Characterizations of Bone-Like Apatite Powder Fabricated Using Modified Simulated Body Fluid

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The objective of this study is to fabricate bone-like apatite (BLAp) powder using the modified simulated body fluid (SBF). The SBF2X and SBF4X groups were prepared by increasing the concentration of inorganic ions by two and four times, respectively, to that of the standard SBF. The mSBF4X group was prepared by particularly increasing the concentrations of calcium and phosphate ions in SBF. Bovine serum albumin (BSA) was added for SBF2X-BSA, SBF4X-BSA, and mSBF4X-BSA groups. Bovine serum albumin (BSA) was added for SBF2X-BSA, SBF4X-BSA, and mSBF4X-BSA groups. BLAp powders were precipitated in these SBFs while being kept at 60°C. Micro-morphology of BLAp powders showed tens of micrometers-sized rounded clusters which composed with sheet-like nano crystallites. The radius of BLAp clusters were decreased by increasing the concentration of inorganic ions and by incorporating the BSA. The hydroxyapatite crystalline structure was dominant for all sample groups. Further, octacalcium phosphate structure was detected in the mSBF4X group. However, these peaks were decreased in mSBF4X-BSA. FT-IR spectra demonstrated that BSA was co-precipitated in BLAp crystallites, and the amount of BSA was higher in the mSBF4X-BSA group than in the SBF4X-BSA group.

Keywords: Bone-Like Apatite, Simulated Body Fluid, Hydroxyapatite, Co-Precipitation.

1. INTRODUCTION

Hydroxyapatite (HAp; Ca10(PO4)6(OH)2), a major component of natural bone, has been used as a coating material on implant surface and as well as bone substitutes.1,2 In order to enhance bone regeneration, various methods have been developed to directly deliver the biological bone growth factors to the biomaterial-implanted region.3,4 However, it is difficult to incorporate biological factors into HAp that is synthesized chemically in different physiological environments and thermally treated at high temperature.

Furthermore, the biological factors only adsorbed on the surface of biomaterials were released rapidly, making it inefficient to achieve the biological effects.5 Bone-like apatite (BLAp) which has similar characteristics of natural bone can be coated by soaking in simulated body fluid (SBF) which has physiological environment.6 Furthermore, organic materials such as bone growth factors can be incorporated into BLAp by mixing the biological factors with SBF.7 This BLAp film coated on biomaterials could provide slow release of biological factors in the body fluid because organic materials chemically bound to the three-dimensional crystal lattice of the BLAp could be released by the dissolution of BLAp.8 In this study, we fabricated BLAp powder having similar characteristics of natural bone using the modified SBF. Bovine serum albumin (BSA) was used as a model protein incorporated into the BLAp powder. After changing the concentrations of inorganic ions in SBF, effects on the characteristics of BLAp powders were investigated.

2. EXPERIMENTAL DETAILS

Modified SBFs which had a higher concentration of inorganic ions compared with that of standard SBF (SBF1X)6 were used in order to fabricate the precipitated BLAp powders. SBF2X and SBF4X were prepared by increasing the concentrations of inorganic ions by two and four times, respectively, to those of SBF1X. The mSBF4X was prepared by increasing the concentrations of CaCl2 and KH2PO4 by four times to those of SBF1X. Bovine serum albumin (BioShop, lot no. 9D11406, Canada) was added to SBF2X, SBF4X, and mSBF4X in order to fabricate the
BSA incorporated BLAp powders. The BSA concentration was 1 mg/mL.

SBF2X and SBF2X-BSA were kept in an electric furnace at 60 °C for 3 days and other SBFs were kept for 1 day. Precipitated powders were filtered and dried at room temperature. Six BLAp powders, namely, SBF2X, SBF4X, mSBF4X, SBF2X-BSA, SBF4X-BSA, and mSBF4X-BSA, were fabricated in this study.

All groups were weighed using an electric balance. The micro-morphology of the powders was observed using scanning electron microscopy (SEM; Hitachi S-4700, Japan). The incorporation of BSA into BLAp was examined by FT-IR spectroscopy (Spectrum 400, PerkinElmer, UK) with an attenuated total reflectance (ATR) accessory. The crystalline structure was analyzed using an X-ray diffractometer (XRD; PANalytical, X’Pert PRO, Netherlands) with Cu Kα radiation. The morphology and structure of the nano-crystallites for mSBF4X and the mSBF4X-BSA groups were examined by high resolution transmission electron microscopy (HRTEM; Techni F20, Philips, Netherlands) operated at 200 kV. The TEM specimens were prepared by dispersing powders in water and drying on a carbon-grid.

3. RESULTS AND DISCUSSION

The amount of precipitated powders was measured as 97, 461, 199, 110, 456 and 414 mg for the SBF2X, SBF4X, mSBF4X, SBF2X-BSA, SBF4X-BSA and mSBF4X-BSA groups, respectively. The higher the concentration of inorganic ions in SBF, the higher the amount of BLAp powders was produced.

Figure 1 presented the SEM images of fabricated BLAp powders using the modified SBFs. The SBF2X group that was kept for 3 days showed clusters sized approximately 100 μm in diameter, while the SBF4X group that was kept for 1 day demonstrated round clusters under 10 μm in size. The clusters were composed with sheet-like nanocrystallites, as shown in the magnified image of Figure 1.

