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Patterned $CaCO_3$

Self-Organized Calcium Carbonate with Regular Surface-Relief Structures[**]

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Organisms produce complex nanostructures consisting of inorganic and organic components. These materials called biominerals form a variety of self-organized and hierarchical structures.[1,2] Calcium carbonate ($CaCO_3$) is one of the most abundant biominerals formed by organisms. $CaCO_3$-based biominerals such as nacre of mollusk shells and coccoliths have complex structures on submicrometer length scales. However, it is not easy to synthesize such elaborate structures in artificial systems. In biomineralization processes, organic matrices interacting with inorganic components play key roles in forming controlled structures. Synthetic approaches to a morphological control of $CaCO_3$ by using organic templates and/or additives led to the formation of a variety of shapes such as hollow spheres,[3] hollow porous spheres,[4] spherules with complex surface structures,[5] sponge-like structures,[6] thin films,[7–9] and helices.[9a] Micropatterning of $CaCO_3$ crystals was achieved by using template-directed systems.[10] Such bioinspired self-organization processes may result in the fabrication of highly functionalized and environmentally friendly materials.[2,11] Our strategy is to obtain $CaCO_3$-based functional composites with self-organized ordered structures by tuning the chemical structures of organic matrices.

Herein we report novel $CaCO_3$ composites with regular surface-relief structures, which have never been seen in natural biominerals nor synthetic materials based on $CaCO_3$. These self-organized $CaCO_3$ crystals with submicrometer-scale periodicity are formed from solution on the thin matrix of a hydrophobically modified polysaccharide in the presence of poly(acrylic acid) (PAA).

We previously reported that $CaCO_3$ thin films with smooth surfaces were formed by the cooperation of solid natural polysaccharides and acidic polymers.[7] We used chitin,

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chitosan, and cellulose as thin film matrices. They are insoluble in water and provide solid surfaces for $CaCO_3$ crystallization. On the other hand, biominerals are often formed on soft surfaces such as lipid bilayers.[1a,b] We expected that the use of soft surfaces such as polymer gels is more effective for the morphological control of $CaCO_3$ because they can interact with inorganic components more easily. We have chosen cholesterol-bearing pullulans[12] as soft gel matrices for $CaCO_3$ crystallization (Figure 1). Pullulans are known to form hydrogels in water; these hydrogels form because of the association of the cholesteryl groups that function as physical cross-linking points.[12b,c] The formation of stable hydrogels was expected because of the high hydrophobicity of the cholesterol.

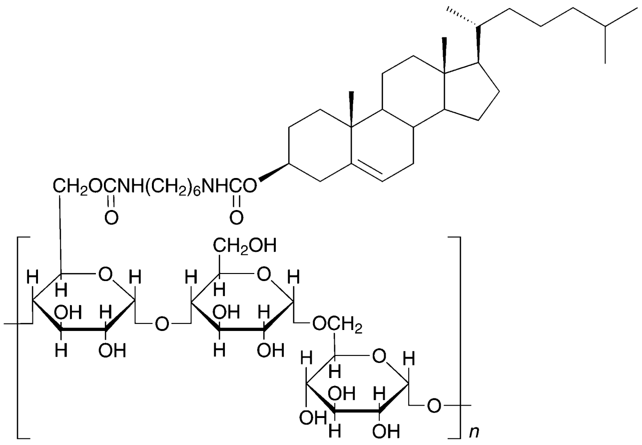

Figure 1. Structure of cholesterol-bearing pullulans (CHPs).

Pullulans that have three and eight cholesteryl groups per 100 glucose units (CHP-3 and CHP-8) were synthesized. These CHPs were spin coated on glass substrates to give thin matrices for crystallization. They were then immersed in aqueous solutions of calcium chloride ($[Ca^{2+}] = 10$ mM) containing PAA. $CaCO_3$ crystallization was induced by the slow diffusion of ammonium carbonate vapor into calcium chloride solution for two days.[13]

Surprisingly, $CaCO_3$ films with periodic surface-relief structures developed on the CHP-3 matrix in the presence of PAA (2.4×10^{-3} wt%) at 20°C, as shown in the scanning electron microscopy (SEM) images (Figure 2a–e). The average distance between grooves is about 800 nm over the whole region of the patterned crystals and the height of the surface-relief structures is about 300 nm, as revealed by atomic force microscopy (AFM) (Figure 3). Since such relief structures function as diffraction gratings, the crystals appear to be iridescent (see Supporting Information).

