SBF⁻. Furthermore,

a peak of TCP was observed on specimens with m-SBF⁻.

DISCUSSION

The present study evaluated the relationship between coatings on titanium and the electrolyte composition of artificial body fluids prepared by the discharging method.

SBF

The SEM observation demonstrated that extremely small amounts of particles were deposited on the surface of specimens with SBF. Although Ca and P were detected on specimens with SBF in the XPS study, crystalline phases of these deposits were not identified. The peak of the C1s spectra of 285.0 eV (C-H) of specimens with SBF was obviously higher than that of the other specimens, indicating that coatings on specimens with SBF were contaminated

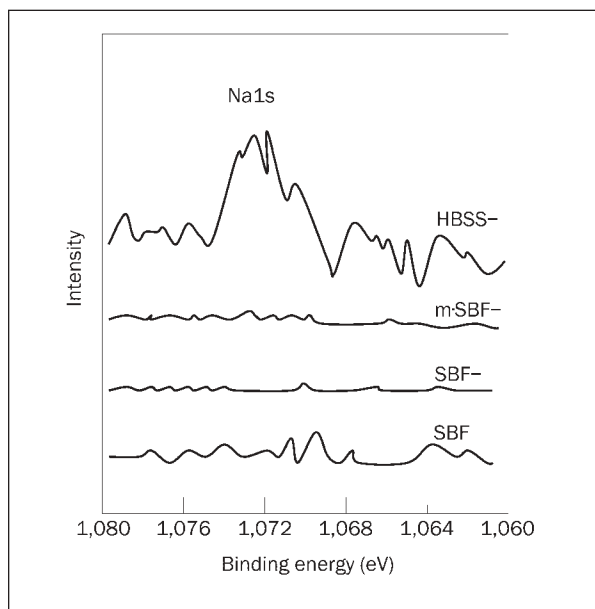


Fig 3 (Left) High-resolution spectra of Na1s on a typical specimen.

Fig 4 (Below) FTIR of the specimen shown in Fig 3.

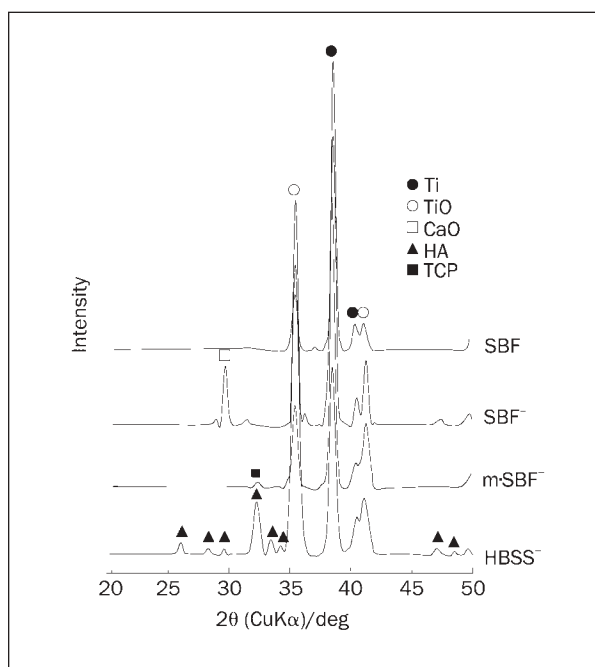
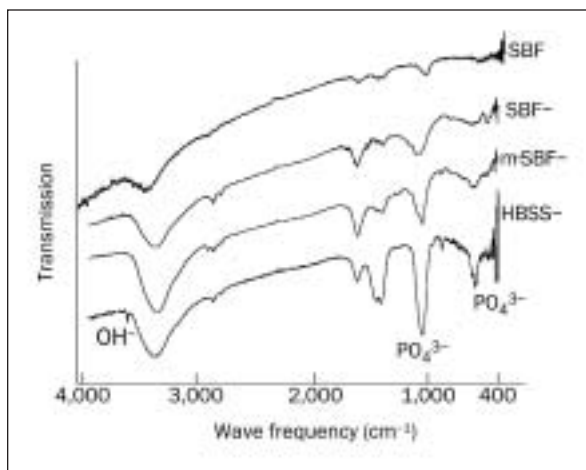


Fig 5 XRD patterns of the specimen shown in Fig 3.

with carbon compounds. Since only the SBF contained pH buffer [$\text{H}_2\text{NC}(\text{CH}_2\text{OH})_3$], this contamination might be the result of organic molecules contained in the pH buffer inhibiting the formation of stable coatings on the specimen.

Ban and Maruno suggested that the addition of a pH buffer greatly increases the electrochemical products.¹¹ However, since the present specimens were prepared with a larger cathodic potential than

that used in their study, the pH buffer inhibited the synthesis of HA on the specimen.

