# Hydroxyapatite Formation on/in Poly(vinyl alcohol) Hydrogel Matrices Using a Novel Alternate Soaking Process

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A novel method of hydroxyapatite (HAp) formation on/in a three-dimensional hydrogel matrix was developed. This method is based on the widely-known wet synthesis of HAp and is alternate soaking process in $CaCl_2$/Tris-HCl (pH 7.4) and $Na_2HPO_4$ aqueous solutions. Using a poly(vinyl alcohol) (PVA) gel as a model matrix, PVA-HAp composites could be prepared and characterized.

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Tissue engineering is the technology of the remodeling of living organisms in vitro and involves the architecture of artificial cellular scaffolds which mimics extracellular matrix. Hard tissue, such as bone and teeth are hybrid materials which are composed of inorganic hydroxyapatie (HAp) within collagen (type I) matrix. For hard tissue engineering, it is necessary to create polymer-HAp composites in order to substitute large bone defects. Some researchers reported polymer-HAp composites that were prepared by mixing,[1,2] and coating method[3,4] for use in biomedical field. However, polymer-HAp composites like bone structure and functions have not been obtained yet. In order to develop a novel material that mimics bone structure, we have studied the preparation and characterization of the polymeric hydrogel-HAp composites.[5,6] In the hydrogel-HAp system, HAp crystal and polymer chains are entangled with each other and their mechanical properties as well as the biological activities of the hydrogel-HAp composites are unique. In a previous study, we used the biomimetic process, which was developed by Kokubo et al.[7] for HAp formation on/in hydrogels. The biomimetic process can effectively prepare HAp composites on the surface of various kinds of materials; however, it takes a long period of time to form a large amount of HAp on/in hydrogels using this process.

In order to overcome these obstacles, a novel HAp formation process on/in hydrogels was developed. This process was based on the widely-known wet process of HAp preparation[8] in which it is done by alternate soaking in two kinds of solutions. In this study, using poly(vinyl alcohol) (PVA) gel as a model matrix, a novel process was introduced and studies on characterization of PVA gel-HAp composites were done.

Poly(vinyl alcohol) (Wako, an average degree of polymerization of 2000 and a saponification degree of 99.5 mol%) gel (crosslinker content; 1 mol% glutaraldehyde for PVA monomer unit) was prepared following procedures that had been described.[9] Calcium phosphate formation in PVA gels was performed as follows (Figure 1): Step 1: a disk shape PVA gel (1 cm in diameter and 1 mm in thickness, swelling ratio 10.4) was soaked in 10 ml of the $CaCl_2$ (Nacalai) (200 mM) / Tris (Sigma)-HCl (pH 7.4) (Ca solution) at 37 °C for 2 h. When the PVA gel was removed from the solution, the excess moisture on the surface of the gel was removed by blotting on filter paper. Step 2: The gel was subsequently soaked in the $Na_2HPO_4$ (Wako) (120 mM) aqueous solution (P solution) at 37 °C for 2 h. The formation of calcium phosphate on/in the PVA gel was proceeded by soaking it alternately in these solutions. The calcium phosphate which was formed on/in PVA gels was analyzed by X-ray diffraction (XRD) with a Geigerflex 2013 (Rigaku Co., Tokyo, Japan) at the scanning angles ranging from 50° to 10° at 2°/min.