The crystallization of $CaCO_3$ starts from multiple nucleation sites on the surface of the CHP-3 matrix. Figure 2a shows an area around a starting point of crystallization. In the initial stage of crystal growth, a bow-tie-shaped crystal is formed as shown in the inset of Figure 2a. The periodically patterned crystals then develop outwards on the CHP-3 matrix until they collide with each other (Figure 2b). Figure 2c shows a magnified image of the patterned crystals. They are composed of calcite particles of about 30 nm in size.[14] At the front of the patterned crystals, we see needle-like crystals (Figure 2d), which are covered with nanoparticles composed of mature patterned structures. Electron diffraction analyses of the patterned crystals indicate that they are locally oriented polycrystalline calcite, although the overall crystallographic orientation is not unidirectional (see Supporting Information). A cross-sectional image of the fractured thin film crystals clearly demonstrates the formation of regular relief structure (Figure 2e), while the other side facing the CHP-3 matrix is smooth.

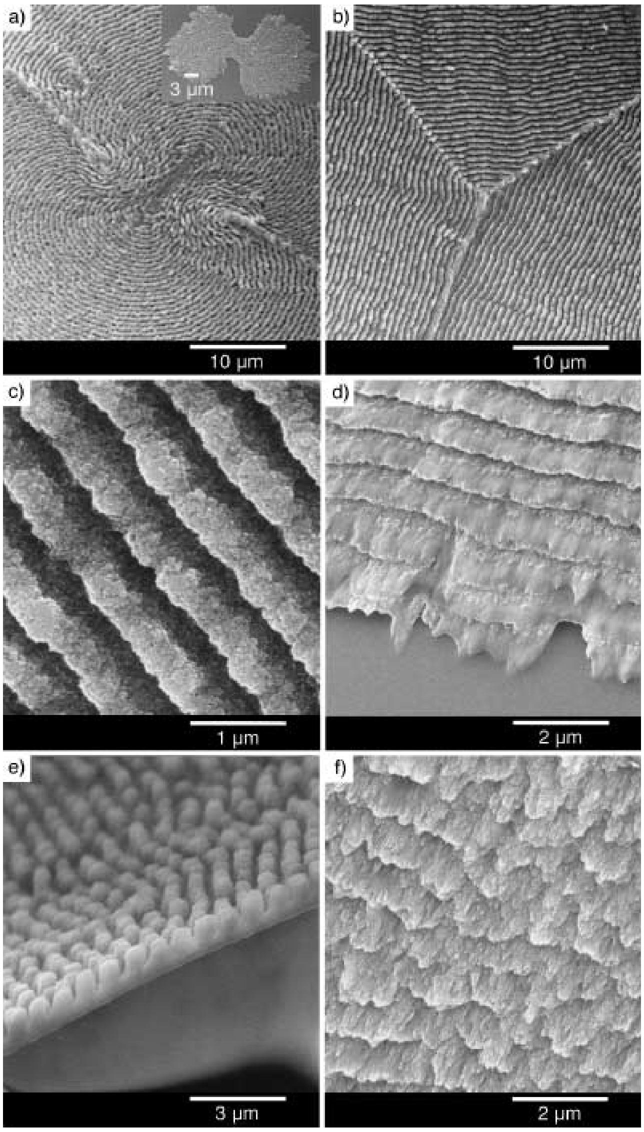

Figure 2. SEM images of patterned $CaCO_3$ crystals grown on CHP-3 matrices, 20°C, 2 d, in the presence of PAA: a–e) 2.4×10^{-3} wt% and f) 5.0×10^{-3} wt%. a) An area around a starting point of crystallization (inset: $CaCO_3$ crystals obtained in the initial stage of crystal growth, 14 h); b) a boundary among patterned films; c) a magnified image; d) a front of patterned crystals; e) a cross-sectional image.

