Biomineralization Approach as Osteoconductive Materials for Orthopaedic and Dental Application

Junji Watanabe[1,2] and Mitsuru Akashi[1,2]
[1] Department of Applied Chemistry, Graduate School of Engineering, and
[2] 21st COE "Center for Integrated Cell and Tissue Regulation", Osaka University, Osaka 565-0871, Japan

Introduction: Hydroxyapatite (HAp) and calcium carbonate ($CaCO_3$) are major biomineral compounds. In particular, HAp crystals contribute mechanical properties in our human bone, and also $CaCO_3$ is a key material such as forming a coral and shells in the sea. In orthopaedic and dental application, biomineralization approach is good candidate to form fine implants not only modification of their interfaces but also preparation of biomineral composites. Originally, Taguchi et al. discovered a pioneering biomineralization study based on an alternate soaking process in 1998 [1]. Moreover, we have found an electrophoresis process for biomineralization [2]. In this paper, our recent investigation regarding innovative biomineralization is summarized and improved. In particular, we focused on a woven-mesh and a hydrogel for HAp formation.

Methods: In the case of typical alternate soaking process, a specimen was alternately soaked in $CaCl_2$/Tris-HCl solution (200mmol/L, pH 7.4) and Na_2HPO_4 solution (120mmol/L) [1]. In this study, a small amount of ethanol was added to their solutions to improve the alternate soaking process. As a substrate, a woven-mesh, which was made of polyester, was used for HAp formation. The woven-mesh was alternately soaked in each solution, which contained ethanol at 0, 10, and 25v/v%. The soaking time was 30 seconds in each solution, and typically repeated for 10 cycles. On the other hand, an agarose hydrogel was used for HAp formation by electrophoresis process. The agarose gel was swelling by Tris-HCl solution (10mmol/L). Each ionic solution was poured into the cathode and anode sides, respectively, as previously reported method [2]. Then, the electrophoresis was carried out for 30 minutes at 100 V. For the characterization, scanning electron microscopy (SEM) was used to observe HAp particles. X-ray diffraction (XRD) patterns were also monitored to estimate crystalline structure.

Results/Discussion: In this study, the alternate soaking process was a little bit improved. Surface wettability of the woven-mesh was so poor that the HAp particles could not be effectively formed. Because surface tension of each ionic aqueous solution was so high, the surface tension was regulated by addition of ethanol, which mixing ratio was 0-25 v/v%. Figure 1 shows SEM observation of the woven-mesh. In the case of bare woven-mesh, porous structure was observed. If the aqueous solutions were used for alternate soaking process, few HAp particles were formed on the mesh (Figure 1 (b)). On the other hand, a large amount of HAp particles were prepared by using ethanol-mixed solutions (Figure 1 (c) and (d)). Moreover, the HAp was formed not only their surface but also inside the woven-mesh. This result indicated that surface wettability was dominant factor for HAp formation, particularly alternate soaking process.

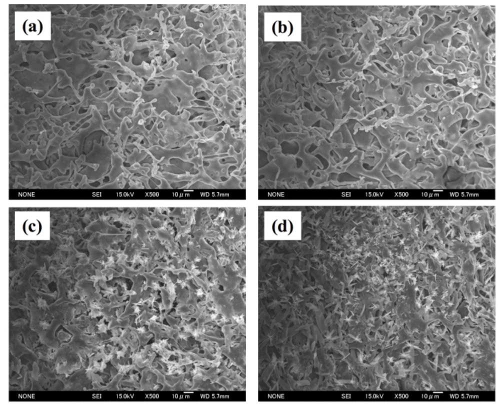

Figure 1 Biomineralization of woven-mesh by improved alternate soaking approach (10cycles); (a) bare woven-mesh, (b) 0v/v% ethanol, (c) 10v/v% ethanol, and (d) 25v/v% ethanol. Scale bars indicate 10μm.

In the electrophoresis process, the formation of HAp was dependent on the concentration of calcium and phosphate ions, summarized in Figure 2. The formed HAp in the agarose gel was dissolved using hydrochloric acid, and the soluble calcium ions were evaluated using a calcium detection kit. In the case of 10 mmol/L, the total amount of calcium was roughly 90ng per 1mg of HAp-formed agarose hydrogel when the equilibrium swelling state was reached. If the concentration was over 20 mmol/L, the calcium content was almost the same at 130ng/mg-wetgel. This result indicates that the HAp formation was dependent on the concentration of each solution because the formed HAp showed resistance in the electrophoresis circuit. From the XRD results, the patterns attributed to HAp was observed. Furthermore, we could apply the improved processes to not only HAp formation but also $CaCO_3$ crystal formation.

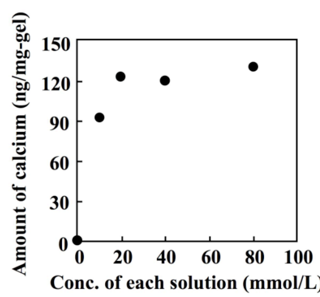

Figure 2 Change in calcium content in agarose gel. The amount of calcium was normalized with 1 mg of wet gel.

Conclusions: In this study, we developed a novel preparative method for HAp formation. The resulting HAp composite has an excellent osteoconductivity and is capable of being scaffold for orthopaedic and dental implants.

Acknowledgement: Part of this study was financially supported by a Grant-in-Aid for 21st Century COE from MEXT of Japan.

References: [1] Taguchi T, Kishida A, and Akashi M. Chem Lett. 1998;27:711-712. [2] Watanabe J, and Akashi M. Biomacromolecules 2006;7:in press.