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Preparation of Thin-film Hydroxyapatite/Polymer Hybrids

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Thin-film hybrids of hydroxyapatite (HAP)/chitosan form in a supersaturated solution of HAP in the presence of poly(acrylic acid) (PAA). The cooperative interactions between chitosan, PAA, and calcium ion on the crystallization of calcium phosphate have been examined. In the presence of PAA, the crystallization of calcium phosphate occurs in the chitosan matrix, resulting in the formation of the hybrid thin films. Observation of the films by electron diffraction and X-ray diffraction confirm the deposition of hydroxyapatite.

Living organisms produce inorganic/organic hybrids for use as hard tissues.[1] These biominerals such as shells, teeth, and bones are formed through cooperative interactions between biomacromolecules and inorganic substances. Biomacromolecules control the nucleation and crystal growth of the inorganic component resulting in the fabrication of inorganic/organic hybrids with elaborate structures. High mechanical properties are induced by the hierarchical structures so produced as in the nacre of seashells and spicules of sea urchin. For examples, the layered structures of a seashell make it more than 1000 times stronger than the corresponding single crystals.[2]

The development and preparation of biomineralization-inspired hybrid materials is an important approach to developing new advanced materials.[3,4] Thin-film $CaCO_3$ crystal/polymer hybrids are one such example. The combination of insoluble polymer matrices with soluble additives is essential to the formation of these thin-film hybrids.[4–6] The polymers serve as templates for the growth of calcium carbonate crystals and affect the structure and crystallographic orientation of the $CaCO_3$/polymer hybrid materials.

Bone and teeth, two of the most important biomaterials, consist of calcium phosphate in the form of hydroxyapatite (HAP). The syntheses of HAP/polymer hybrid materials have been extensively studied for use as a regenerative medical material.[3,7–17] Kokubo and co-workers have reported on the crystallization of HAP on an activate inorganic substrate using a simulated body fluid.[9] HAP/polymer hybrids have also been prepared by an alternative soaking technique by Akashi et al.[10] However, only limited examples of a smooth and homogeneous thin-film HAP/polymer hybrids have been reported.[11–15]

This paper describes the production of thin HAP/polymer films through a self-organization process inspired by biomineralization. The formation of $CaCO_3$/polymer hybrids were used to control the crystallization of HAP.[5] These thin films of HAP/polymer hybrids are particularly useful for osteoblast culture because of their outstanding mechanical properties. It is expected that the hierarchical structures of the thin-film hybrids will lead to improved mechanical properties, such as high flexibility and high fracture toughness.

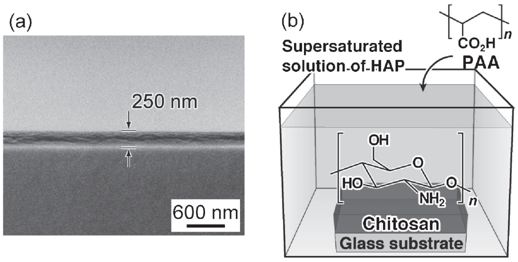

Figure 1. (a) Cross-sectional SEM image of a chitosan matrix. (b) Schematic illustration of the experimental process for the crystallization of HAP.

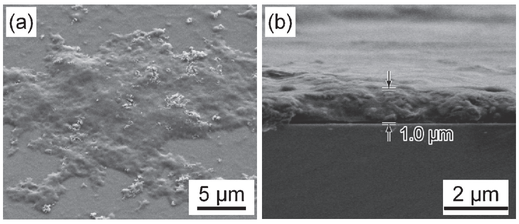

Figure 2. Scanning electron micrographs of the HAP/chitosan hybrids formed in the presence of PAA (3×10^{-3} wt %). (a) Top and (b) cross section of the thin-film hybrid.