The regularity of the surface-relief structure of $CaCO_3$ is influenced by the concentration of PAA. The best concentration for the development of the regularly patterned crystals

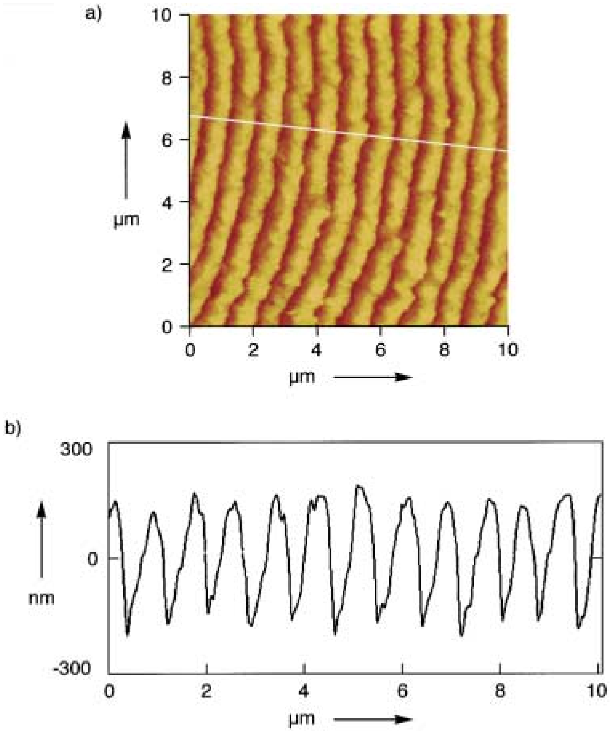

Figure 3. AFM images of patterned $CaCO_3$ crystals developed on the CHP-3 matrix in the presence of PAA (2.4×10^{-3} wt%), 20 °C, 2 d: a) top down view; b) cross-sectional image of the white line region depicted in (a).

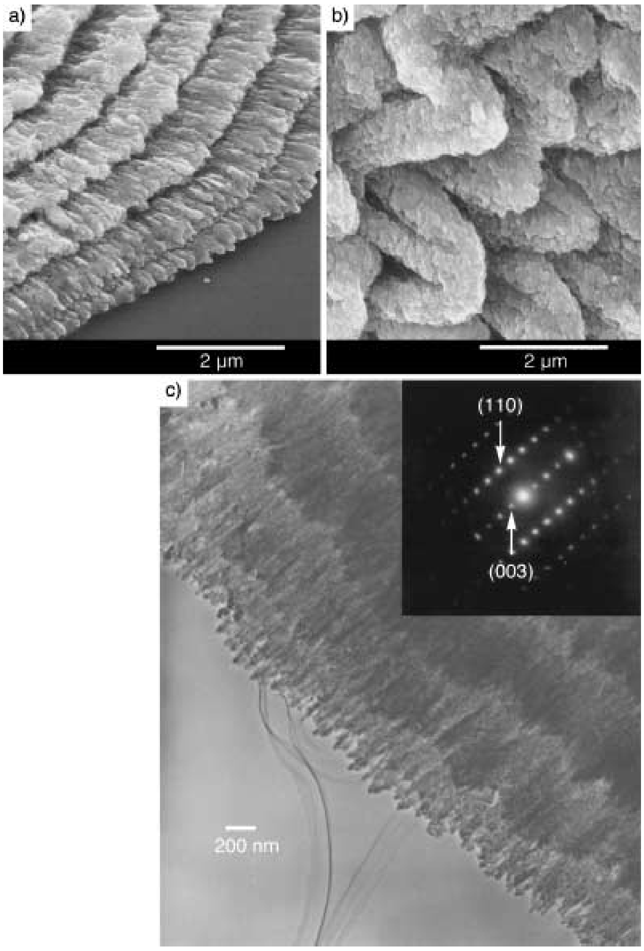

Figure 4. Effects of crystallization temperature. a,b) SEM images of $CaCO_3$ crystals grown on the CHP-3 matrices in the presence of PAA (2.4×10^{-3} wt%): a) 10 °C, 2 days; b) 50 °C, 4 h. c) TEM image of the patterned $CaCO_3$ crystals grown on the CHP-3 matrix in the presence of PAA (2.4×10^{-3} wt%), 10 °C, 2 days. The inset shows a selected-area diffraction pattern corresponds to the needlelike crystals. The crystals show the preferential orientation growth in length along the *c* axis of calcite.

is around 2.5×10^{-3} wt%. Less-ordered structures are deposited when the concentration of PAA increases as shown in Figure 2 f (PAA concentration: 5.0×10^{-3} wt%). For higher concentrations (more than 6.0×10^{-3} wt%), no crystals are obtained because of the inhibiting effect of PAA,[7b] whereas for lower concentrations of PAA (less than 1.0×10^{-4} wt%), rhombohedral crystals of calcite and aggregated crystals of vaterite are formed on the matrix.