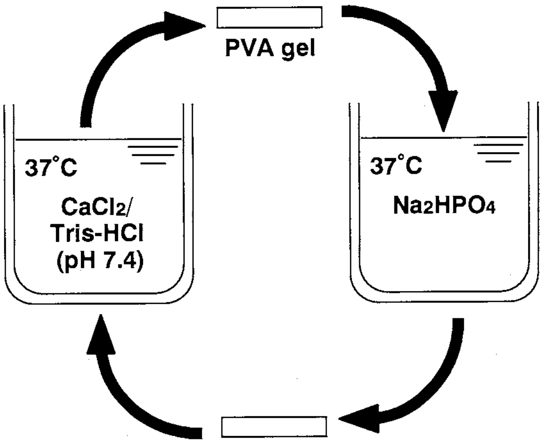

**Figure 1.** Experimental procedures of an alternate soaking process used in this study.

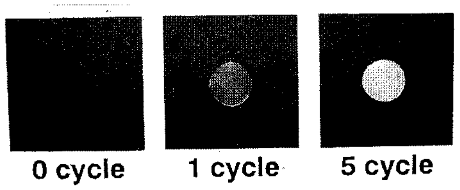

**Figure 2.** Photographs of PVA gels after various reaction cycles.

Figure 2 shows a photograph of the PVA gels before and after 1 or 5 reaction cycles. It is clear that the PVA gel became opaque with an increase in the reaction cycles. A slight decrease in the diameter of the gel was observed after 5 reaction cycles.

After each cycle, the PVA gels were freeze-dried and the calcium phosphate that had formed on/in the PVA gels was characterized by X-ray diffraction. The results are shown in Figure 3. Broad peaks were found around 31.8, 32.2, 32.9, and 25.9° and were assigned to the main peaks of the hydroxyapatite (HAp) crystals.[10] The intensity of the peaks increased with an increase in the reaction cycles. HAp is

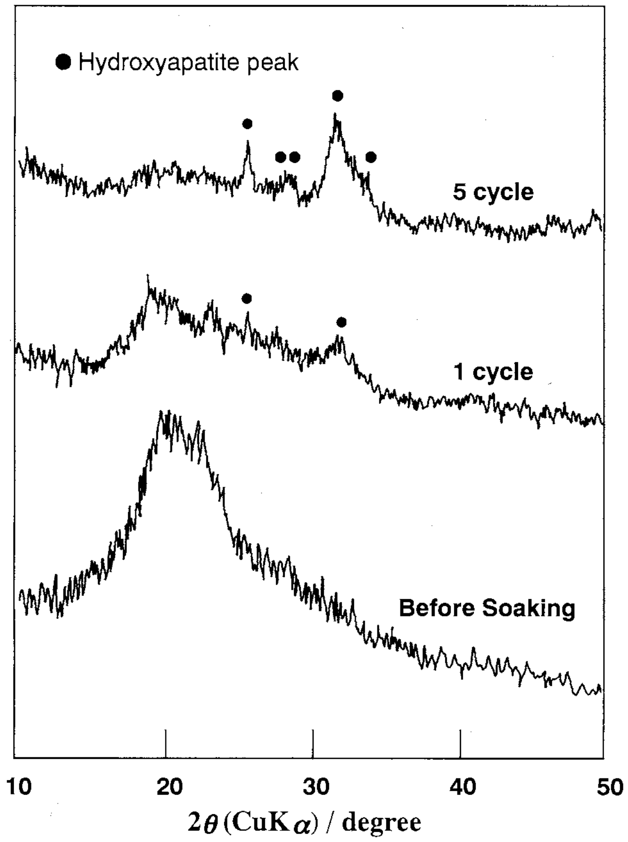

**Figure 3.** X-ray diffraction patterns of PVA gels after variou reaction cycles.

thermodynamically the most stable calcium phosphate in a neutral or basic environment;[10] thus, most of the calcium phosphates which were formed on/in PVA gels were to be HAp. It can be also seen from Figure 3 that the crystallinity of the HAp which had formed on/in the PVA gels was relatively low. The crystallinity of the HAp that was prepared by the wet process can be controlled by the reaction temperature;[11] thus, PVA-HAp composites with various crystallinities of HAp can be obtained by changing the temperature of the Ca and P solutions.

In conclusion, the HAp crystals could be formed on/in a hydrogel by a novel alternate soaking process using a PVA gel as a model matrix for a fairly short amount of time (5 cycles correspond to 20 h). This process can also be used for many kinds of synthetic or natural polymer hydrogels such as poly(acryl amide), poly(ethylene glycol), poly(γ-glutamic acid), agarose, and collagen. These hydrogel-HAp composites are expected to be useful not only as bone-substitute materials but also as soft-tissue adhesive materials. Further research on preparation and functions of hydrogel-HAp composites using this novel alternate soaking method is now in progress.

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