A Tunable Hydroxyapatite Formation in Agarose Hydrogel as Biomineral Functions

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Introduction: Biominerals such as hydroxyapatite (HAp) and calcium carbonate are common in nature; for example, vertebrate animals have bones and teeth, and coral reefs and shells consist of mineral calcification. Ideal bone substitutes for human bone are required to have excellent osteoconductivity and osteoinductivity, and to completely replace newly generated bone. Kokubo reported pioneer work regarding HAp formation onto biomaterials using simulated body fluid (SBF) [1]. As an alternative approach, Akashi *et al*. discovered a novel alternate soaking process to prepare the HAp composite on/in agarose hydrogel, using calcium chloride and disodium hydrogenphosphate aqueous solutions [2]. The HAp-agarose composite, which was prepared by alternate soaking process, showed excellent osteoconduction as fillers for tooth-extraction sockets in the jaws of *Macasa fascicularis* monkeys [3]. So, we thought the HAp-agarose composite might be a hopeful as a biodegradable bone-graft material with excellent osteoconductivity. In this study, we focused on a tunable formation of the HAp-agarose composite using electrophoresis approach. The favorable characteristics are as follows; i) quick formation of HAp (within 30 min), ii) tunable physical properties such as HAp concentration, diameter, and crystallinity, and finally iii) regulation of biological functions such as protein adsorption and cell adhesion. Particularly, the physical properties and biological functions are discussed.

Methods: An agarose hydrogel was then set in the electrophoresis apparatus. Typically, the calcium chloride aqueous solution and disodium hydrogenphosphate aqueous solution were then set in the apparatus on the cathode and anode sides, respectively. After the electrophoresis, an agarose hydrogel with a turbid band was obtained, and the band was used for further characterization. Scanning electron microscopy was used after the vapor deposition of osmium tetraoxide to observe inside the hydrogel. X-ray diffraction patterns were monitored to estimate crystalline structure. As a preliminary test *in vivo*, New Zealand white rabbits were used for the animal experiment. A drill hole was made on the medial femoral condyle and the HAp-agarose composite was implanted into the hole. After the given weeks, the bone regeneration was evaluated with micro CT, and histological evaluations were also performed.

Results / Discussion: The electrophoresis approach is capable of uniformly forming HAp in the agarose hydrogel. The calcium and phosphate ions compulsorily intruded into the agarose hydrogel, and HAp-agarose composite could be prepared (Figure 1). Figure 2 shows the SEM micrographs obtained upon changing the preparative conditions. In the case of agarose, no depositions were observed in the agarose gel network (Figure 2 (a)). On the other hand, there were many depositions which were entangled with the agarose gel network (Figure 2 (b)). Furthermore, significant differences were observed in each SEM observation, if the concentration of the solutions was changed from 10 to 40 mmol/L. If one zooms in on the depositions, many particles with a fine morphology can be observed. The diameter was roughly 1.5 μm, and the surface was covered with needle-shaped crystals, much like a sea urchin and/or spherical moss. Ma *et al*. reported HAp crystals with a similar morphology [4]. We also measured crystalline structure with XRD. The obtained patterns were broad due to the lower content of HAp crystals in the agarose hydrogel. However, typical peaks were observed at 25.8° and 31.7°, which were attributed to HAp crystals (002) and (211). The peak intensity was independent of the concentration of each ionic solution. After 1 and 2 weeks the bone regeneration made progress, and after 4 weeks satisfactory bone regeneration was observed in the HAp-agarose composite. The preliminary result suggested that this HAp-agarose composite had excellent osteoconductivity and favorite scaffolds for bone regeneration.

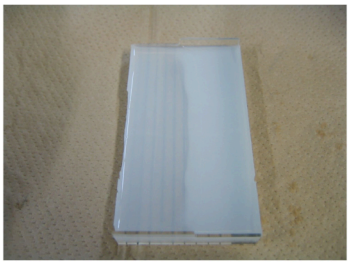

Figure 1 HAp-agarose composite by electrophoresis approach.

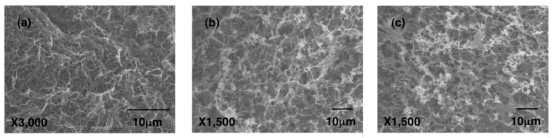

Figure 2 Scanning electron microscope observations of (a) agarose hydrogel, and HAp-agarose composite by changing concentration of calcium and phosphate solutions ((mmol/L)/(mmol/L)); (b) 10/10, and (c) 40/40.

Conclusions: We developed a novel preparative method for a HAp-agarose composite using the electrophoresis approach. The HAp was formed homogeneously in the agarose hydrogel within 30 min. The formed HAp-agarose composite has a potential for bone substitute with excellent osteoconductivity and favorite scaffolds for bone regeneration.

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