The crystallization temperature greatly affects the morphology of the patterned crystals. Figure 4 a and b shows the surfaces of $CaCO_3$ films crystallized at 10 and 50 °C, respectively. At 10 °C, needlelike crystals of calcite that are preferentially oriented in the crystallographic *c* axis are grown (Figure 4 c). However, the deposition of thin film crystals on the matrix is sporadic owing to the higher solubility of $CaCO_3$ at lower temperatures. At 50 °C, random corrugations are formed on the surface of the calcite films (Figure 4 b). The formation of this random structure may be attributed to the rapid crystallization at higher temperatures because of the increase of the sublimation rate of ammonium carbonate and the lower solubility of $CaCO_3$.[15]

The proper degree of substitution in the matrix is important for the periodic pattern formation. When more hydrophobically modified pullulan CHP-8 (containing eight cholesteryl moieties per 100 glucose units) is used as a matrix in place of CHP-3, $CaCO_3$ films with less regular surfaces are developed on the CHP-8 matrix in the presence of PAA at 20 °C (see Supporting Information). Unmodified pullulan does not function as a thin film matrix because it is soluble in water. The presence of the unmodified pullulan in solution has no effect on crystallization; rhombohedral crystals of calcite and aggregated crystals of vaterite are formed in the presence of unmodified pullulan without PAA. In the presence of both unmodified pullulan and PAA (2.4×10^{-3} wt%), crystallization is inhibited.

It is difficult to fully understand the mechanism of the formation of the patterned crystals. We assume that the periodic pattern formation in the present study belongs to a self-organization process in the reaction-diffusion systems,[16,17] in which competition between precipitation and ion diffusion occurs. Such behavior is usually observed in gel media.[17] In the present material, the pullulan matrix is swollen with a calcium chloride solution to form the hydrogel as a result of the association of the cholesteryl group.[12b,c] When the CHP matrix is placed in a solution containing PAA, PAA may be adsorbed on the surface and incorporated into the matrix through the interaction between the CHP and PAA (see Supporting Information). The interaction of PAA with calcium ions leads to a high local concentration of calcium ions in/on the matrix. When the calcium ions react with carbonate ions, the deposition of $CaCO_3$ occurs. These reactions induce a rapid decrease of ion concentration. The deposition is no longer sustained owing to the lack of ions because efficient ion transport to the reaction sites is prevented by the influence of the gel matrix. After some time, the high ion concentration recovers and the $CaCO_3$ is deposited once more. The cycling of these processes may result in the pattern generation.

The SEM image of the CHP matrix obtained after the dissolution of $CaCO_3$ grown on the matrix shows that the periodic pattern formation of $CaCO_3$ accompanies the deformation of the CHP matrix (see Supporting Information). One possible explanation on this behavior is that the transformation from amorphous to crystalline states that accompanies the matrix deformation occurs after patterned amorphous $CaCO_3$ is deposited (Figure 2d, and Supporting Information). Amorphous-to-crystalline transformation was often observed in the presence of acidic polymers.[8b,9b,18] Aizenberg and co-workers reported that micropatterned substrate-induced millimeter-sized single calcite crystals were a consequence of a relaxation of the tensile stress of the crystals on transforming from amorphous to crystalline states.[10d] This effect suggests the existence of a relationships between the formation of the crystals and tensile stress. In our system, the transformation the tensile stress of the crystals, which is increased by the transformation from amorphous to crystalline states, is relaxed, thus leading to the enhancement of the patterns formed in the amorphous states of $CaCO_3$.

In conclusion, we have succeeded in preparing periodically patterned calcium carbonate films for the first time. These self-organized $CaCO_3$ films with regular surface-relief structures are formed on the thin matrix of cholesterol-modified pullulan from an aqueous solution containing poly(acrylic acid). Such spontaneous pattern generation with remarkable regularity in the submicrometer length scale is unusual, even in other materials. The use of organic matrices in the fabrication of hybrid materials offers the potential to control structures in simple and mild synthetic conditions.

Experimental Section

Cholesterol-bearing pullulans (CHPs) were synthesized according to literature procedures.[12a] Details can be found in the Supporting Information. CHP matrices were prepared by spin coating of their 2 wt% DMSO solution on glass substrates. They were annealed for 1 h at 180 °C to remove the solvent.