HAP was crystallized from supersaturated solution in the presence of poly(acrylic acid) (PAA) (weight-average molecular weight: 5000).[7c] The chitosan matrix was prepared by spin coating onto a glass substrate. The thickness of the obtained matrix was about 250 nm in the dried state (Figure 1). The supersaturated solution was prepared as follows. HAP was suspended in deionized water. Carbon dioxide gas was bubbled into a stirred suspension for 5 h at 3 °C. The remaining solid HAP was then removed by filtration. The concentration of calcium ions in the solution was determined by ethylenediaminetetraacetate (EDTA) back-titration using a calcium standard solution. After the addition of poly(acrylic acid), the concentration of Ca^{2+} was adjusted to 1.0×10^{-3} mol L^{-1}. The resulting solution was transferred into vessels containing the chitosan matrices. After 12 h at 25 °C in a water bath, the resulting hybrids were collected. The pH increased gradually from 5.2 to 7.6 with incubation time as the CO_2 gas diffused out of the solution. The obtained hybrids were washed with deionized water and dried on a clean bench.

In the presence of PAA (3.0×10^{-3} wt %), the crystallization of calcium phosphate proceeded in the chitosan substrates and thin-film hybrids were formed (Figure 2). The size of these film hybrids was about 10 µm. A cross-sectional SEM image of the thin-film hybrids shows that the crystals formed not only on the surface of the chitosan matrix but also inside the chitosan

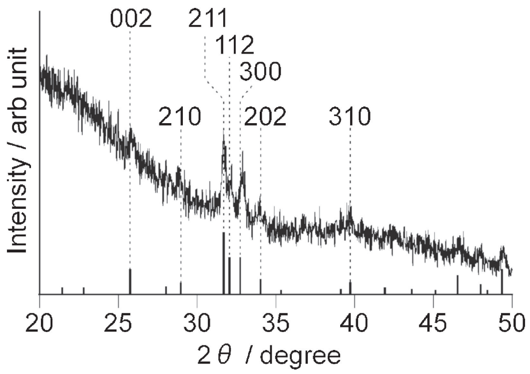

Figure 3. X-ray diffraction pattern of the as-prepared sample. The positions of peaks from stoichiometric HAP from Powder Diffraction File No. 9-432 are also shown for comparison.

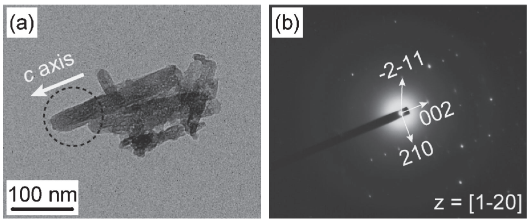

Figure 4. (a) TEM image and (b) the electron diffraction image of the block-shaped crystals in the indicated area as formed in the chitosan matrix in the presence of PAA.

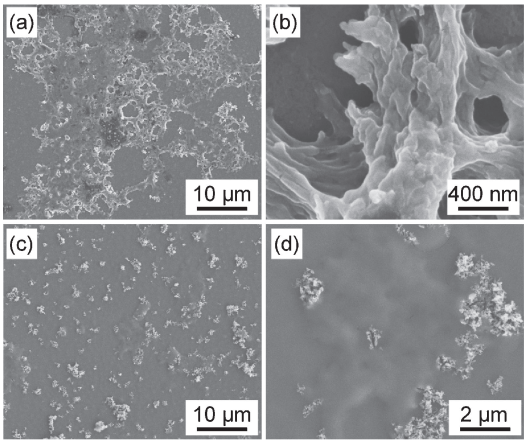

Figure 5. SEM images of HAP/chitosan hybrids formed on chitosan in the presence PAA grown for 3 h (a and b) and 24 h (c and d). [PAA] = 3.0×10^{-3} wt %.

matrix (Figure 2b). The thickness of the hybrid material was about 1.0 μm. From the SEM observation of the fractured surface, the hybrid material was made up of assemblies of block-shaped crystalline particles about 0.2 μm in size. Each nanocrystal was randomly orientated in the chitosan matrix.[18]

The samples were characterized by X-ray diffraction measurement (Figure 3) to examine the phase of the calcium phosphate crystals. The peak at $2\theta = 26°$ in the XRD pattern of the hybrid has been ascribed to the 002 diffraction peak of HAP. The peaks observed at around 32° are also attributed to the 211, 112, and 300 reflections of HAP. This is consistent with JCPDS, No. 9-432. A broad reflection at 20° in the pattern indicates the existence of chitosan. No reflections for other crystals such as calcium carbonate were observed in the diffraction pattern.