SBF⁻

Deposits were sparsely observed on the specimens with SBF⁻ in the SEM observation. Since the SBF⁻ did not contain pH buffer, a larger quantity of coating was observed on the specimens with SBF⁻ than on the specimens with SBF. Since the Ca/P ratio on the specimens with SBF⁻ was excessively high, and the peak of CaO was observed on specimens with SBF⁻, the chemical composition of the deposits was CaO.¹⁴

The findings obtained from SBF and SBF⁻ suggest that the high Ca/P chemical ratio of a typical SBF (2.5) is insufficient to form HA on a titanium surface by this method, even if the organic pH buffer is removed from the SBF. Therefore, it was not possible to prepare stable coatings on specimens with either SBF or SBF⁻.

m-SBF⁻

Uniform dense granular coatings were observed on specimens with m-SBF⁻. Since the Ca/P ratio of m-SBF⁻ was same as HBSS⁻, the Ca/P ratios of the coatings with m-SBF⁻ and HBSS⁻ were roughly equivalent, although the calcium contents of m-SBF⁻ were the same as those of SBF and SBF⁻. Nevertheless, the crystalline phases of HA were not observed on specimens with m-SBF⁻.

Since the Ca/P ratio of the coatings on the specimens with m-SBF⁻ was 1.59, the peaks attributed to OH⁻ groups were not detected although those of the PO₄³⁻ groups were, and a peak of TCP was detected in the XRD study, these coatings were

TCP. Furthermore, since the crystalline phases were not clearly detected in the XRD study, the chemical state of these coatings was of low crystallinity.

The findings obtained from SBF⁻ and m-SBF⁻ indicate that the Ca/P chemical ratio of m-SBF⁻ (1.5) is appropriate to form calcium phosphate coatings on a titanium surface using this method. However, HA was not formed, even with m-SBF⁻.

HBSS⁻

In the XPS study, since the Ca/P ratio of the coatings on the specimens with HBSS⁻ was 1.71, which was consistent with that of bone,¹⁰ the Ca/P chemical composition of HBSS⁻ was suitable. The XRD and FTIR studies also indicated that the crystalline phases of the coatings on the specimens with HBSS⁻ were similar to those of bonelike HA. In addition, the XRD and FTIR studies also demonstrated that HA coatings with high crystallinity were prepared on specimens with HBSS⁻.

These findings suggest that the chemical composition of HBSS⁻ is suitable for forming crystalline HA coatings on a titanium surface. Since HA was not identified on specimens with m-SBF⁻, it is insufficient to nucleate HA with a solution with a Ca/P ratio of only 1.5. On the other hand, because it has been assumed that preferential calcium adsorption is available to form HA on a titanium surface using electrochemical methods, typical SBF is commonly used in electrochemical depositions.

Sodium was detected only on the specimens with m-SBF⁻ and HBSS⁻ in the XPS study. Considering that HA was detected only on the specimens with HBSS⁻, a specific chemical composition ratio of Na, Ca, and P appears essential to nucleate HA coatings on the titanium surface. Wei and associates indicated that a titanium surface pretreated with sodium hydroxide aqueous solution increased biomineralization, because the released Na⁺ accelerated HA nucleation.¹⁵ Another study also demonstrated that a titanium surface pretreated with glow discharge plasma could enhance calcium phosphate deposition, because preferential sodium adsorption was increased.¹⁶ Therefore, we concluded that preferential sodium adsorption followed by phosphate and calcium ions can nucleate HA coatings on specimens with HBSS⁻.

Accordingly, since the ratio of Ca and P to Na contained in m-SBF⁻ was relatively high, TCP was coated on specimens with m-SBF⁻, although the Ca/P chemical ratio was equivalent to HBSS⁻.

CONCLUSIONS

The properties of coatings formed on titanium plates by discharging in typical and modified artificial body fluids were evaluated.

- Solutions containing organic pH buffer were unable to form stable coatings on a titanium surface in this study.
- Coating properties depend on the Ca/P ratio of the applied solution. A 1.5 ratio of Ca/P allowed the preparation of a calcium phosphate coating on a titanium surface, whereas a 2.5 Ca/P ratio was inappropriate.

TCP was prepared on a titanium surface in a solution with high ion concentrations of calcium (2.5 mmol/L) and phosphorus (1.67 mmol/L)—a Ca/P chemical ratio of 1.5.

Crystalline HA was observed on a titanium surface in a solution with low ion concentrations of calcium (1.26 mmol/L) and phosphorus (0.83 mmol/L) and a Ca/P chemical ratio of 1.5.

From this study, it may be concluded that HBSS⁻ is suitable for forming crystalline HA coatings on titanium surfaces under the conditions of the described discharging method.

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