In the synthesis of colloidal nanocrystals, the critical size of crystalline nuclei is smaller in high

![Figure 1](image-url)
monomer concentration compared with low monomer concentration. The nanocrystals grow more rapidly and show narrower distribution of size in high concentration. Similarly, in this study, it was inferred that the number of crystalline nuclei formed during the initial growing stage was higher in the SBF4X group than in the SBF2X group because the critical size of crystal nucleation would be smaller in higher ion concentration conditions. Therefore, SBF4X group produced smaller and more homogeneously sized BLAp clusters with greater amount of powder compared to SBF2X group.

The clusters in the SBF2X-BSA group incorporated with BSA were smaller than those in the SBF2X group. The SBF4X-BSA group clearly indicated that the size of clusters decreased by incorporating BSA. The flake-like crystallites in the mSBF4X group indicated that the growing mechanism of BLAp crystallites was different to that in SBF4X group. However, the morphology of the mSBF4X-BSA group was similar to that of the SBF4X group. Also, the amount of mSBF4X-BSA powder was much higher than that of the mSBF4X group. Therefore, it was inferred that adding BSA to SBF affected the formation of BLAp crystallites in the mSBF4X-BSA group to increase.

Several studies have reported that BSA inhibits BLAp coating on materials and decreases its crystallinity. However, the amount of powder for the mSBF4X-BSA group was two times more than that for the mSBF4X group. According to this result, BSA could play a role as an accelerator in forming BLAp powders in the mSBF4X group.

A typical HAp crystalline structure was dominant in the SBF2X, SBF4X, SBF2X-BSA and SBF4X-BSA groups, as shown in Figure 2. Moreover, a weak XRD peak of sodium chloride was observed in all groups. The intensity of (211) the HAp peak observed in the SBF2X and SBF4X groups decreased by incorporating BSA. According to the result, adding BSA to SBF affected the growing direction of BLAp crystallites in SBF. Song et al. reported that the incorporation of BSA into BLAp induced BLAp crystallites to grow in the c-axis direction when BLAp film was coated onto the titanium surface.

The octacalcium phosphate (OCP; Ca₈(HPO₄)₂(PO₄)₄) crystalline structure was observed with the HAp structure in the mSBF4X group. OCP crystallites are one of the HAp precursors and can be formed in the high concentration of Ca ions. This structure resulted in different SEM images of the mSBF4X group compared to those of other groups, as shown in Figure 1. However, the OCP structure was decreased and the HAp structure was dominant for the mSBF4X-BSA group. This result confirmed that BSA played a role as an accelerator during the growth of HAp crystallites in mSBF4X. The BSA, having an isoelectric...
point (pI) of 4.7, is negatively charged in SBF. Therefore, it can bind to Ca\(^{2+}\) ions easily, thereby helping the HAp nuclei at the initial growing stage.

TEM images (Fig. 3) showed that plate-like crystallites of over 100 nm wide were observed in the mSBF4X group, while plate-like crystallites which have tens nanometer in width and several nanometers in thickness were observed in the mSBF4X-BSA group. Selected area electron diffraction patterns (SAED) portrayed a ring shape of the HAp structure, indicating that apatite crystallites had low crystallinity.

Figure 4 displayed the FT-IR spectra of the SBF4X, SBF4X-BSA, mSBF4X and mSBF4X-BSA groups. Two sharp peaks of 554 and 600 cm\(^{-1}\) and a broad peak of 1020 cm\(^{-1}\) were attributed to the phosphate group (PO\(_4\)) of the hydroxyapatite structure. The peaks of 525, 628 and 908 cm\(^{-1}\), as shown in the mSBF4X group, were attributed to the HPO\(_4\) band originated from the OCP structure. The peaks of 1404 and 1450 cm\(^{-1}\) were characteristic peaks of carbonate (CO\(_3\)). Finally, the two broad peaks of 1530 and 1640 cm\(^{-1}\) were attributed to the amide group of BSA.

The results of FT-IR revealed that BLAp powders of SBF4X, SBF4X-BSA and mSBF4X-BSA groups had carbonate apatite similar to natural bone. The OCP structure was observed in the mSBF4X group, which agreed well with the XRD patterns in Figure 2. The BSAs were co-precipitated in the SBF4X-BSA and mSBF4X-BSA groups. The relative peak intensity of the amide and phosphate groups in the mSBF4X-BSA group was higher than that in the SBF4X-BSA group. Hence, it was inferred that the amount of BSA incorporated in BLAp was higher in mSBF4X-BSA than in SBF4X-BSA.

4. CONCLUSION
The BLAp powders composed of variously sized HAp clusters and incorporating organic material (BSA) were fabricated by changing the concentration of inorganic ions in SBF. The size of BLAp clusters decreased as the concentration of inorganic ions in SBF increased. The OCP structure was observed in the mSBF4X group. However, HAp structure was dominant and the amount of powder increased by adding BSA to mSBF4X. In conclusion, BSAs played a role in growing the nuclei of BLAp crystallite and they were efficiently co-precipitated in mSBF4X.

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References and Notes

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