The TEM image of the block-shaped crystals grown in the matrix is shown in Figure 4 and reveals the crystallographic characteristics of the calcium phosphate. The bright-field TEM image and the electron diffraction image of the selected area, reveal that the block-shaped crystals were HAP and had developed preferentially along the *c* axis (Figure 4b). The PAA seems to bind to specific crystallographic planes of the growing HAP crystals. These surfaces are blocked from further growth resulting in the formation of block-shaped crystals.

These observations clearly indicate that the calcium phosphate grown in the chitosan matrix is HAP. The thin-film HAP/chitosan hybrids are formed through self-organization. In the absence of PAA, spherical aggregates of HAP crystals were formed on the chitosan matrix after 84 h.[18] It shows that the chitosan matrix alone has no significant effect on the crystallization of thin-film calcium phosphate. The glass substrate shows almost no interaction with PAA, with no crystallization being observed after 12 h. Because the crystallization of HAP is suppressed by PAA in the solution as observed for $CaCO_3$,[5b,5c] HAP crystals grow only in the chitosan matrix. A reasonable mechanism for this process involves the carboxy groups of the adsorbed PAA interacting with calcium ions. This would result in a local increase in the concentration of calcium ion on the surface of the chitosan matrix. These results show that the combination of PAA and chitosan matrices is a critical condition for the formation of the thin-film hybrids.

HAP crystals isolated from the chitosan matrix were characterized by attenuated total reflection infrared (ATR-IR) measurement.[18] In the ATR-IR spectra, three peaks are observed at 550, 601, and 1026 cm^{-1} in the phosphonyl band region. The 550 and 601 cm^{-1} peaks are due to P–O bending vibrations. The 1026 cm^{-1} peak is due to the P=O stretching band. These peaks indicate the presence of PO_4^{3-} in the structure of the apatite crystals. The additional broad bands at 1460 and 1550 cm^{-1}, which are due to the carbonyl groups of PAA, probably relate to a chelation complex of the carboxy groups of PAA with calcium ions. The weak absorption band at 875 cm^{-1} is probably due to CO_3^{2-}, which came from the CO_2 dissolved in the solution.

Time-resolved experiments were performed to help understand the formation process of the thin-film HAP hybrids (Figure 5). SEM images of hybrids after incubated for 3 h (Figures 5a and 5b) show that the HAP crystal nucleation had started in the chitosan matrix and that crystals had grown along the chitosan fibrils. The crystals continued to grow and evenly filled the matrix after 12 h. However, after incubating for 24 h, the thin film was broken by internal stress from the growth of the HAP crystals (Figures 5c and 5d). The effects of PAA concentration on the formation of thin-film hybrids have been examined. The thin-film hybrids were obtained over a PAA concentration range of 1.0×10^{-3} to 5.0×10^{-3} wt %. The best concentration for the development of the thin films was around 3.0×10^{-3} wt %. At higher concentrations (more than 1.0×10^{-2} wt % of PAA), no hybrid material was obtained because of the inhibiting effect of PAA. This resulted in the formation of spherical aggregates of HAP on the chitosan matrix. For lower concentrations (less than 1.0×10^{-3} wt %), a large amount of rod-like crystals of HAP and aggregates were formed on the matrix.

In conclusion, thin-film hydroxyapatite/chitosan hybrids were obtained through the cooperative interactions of insoluble and soluble polymers. Calcium ions were condensed in the chitosan matrix by the carboxy groups of PAA adsorbed on the surface of the chitosan matrix, which induces the HAP nucleation and crystal growth in the matrix as well as the formation of thin-film calcium carbonate/polymer hybrids. We believe this approach gives more useful HAP/polymer hybrids with a hierarchical structure, because the polymer serves as a template,[19] causing the nucleation and crystal growth of HAP. The crystallization processes of HAP controlled by polymer templates have not yet been fully understood. Further understanding of the mechanism of molecular control for crystallization will enable us to develop new functional HAP/polymer hybrid materials that have great potential for applications as biomaterials and structural materials with significant mechanical properties.

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