

Formation of calcium phosphate having a hierarchically laminated architecture through periodic precipitation in organic gel

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Hierarchically laminated calcium phosphate was produced through Liesegang periodic precipitation in a gel matrix of poly(acrylic acid) containing phosphate anions by diffusion of calcium cations.

Hard tissues of vertebrates, such as bone and teeth, usually have a three-dimensionally complex structure of hydroxyapatite (HAp) organized with organic matrices. The production of artificial bones having a hierarchical structure similar to natural products is technologically important for medical and dental applications. Bone-like HAp coatings on the surface of various organic substrates were achieved by mimicking biological processes in simulated body fluid (SBF).^{1–7} The biomimetic approach has been utilized to produce composite materials of HAp with collagen,^{2,3} cellulose⁴ and other organic polymers.^{5–7} An alternate soaking process in calcium and phosphorous solutions was reported as a method providing HAp formation in a three-dimensional organic matrix.⁸ A well-ordered composite of HAp and collagen was also prepared through self-organization by the interplay at the organic–inorganic interface.⁹ Recently, Bertozzi *et al.* showed a new approach to fabricating three-dimensional bone-like composites by the calcification of hydrogels.¹⁰ However, highly-tailored HAp-polymer composites having a hierarchical architecture from nanoscale structures to macroscopic morphology have not been produced. The development of a fabrication technique for hierarchically layered HAp structures similar to bones will have an especially significant impact on medical applications.

Liesegang rings are periodic bands produced during the precipitation of inorganic salts in gels.^{11–13} A far-from-equilibrium system providing a layered structure is interesting with regard to natural periodic phenomena in geological and biological minerals^{13,14} and modern thermodynamics.¹⁵ On the other hand, there are few studies on the application of this phenomenon to the preparation processes of functional materials.¹⁶

This study provides a novel preparation technique for laminated architectures of calcium phosphate using periodic precipitation in a non-equilibrium reaction system with diffusion of reactants in an organic polymer matrix. We successfully prepared calcium phosphate–poly(acrylic acid) composites having a hierarchically laminated structure. Moreover, macroscopically porous HAp was produced by removing organic components from the composites *via* a firing process. The architecture of the products was changed from a layered structure into a cellular body by controlling the concentration of the reactants.

Poly(acrylic acid), a typical water-absorbing polymer, was useful to provide an aqueous gel matrix for the precipitation reaction between calcium cations and phosphate anions. Calcification was achieved by diffusion of calcium ions into a hydrogel of poly(acrylic acid) containing a phosphate solution.¹⁷ The organic polymer gels were prepared by mixing 5 g acrylic acid, 0.1 g *N,N'*-methylene bis-acrylamide and 0.15 g ammonium peroxosulfate in 45 g water. We added diammonium hydrogenphosphate (NH₄)₂HPO₄ into the mixture and prepared 1.2 M phosphate solution as the standard condition. Gelation of the mixture occurred after it had been kept at 60 °C

for 2 h. The same volume of 4.0 M Ca(NO₃)₂ aqueous solution adjusted to the physiological pH of 7.0 with α,α,α -tris(hydroxymethyl)methylamine–HCl was loaded on the gel in a cylindrical vessel at 25 °C. The gel body of 10 mm in thickness was solidified with diffusion of calcium cations in exchange for protons within 72 h. The organic gel was totally solidified and converted into an opaque composite material through precipitation of calcium phosphate in the gel matrix (Fig. 1a). The shape of the solidified composites with the calcification was slightly deformed after a drying process although untreated gels obviously shrank by the evaporation of water.

Morphological characterization was carried out using a field emission scanning electron microscope (FESEM, Hitachi S-4700). A cross-sectional view of the composite in Fig. 1 was produced by diffusion of calcium ions. We observed periodic bands arranged perpendicularly to the direction of the diffusion in the gel matrix (Fig. 1a and 1b). In the course of the diffusion, discrete precipitation zones form in the gel matrix, which are denoted as Liesegang rings. These laminated structures were ascribed to periodic precipitation produced during the diffusion of calcium cations in the hydrogel although such a fine band structure with micrometre-scale periodicity has rarely been reported. Moreover, we recognized the presence of submicron-scale thin plates in the micrometre-scale bands (Fig. 1c). Thus, the products had a hierarchically laminated architecture through a Liesegang phenomenon having double periodicity at micrometre- and submicrometre-scales.

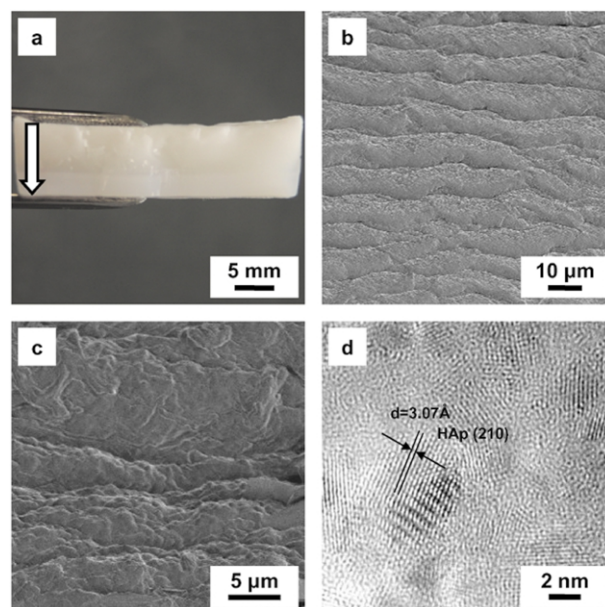


Fig. 1 Cross sectional view of a solidified gel (a), FESEM images of hierarchically laminated bands of calcium phosphate in the gel matrix (b and c) after diffusion of calcium ions ($[Ca^{2+}] = 4.0$ M, $[PO_4^{2-}] = 1.2$ M), and TEM image of laminated bands, which were composed of nanocrystalline calcium phosphates and organic polymer (d). A white arrow indicates the direction of the diffusion of calcium ions.