Purified water obtained from an Auto pure WT100 purification system (Yamato, relative resistivity: maximum $1.8 \times 10^7\,\Omega\,\text{cm}$) was employed for the crystallization of calcium carbonate. Poly(acrylic acid) ($\bar{M}_w = 2{,}000$) was added to calcium chloride aqueous solution ($[Ca^{2+}] = 10\,\text{mM}$). The solution was transferred to vessels containing CHP matrices. The vessels were then placed in a closed desiccator together with a vial of ammonium carbonate.[13] An incubator (Fukushima) was used to maintain a constant crystallization temperature (10–50 °C). The pH value of the solution increased from 4.3 to 9.1 during the crystallization experiment.

SEM images were obtained using a Hitachi S-900S field-emission SEM operated at 5–10 kV. Samples were platinum coated by using a Hitachi E-1030 ion sputter. TEM images were taken with a JEOL JEM-4000FXII at 400 kV. AFM measurements were carried out by a Digital Instruments Nanoscope III system with standard Si tips. Fourier transform infrared spectra were recorded on a Jasco FT/IR-660Plus spectrometer using KBr pellet.

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[1] a) S. Mann in *Biomineralization* (Eds: R. G. Compton, S. G. Davies, J. Evans), Oxford University Press, Oxford, **2001**; b) L. Addadi, S. Weiner, *Angew. Chem.* **1992**, *104*, 159–176; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 153–169; c) S. V. Dorozhkin, M. Epple, *Angew. Chem.* **2002**, *114*, 3260–3277; *Angew. Chem. Int. Ed.* **2002**, *41*, 3130–3146.

[2] a) S. Mann, G. A. Ozin, *Nature* **1996**, *382*, 313–318; b) G. A. Ozin, *Acc. Chem. Res.* **1997**, *30*, 17–27.

[3] a) H. Cölfen, M. Antonietti, *Langmuir* **1998**, *14*, 582–589; b) L. Qi, J. Li, J. Ma, *Adv. Mater.* **2002**, *14*, 300–303.

[4] D. Walsh, S. Mann, *Nature* **1995**, *377*, 320–323.

[5] a) S.-H. Yu, H. Cölfen, J. Hartmann, M. Antonietti, *Adv. Funct. Mater.* **2002**, *12*, 541–545; b) J. Küther, R. Seshadri, W. Tremel, *Angew. Chem.* **1998**, *110*, 3196–3199; *Angew. Chem. Int. Ed.* **1998**, *37*, 3044–3047.

[6] a) D. Walsh, B. Lebeau, S. Mann, *Adv. Mater.* **1999**, *11*, 324–328; b) R. J. Park, F. C. Meldrum, *Adv. Mater.* **2002**, *14*, 1167–1169.

[7] a) T. Kato, *Adv. Mater.* **2000**, *12*, 1543–1546; b) T. Kato, T. Suzuki, T. Amamiya, T. Irie, M. Komiyama, H. Yui, *Supramol. Sci.* **1998**, *5*, 411–415; c) T. Kato, T. Suzuki, T. Irie, *Chem. Lett.* **2000**, 186–187; d) A. Sugawara, T. Kato, *Chem. Commun.* **2000**, 487–488; e) N. Hosoda, T. Kato, *Chem. Mater.* **2001**, *13*, 688–693.

[8] a) S. Zhang, K. E. Gonsalves, *Langmuir* **1998**, *14*, 6761–6766; b) G. Xu, N. Yao, I. A. Aksay, J. T. Groves, *J. Am. Chem. Soc.* **1998**, *120*, 11977–11985.

[9] a) L. A. Gower, D. A. Tirrell, *J. Cryst. Growth* **1998**, *191*, 153–160; b) L. B. Gower, D. J. Odom, *J. Cryst. Growth* **2000**, *210*, 719–734.

[10] a) J. Aizenberg, A. J. Black, G. M. Whitesides, *Nature* **1999**, *398*, 495–498; b) J. Aizenberg, A. J. Black, G. M. Whitesides, *J. Am. Chem. Soc.* **1999**, *121*, 4500–4509; c) J. Aizenberg, *J. Cryst. Growth* **2000**, *211*, 143–148; d) J. Aizenberg, D. A. Muller, J. L. Grazul, D. R. Hamann, *Science* **2003**, *299*, 1205–1208.

[11] a) E. Dujardin, S. Mann, *Adv. Mater.* **2002**, *14*, 775–788; b) T. