Comparison of apatite-coated titanium prepared by blast coating and flame spray methods—Evaluation using simulated body fluid and initial histological study—

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It has previously been demonstrated that apatite may be coated on the surface of titanium (Ti) at room temperature when the titanium is blasted with apatite powder. This method is known as the blast coating (BC) method. In this study, the osteoconductivity and tissue response to Ti implants blast-coated with apatite (BC implants) were evaluated using apatite-coated Ti implants produced using the flame spraying (FS) method (FS implants) and pure Ti implants as a control. Initial evaluation using simulated body fluid demonstrated higher osteoconductivity in BC implants than in FS implants. Therefore, specimens were implanted in rat tibias for 1, 3 and 6 weeks. At one week after implantation, BC implants showed much higher bone contact ratio when compared with FS implants; the bone contact ratio of BC implants was 75.7%, while the FS and pure Ti implants had ratios of 30.8% and 5.5%, respectively. The difference in bone contact ratio between BC and FS implants decreased with implantation time and the ratios were equal after 6 weeks. In conclusion, BC implants show higher osteoconductivity than FS implants, and thus BC implants are beneficial for early fixation of implants to bone tissue.

Keywords: Apatite, Titanium, Blast coating, Simulated body fluid

INTRODUCTION

Apatite [$\text{Ca}_{10-x}(\text{HPO}_4)_x(\text{PO}_4)_{6-x}(\text{OH})_{2-x}$] coatings on the surface of titanium (Ti) implants have been widely employed in order to achieve better osteoconductivity. For coating, the plasma spraying and flame spraying (FS) methods are generally used because they ensure tight adhesion between the apatite coating and the Ti surface¹. However, these methods employ very high temperatures during the coating process and can result in various problems, including (1) changes in structure², (2) formation of apatite with extremely high crystallinity³, and (3) long-term dissolution and scraping off of the coating layer⁴⁻⁸. To avoid shortcomings in the apatite coating methods using high temperatures, many alternative room temperature coating methods were studied extensively including ion beam sputtering, dipping, electrophoretic deposition and electrochemical deposition. However, none of the above methods provided a tight bonding between the apatite and Ti.

The blast coating (BC) method was therefore devised based on a paper reporting that abradant sticks to Ti when sandblasted⁹. Using this principle, fabrication of Ti with apatite powder stuck to its surface was attempted. Interestingly, it was found that the Ti surface was coated with apatite when Ti was blasted with apatite powder¹⁰. An initial study in rats demonstrated that the coating layer prepared by the BC method did not scrape off, even after the implant procedure, and that the implant had better osteoconductivity when compared with pure Ti¹¹. One of the key advantages of the BC method is that coating can be performed at room temperature. It has

been reported that apatite sintered at lower temperature shows better osteoconductivity¹². Therefore, BC may allow fabrication of apatite-coated Ti implants with higher osteoconductivity.

Basically, osteoconductivity is evaluated based on implant studies using experimental animals. Fortunately, it has been reported that initial screening can be performed using simulated body fluid (SBF) having almost the same ion concentrations as human blood plasma, as SBF can accurately reproduce *in vivo* changes on the implant surface^{13,14}. Although the detailed mechanisms of the appearance of osteoconductivity have not been clarified to date, the formation of so-called biological apatite on the surface of osteoconductive materials *in vivo* is thought to be important in the bonding of implants with bone¹³. Therefore, SBF is thought to be useful to understand the physicochemical reaction between the material surface and inorganic components in blood plasma.

In the present investigation, we hypothesized that implants prepared by the BC method at room temperature would show higher osteoconductivity than implants prepared by the flame spraying (FS) method at 3,000°C, and that more precipitates would appear on the implant with higher osteoconductivity when immersed in SBF. To evaluate this hypothesis, the decreases in Ca^{2+} and HPO_4^{2-} concentrations in SBF were preliminarily measured. Furthermore, in an *in vivo* study, bone contact ratio was used as an index of osteoconductivity to compare apatite-coated titanium prepared by the BC and FS methods in experimental animals in order to confirm the results obtained in the *in vitro* study.

MATERIALS AND METHODS

Characterization of apatite powder and coated apatite

For characterization, X-ray diffraction (XRD) and Fourier transformed infrared spectroscopic (FT-IR) analyses were performed. For XRD analysis, patterns were recorded with a vertically mounted diffractometer (Rigaku Denki Co., Tokyo, Japan) using counter-monochromated CuK α radiation generated at 30 kV and 10 mA. For FT-IR analysis, the KBr method was employed with an FT-IR spectrometer (Spectrum 2000LX; Perkin-Elmer Co. Ltd., Kanagawa, Japan).

For elemental analysis of apatite powder, carbon content was measured using a CHN coder (Yanako, CHN coder MT-5, Kyoto, Japan).

Preparation of BC, FS discs and implants

Pure Ti discs of grade 4 (10 mm diameter $\times$ 2 mm height) kindly donated by the manufacturer (Kyocera, Kyoto, Japan) were washed in acetone for 10 min. The discs were then mounted in a holder that allowed a defined distance of 2 mm between the titanium surface and the sandblaster nozzle, and were blasted with apatite powder (HAP-BN; Central Glass, Tokyo, Japan) for 10 s using a sandblaster (Hi-blaster SHB-20, Shofu, Kyoto, Japan) at a compressive pressure of 0.54 MPa. Apatite coating was performed on only one side of the disc. After coating, discs were cleaned ultrasonically in distilled water in order to remove adsorbed apatite powder. It was confirmed that the titanium surface was coated completely and homogeneously by SEM observation¹⁰. FS discs were prepared at 3,000°C by the manufacturer (Kyocera). The thickness of coating layer of FS disc was 20–30 μ m. BC and FS implants with a diameter of 2 mm and a length of 5 mm for histological study were prepared in the same manner. Surface roughness (Ra) (Surfcorder SE 1700, Kosaka Laboratory Ltd., Tokyo, Japan) of the fabricated Ti, FS and BC implants was 1.67 $\pm$ 0.08 μ m, 3.91 $\pm$ 0.13 μ m and 1.30 $\pm$ 0.12 μ m, respectively (measurement length of 2.0 mm).

Screening test using SBF

SBF having an electrolyte composition similar to human plasma was prepared from reagent grade NaCl, NaHCO₃, KCl, K₂HPO₄ $\cdot$ 3H₂O, MgCl₂ $\cdot$ 6H₂O and CaCl₂ (prepared from CaCO₃ and HCl), as described previously¹³. The respective ion concentrations (mmol/L) of blood plasma and SBF are as follows: Na⁺ (142.0 and 132.0); K⁺ (5.0 and 5.0); Ca²⁺ (2.5 and 2.5); HCO₃⁻ (4.2 and 27.0); and HPO₄²⁻ (1.0 and 1.0). SBF was buffered at pH 7.4 at 37°C with 50 mmol/L HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pK_a=7.5) and NaOH. Five discs of each type were immersed in 200 mL of SBF and left to stand at 37°C for 14 days.

After immersion, five discs were removed from SBF, rinsed in distilled water and dried at 50°C for 24 h. Specimens were sputter-coated with gold for morphological observation by scanning electron microscopy (SEM; DS-720, Topcon Co., Tokyo, Japan).

For quantitative analysis, aliquots of SBF were withdrawn at regular intervals, filtered through a 0.20 μ m filter and analyzed for Ca²⁺ and HPO₄²⁻. Ca²⁺ concentration was measured using an atomic absorption spectrophotometer (Z-8000, Hitachi, Ltd., Tokyo, Japan). Ca²⁺ levels were obtained by means of a standard curve constructed with 3, 6, 10 ppm. HPO₄²⁻ concentration was measured using a UV spectrophotometer (DU640, Beckman, USA) at wavelength 890 nm. Data were measured in three independent groups and averaged values were used.