Structural and compositional determination of the products was performed with a Rigaku RAD-C X-ray powder diffractometer with Cu K α radiation (XRD), field emission transmission electron microscope (TEM, Philips TECNAI-F20) equipped for energy-dispersive X-ray analysis (EDXA), and Fourier-transform infrared spectrometer (FT-IR, BIO-RAD FTS-165). The results of the determination techniques suggest that the white precipitation bands mainly consisted of nanocrystalline HAp and also contained a small amount of dicalcium phosphate anhydrate and amorphous calcium phosphate. Especially, a TEM image (Fig. 1d) clearly indicates that the hierarchically laminated bands in the composite products were composed of nanoscale HAp particles without specific crystallographic orientation.

After firing the composites at 700 °C in air, layered porous structures consisting of calcium phosphate were obtained through the combustion of organic compounds. As shown in Fig. 2a, the architecture of the products was basically derived from the precipitation bands in the gel matrix, whereas the layer thickness and interlayer distance decreased slightly with removal of the organic compounds. Since each layer was composed of submicron-scale thin plates (Fig. 2b), the porous products also had a hierarchical laminated architecture. As the concentration of phosphate ions in the gel matrix decreased, the discrete precipitation bands blurred and an opening between the layered films increased with the formation of cross-linkages (Fig. 2c). Finally, we obtained a cellular porous architecture consisting of curved thin films (Fig. 2d). This morphological change is ascribed to weakening the periodicity of precipitation bands with a decrease in the degree of supersaturation on a

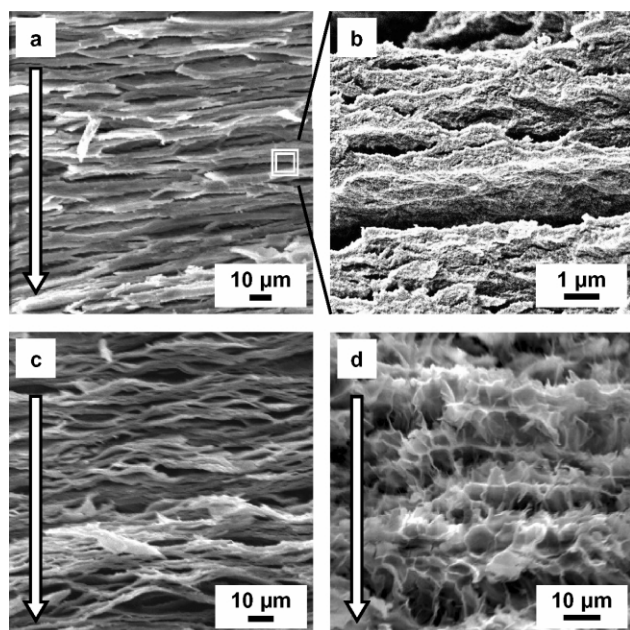


Fig. 2 Scanning electron microscope images of layered structures of HAp after calcination at 700 °C in air for 12 h. A layered HAp structure (a) was prepared with the standard condition ($[Ca^{2+}] = 4.0$ M, $[PO_4^{2-}] = 1.2$ M). Each layer was composed of submicron-scale thin films (b). Porous HAp consisting of corrugated thin films (c) and a cellular structure (d) were obtained with precursor concentrations lower than the standard condition (c: $[Ca^{2+}] = 1.7$ M, $[PO_4^{2-}] = 1.0$ M, d: $[Ca^{2+}] = 5.0$ M, $[PO_4^{2-}] = 0.6$ M). White arrows indicate the direction of the diffusion of calcium ions.

Liesegang phenomenon. The influence of the calcium concentration on the morphology was relatively small.

The crystallization and grain growth of the precipitated calcium phosphate resulted from the calcination process. The crystal phase of the layered calcium phosphate depended on the concentration of phosphate ions in the gel matrix. Layered HAp was successfully obtained at a relatively low concentration below 1.2 M. In this case, the composition of calcium phosphate produced in the composites was almost the same as that of HAp (Ca/P \approx 1.7). The formation of β -tricalcium phosphate (Ca/P = 1.5) and β -calcium pyrophosphate (Ca/P = 1.0) was observed with a high concentration range in excess of 2.0–4.0 M. In contrast, the crystal phase was not influenced by the concentration of calcium ions. A moderate reaction condition with a low phosphate concentration was required for the formation of periodic bands consisting of calcium phosphate having a composition that was similar to that of HAp. The crystallographic orientation of HAp grains in the layered structures was random. Thus, the platelike morphology of HAp is simply ascribed to the band structures due to the periodic precipitation in the gel rather than the crystallographic property of calcium phosphate.

Laminated HAp–polymer composites and porous HAp with a hierarchically layered structure have potential as new types of biocompatible organic–inorganic composites and porous inorganic materials, respectively. Thus, this technique, which uses Liesegang periodic precipitation phenomena in a far-from-equilibrium system, is valuable as a novel route for the nano- and micrometre-scale fabrication of functional materials.

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