MORPHOLOGY OF HYDROTHERMAL-ELECTROCHEMICALLY DEPOSITED APATITE AND ITS BIOACTIVITY *IN VITRO*

SEIJI BAN, SHIGEO MARUNO* and JIRO HASEGAWA

Department of Dental Material Science, School of Dentistry, Aichi-Gakuin University, Chikusa-ku, Nagoya 464-8650, Japan

*Electrical and Computer Engineering, Nagoya Institute of Technology, Showa-ku, Nagoya 466-8555, Japan

Abstract Needle-like apatite crystals were formed on pure titanium plate by a hydrothermal-electrochemical method at 100-200°C in an electrolyte containing calcium and phosphate ions. The width and length of the apatite needles increased with the electrolyte temperature. The aspect ratio of the apatite decreased with it. Pure titanium plates with the hydrothermal-electrochemically coated apatite at 100-200°C were soaked in a simulated body fluid from 3 to 27 weeks at 37°C. After soaking, the changes in weight of the specimens were measured, and the surfaces of the specimens were characterized. The specimens having fine apatite needles formed at 100°C showed the largest weight gain due to the deposition of the carbonate containing apatite having low crystallinity.

INTRODUCTION

Various coating methods for calcium phosphate on metal substrates have been developed to obtain excellent compatibility of implants with living tissues. In 1992, we reported that the calcium phosphates could be formed on the titanium by an electrochemical process.¹ The morphology and the microstructure of the deposited calcium phosphates were affected by the composition of the electrolyte,^{1,2} the electrolyte temperature,³⁻⁵ the current density,⁵ the current loading time,^{5,6} and the composition of substrate metal.⁷ Needle like apatite was deposited on the electrode by loading a relatively high current density in an electrolyte without magnesium ion at 62-92°C.^{4,5} Plate like apatite and octacalcium phosphate were formed at relatively low current density at 52-92°C in the electrolyte.⁵ Furthermore, we reported that the electrochemical deposition of needle-like apatites on the surface of implants significantly promoted the new bone formation in the early stages of implantation.⁸ Based on these results, in 1998, we developed a novel formation method for needle-like apatite above 100°C in an electrolyte under high pressure, namely hydrothermal-electrochemical coating method.⁹ The size and the crystallinity of the needle-like apatite remarkably increased with the electrolyte

temperature. It is known that the morphology of apatites strongly affect their bioactivity.¹⁰ The present study was designed to study the morphology of the apatite deposited using the hydrothermal-electrochemical method and its effect on bioactivity *in vitro*.

EXPERIMENTAL PROCEDURE

The working electrode was a pure titanium plate (20 x 20 x 0.5 mm) and the counter electrode was a platinum plate (10 x 20 x 0.1 mm). The electrolyte was prepared by dissolving given amounts of reagent-grade chemicals of 137.8 mM of NaCl, 1.67 mM of K_2HPO_4 , and 2.5 mM of $CaCl_2 \cdot 2H_2O$ in distilled water. The solutions were buffered to a pH of 7.2 with 50 mM tris(hydroxymethyl)aminomethane, $(CH_2OH)_3CNH_2$, and an adequate amount of hydrochloric acid. The electrolyte was incubated at 100-200°C using an autoclave and an electric heater. Current was maintained at 12.5 mA/cm² by a DC power supply for 1 h. After the current-loading, the titanium electrodes were rinsed with distilled water and dried at 37°C in air.

The specimens with and without electrochemical coating were immersed in a simulated body fluid (SBF) maintained at 37°C. SBF was prepared by dissolving given amounts of reagent-grade chemicals of NaCl, $NaHCO_3$, KCl, K_2HPO_4 , $MgCl_2 \cdot 6H_2O$, and $CaCl_2 \cdot 2H_2O$ into distilled water and buffered at a pH of 7.2 with 50 mM trishydroxyaminomethane and hydrochloric acid.¹ At 3, 9, 18 and 27 weeks after soaking in the SBF at 37°C, the specimens were thoroughly rinsed with distilled water. After drying at 37°C, the weights of the specimens before and after soaking were determined by an electronic balance meter. Three specimens for each group were used. The morphology of the deposits on the electrode after drying was observed by field emission type scanning electron microscopy (FE-SEM). Furthermore, the deposits were characterized by X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FTIR).