The apatite layers formed on the surface of Ti by the BC and FS methods, as well as the precipitates formed after immersion in SBF, were analyzed using FT-IR spectroscopy. The coating layer and precipitates were scraped off with a blade and subjected to FT-IR spectroscopy using the KBr method.

Histological examination and bone contact ratio

This experiment was performed according to the Guidelines for the Treatment of Experimental Animals at Okayama University Dental School. Seven-week-old male Wistar rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal®; Abbott Co., Chicago, IL) at a dose of 50 mg/kg body weight. After their legs were shaved, an incision was made under sterile conditions and the medial end of the tibia was exposed. A 2.0-mm-diameter hole was made in the tibia using a 2.0-mm twist drill under cooling with normal saline solution, and a pure Ti implant, FS implant or BC implant was inserted into the hole with finger pressure. The surgical wound was closed with 4-0 silk sutures. Four animals were used for each implantation period and for each implant.

Experimental rats were sacrificed at 1, 3 and 6 weeks after surgery and were perfused with a solution of 10% neutral buffered formaldehyde. Implants were excised with surrounding bone. All specimens were dehydrated in a graded alcohol series and were embedded in polyester resin (Rigolac®; Nissin EM Co., Tokyo, Japan). Each undecalcified block was cut along the long axis of the implant into two sections on a slicer using a diamond blade (Exakt BS-3000, Meiwa, Osaka, Japan). Sections were ground to a thickness of 50 μ m, and were stained with toluidine blue for histological examination. The specimens were observed under a light microscope (Nikon M-35FA, Tokyo, Japan). Bone contact ratio was measured as an index of osteoconductivity. Briefly, the percentage of surface area that directly contacted with bone was measured using histological pictures. The contact ratio was measured from four specimens and average values were used.

Statistical analysis was performed with JMP 6.02® software (SAS Institute, Inc., Cary, NC.). Repeated measures ANOVA was used for the bone contact ratio and Ca²⁺ and HPO₄²⁻ concentrations. *P* values of <0.05 were considered to be significant.

RESULTS

Compositional analysis of apatite powder and coated apatite

XRD analysis revealed that apatite powder was pure apatite containing no other phases. By CHN analysis, the values for CO and HO were 0.79 and 0.42, respectively. This demonstrates that apatite powder contains carbon. FT-IR analysis revealed that original apatite powder contained carbonate. No changes were observed after

BC with respect to carbonate content. In contrast, no carbonate was observed after FS. The Ca/P molar ratios of apatite powder before and after BC measured with energy dispersive X-ray spectroscopy were 1.6 ± 0.3 and 1.6 ± 0.2 , respectively. This indicates that there was no compositional change during BC.

Screening test using SBF

In order to understand the ionic behavior near the implant surface and to minimize the number animal

Fig. 1 Scanning electron micrograph of disc surfaces before and after immersion in SBF for 14 days. (a) Ti (before); (b) Ti (after); (c) FS (before); (d) FS (after); (e) BC (before); and (f) BC (after).

studies, a screening test was performed using SBF. FS discs (Fig. 1c) and BC discs (Fig. 1e) were covered with apatite, and were compared with pure Ti (Fig. 1a). FS discs showed pancake-like particles or flattened spherical droplets along with the microcracks (arrows) typical of FS coating. On the other hand, BC discs showed no microcracks and grain size of the coating was much smaller than that of FS discs. In this study, an apatite coating was confirmed, in contrast to apatite particles independently sticking to the Ti surface. No morphological changes were observed, even when pure Ti discs were immersed in SBF for 14 days (Fig. 1b). In contrast, FS and BC discs were covered with precipitate when immersed in SBF for 14 days (Fig. 1d, 1f). The microcracks shown in Fig. 1d, 1f were due to the drying after immersion in SBF. Higher magnification observation revealed that the precipitate was composed of needle-like fine crystals typical of so-called bone-like apatite (data not shown). No differences were observed between FS and BC discs based on SEM observation.

Figure 2 shows the FT-IR spectra of the coating layers (a, c) and the precipitates (b, d) formed after immersion in SBF. The FT-IR spectrum of the coating layer of BC discs (Fig. 2c) showed peaks corresponding to $\gamma_3\text{CO}_3^{2-}$ at 1417 cm^{-1} and $\gamma_2\text{CO}_3^{2-}$ at 873 cm^{-1} . This indicates that the coating layer of BC was a B-type carbonate apatite, in which carbonate ions are located at PO_4^{3-} sites; this is the apatite form found in bone¹⁵. In contrast, the apatite found in FS discs (Fig. 2a) was found to be CO_3 -free apatite. The precipitates formed on the surface of FS and BC discs after immersion in SBF were also found to be B-type carbonate apatite.

Figure 3 shows the changes in Ca^{2+} and HPO_4^{2-} concentrations in SBF. No Ca^{2+} and HPO_4^{2-} concentration changes were observed with pure Ti discs. On the other hand, Ca^{2+} and HPO_4^{2-} concentrations gradually

decreased with immersion time in the case of FS and BC discs. Although significant differences were not observed, decreases in Ca^{2+} and HPO_4^{2-} concentration were more rapid in BC discs when compared with FS discs.

Fig. 2 FT-IR spectrum of the coating layer and precipitate removed from disc surfaces. (a) FS disc (before immersion); (b) FS disc (after immersion); (c) BC disc (before immersion); and (d) BC disc (after immersion).

Fig. 3 Changes in Ca^{2+} and HPO_4^{2-} concentrations (mmol/L) over time in 200 mL of SBF.

Histological examination and bone contact ratio

No inflammatory responses were observed for any of the implants in rat tibias. One week after implantation, new bone formation was observed along the surfaces of all implants. However, new bone did not come into contact with the surface of the pure Ti implant (Fig. 4a). In contrast, new bone directly contacted the surface of the FS and BC implants (Fig. 4b, c). Three weeks after implantation, new bone formation increased along the

surface of all types of implant (Fig. 5). Bone contact ratio was summarized as a function of implantation period (Fig. 6). As shown in Fig. 6, bone contact ratio increased with implantation period, regardless of implant type. However, the rate of increase in bone contact ratio differed with implant type. The slowest bone contact ratio increase was observed for pure Ti; bone contact ratio was approximately 30%, even at 6 weeks. Both apatite-coated implant groups showed higher bone

Fig. 4 Histological photographs at 1 week after implantation. (a) Ti implant; (b) FS implant; (c) BC implant.

Fig. 5 Histological photographs at 3 weeks after implantation. (a) Ti implant; (b) FS implant; (c) BC implant.

Fig. 6 Bone contact ratios of Ti, FS and BC implants.

contact ratios. At 6 weeks, bone contact ratio in both apatite-coated groups was approximately 90%. Although the bone contact ratios of BC and FS implants were the same at 6 weeks, it should be noted that BC implants had a larger bone contact ratio than FS implants at 1 week and 3 weeks after surgery. A significant difference ($p=0.0006$) was observed among the three groups.

DISCUSSION

FTIR analysis revealed that apatite powder contains carbonate before BC and that carbonate ions are present in the coating layer of BC discs before immersion in SBF. The coating layer prepared by the flame spraying and plasma spraying methods do not contain carbonate ions, as these methods employ high temperatures during the coating process. On the other hand, the coating layer prepared by BC method at room temperature ensures the presence of carbonate ions. Therefore, BC method is able to produce apatite coatings similar to those of human bone.

The bioactive material forms a layer of carbonate hydroxyapatite on the surface *in vivo*, and this material becomes connected with bone throughout the layer. Apatite formed on the surface is very similar to bone apatite in both structure and composition. As a result, new bone is formed when osteoblasts actively proliferate and differentiate in the apatite surface. In this way, the implanted material is connected to the surrounding bone. SBF has almost the same ion concentrations as human plasma and is able to reproduce observed *in vivo* surface changes. Therefore, SBF is used to examine the bioactivity of materials *in vitro*. Gu *et al.* reported that the surface of the hydroxyapatite coating was covered with a newly formed layer consisting of small granular structures after 2 weeks of immersion in SBF¹⁶⁾. Other

authors have reported similar findings for pure HA coatings¹⁷⁾. In this study, a similar precipitate was observed when apatite coated titanium discs were immersed in SBF. FTIR analysis revealed that the precipitate was apatite containing carbonic acid groups. Carbonate ions can be located in both the hydroxyl groups and phosphoric groups of hydroxyapatite. It is known that apatite in the human bone is a B-type carbonate hydroxyapatite¹⁵⁾. On FTIR analysis, the CO_3^{2-} absorption bands revealed that the carbonate ions were located in PO_4^{3-} lattice sites, indicating a B-type carbonate hydroxyapatite structure.

In the *in vitro* study using SBF, decreases in Ca^{2+} and HPO_4^{2-} concentration were more rapid in BC discs when compared with FS discs, while in the *in vivo* study, BC implants had a larger bone contact ratio than FS implants from the initial stages. These results suggested that BC implants have better osteoconductivity than FS implants. When pure Ti was immersed in SBF, no precipitate was noted on the surface, and the Ca^{2+} and HPO_4^{2-} concentrations of SBF did not change. The precipitate, however, may appear on Ti when immersed in SBF for longer periods; Ti is an osteoconductive material.

Although the detailed mechanisms were not clarified in this initial comparative study, it is apparent that the nature of the coating led to differences in osteoconductivity. The key difference between the BC and FS methods may be the temperature during coating. Although the FS method employs a lower temperature than plasma spray coating, high temperatures remain indispensable for FS¹⁸⁾. Therefore, changes in structure, formation of extremely high crystallinity, long-term dissolution and scraping off of the coating layer are unavoidable. High-temperature coating also results in residual stress at the interface between Ti and the coating layer due to the different thermal coefficients between Ti and apatite¹⁹⁾. The FS method results in amorphous Ca phosphate regions forming as a result of the very rapid cooling rates that occur assuming apatite powder melting during the FS operation. Microcracks observed in FS implants (Fig. 1c) are also thought to be the result of residual stress.

It should be noted that the apatite found in bone is not stoichiometric hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] but carbonate apatite [$\text{Ca}_{10-x}(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{OH})_{2-x}$], which contains 6–8 wt% carbonate. Unfortunately, carbonate apatite is lost at high temperatures, and decomposes even at the temperatures used for sintering, 800–1,200°C. Thus, carbonate apatite decomposes at the temperature used for FS. To prevent thermal decomposition, stoichiometric hydroxyapatite is used as a spray powder, as stoichiometric hydroxyapatite is relatively stable at high temperatures. However, high temperatures remain a problem for stoichiometric hydroxyapatite. For example, Karashima *et al.*¹²⁾ reported that the osteoconductivity of hydroxyapatite decreases with increasing sintering temperature. On compositional analysis, there were no compositional changes during BC. Therefore, the BC method, which

can coat Ti with apatite at room temperature, may be an ideal coating method with respect to degree of osteoconductivity.

Osteoconductivity is enhanced by implant surface roughness. The BC implant had the smallest surface roughness among these three implant types, but the bone contact ratios in BC implants at 1 and 3 weeks were higher. It is apparent that the higher osteoconductivity of BC implants is beneficial for patients. In the present study, bone contact ratio was the same at 6 weeks, and there was no clear evidence with respect to osteoclastic resorption of the coating layer. In contrast to stoichiometric hydroxyapatite, carbonate apatite is known to be resorbed by osteoclasts. Further evaluation including longer implantation periods and *in vitro* evaluation using osteoblasts and osteoclasts are thus necessary based on this initial evaluation of BC implants.

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