RESULTS AND DISCUSSION

Figures 1 and 2 show the FE-SEM micrographs of the surface and the section of the deposits formed at 100 and 180°C. These deposits were identified as hydroxyapatite crystal needles grown along *c*-axis, and both width and length of the needles increased with the electrolyte temperature.⁹ Most of the needle-like crystals grew up perpendicular

FIGURE 1. FE-SEM micrograph of the surface of the deposits formed at 100 and 180°C.

FIGURE 2. FE-SEM micrograph of the section of the deposits formed at 100 and 180°C.

to the substrate. Judging from the FTIR study, the crystallinity of the apatite also increased with the electrolyte temperature.⁹

Figure 3 shows c/a aspect ratio of the apatite needles deposited using the hydrothermal-electrochemical method at 100-200°C. The length of one side (a) and the longitudinal length (c) of hexagonal crystals were determined by the FE-SEM micrographs of the surface and the section of the deposits. The c/a aspect ratio of the apatite needles decreased with the electrolyte temperature, indicating wider and shorter needle with higher temperature.

Figure 4 shows total surface area of the titanium electrode coated with the apatite needles deposited using the hydrothermal-electrochemical method at 100-200°C. The total surface area of the titanium electrode was calculated as follows. At first, the surface area (A) and the volume (V) of one needle was calculated from the size (a and c) of the needles.

$$A = 6ac + \frac{3\sqrt{3}}{2}a^2 \quad \text{and} \quad V = \frac{3\sqrt{3}}{2}a^2c$$

From the weight gain (W) with the hydrothermal-electrochemical coating, the volume of

FIGURE 3. c/a aspect ratio of the apatite deposited at 100-200°C.

FIGURE 4. Total surface area of the titanium plates with the apatite deposited at 100-200°C.

the one needle, and the density of apatite, the total number (n) of the apatite needle was calculated and subsequently the total surface area (A_{total}) was derived.

$$n = \frac{W}{d \times V} \quad \text{and} \quad A_{total} = n \times A$$

The density of the needle (d) was employed as 3.156 for hydroxyapatite.¹¹

Although the size of the needles increased with the electrolyte temperature, the number of the needles decreased with it. Then, the total surface area significantly decreased with the electrolyte temperature; the surface area of the electrode with apatite needles at 200°C was about 10 times smaller than that at 100°C.

Figure 5 shows the weight gain of the titanium plates coated with the apatites formed at 100-200°C after soaking in SBF for 3-27 weeks at 37°C. The weight gain of the titanium plates with apatites deposited at 100-140°C increased with the soaking time in SBF. The weight gain decreased with the electrolyte temperature at which the needle-like apatites were formed. The specimens with apatite needles formed at 150-200°C showed little weight change.

Our previous study¹² reported that, when the titanium plates with apatite formed at 4-92°C were soaked in SBF for 27 weeks, the weight gain of the plates increased with the electrolyte temperature at which the apatite was formed. The deposits at 4°C and 37°C

FIGURE 5. Weight gain of the titanium plates with the apatite deposited using the hydrothermal-electrochemical method at 100-200°C after soaking in SBF at 37°C.

FIGURE 6. FE-SEM micrograph of the surface of the apatite needle deposits at 100 and 180°C after soaking in SBF for 27 weeks at 37°C.

were granular calcium phosphates having low crystallinity and the size of granules at 37°C was smaller than those at 4°C, whereas the deposits at 62°C and 92°C were needle-like apatites containing carbonate, elongated to *c*-axis, and the deposits at 92°C were wider width and longer than those at 62°C. From these results, it is concluded that the specimens having fine apatite needles formed at 100°C showed the largest weight gain.

Figure 6 shows FE-SEM micrograph of the surface of the deposits at 100 and 180°C after soaking in the simulated body fluid for 27 weeks at 37°C. FE-SEM observation revealed that granular particles precipitated on the edge of apatite needle formed at 100°C and the sizes of the precipitates were similar to the width of the needle.

On the contrary, there were small amount of precipitates on the edge of the needles formed at 180°C. These precipitates were identified with carbonate containing apatite having low crystallinity. It is possible that these precipitates were formed from the super-saturated calcium phosphate ions in SBF due to the dissolution of the apatite needles.

Therefore, it is concluded that the fine needle-like apatite formed at low temperature has higher bioactivity *in vitro* than those at high temperature, because the bioactivity depends on solubility and relative surface area. It implies that the fine apatite needles formed at 100°C can most effectively act as nucleation sites for biological apatite formation *in vivo*.

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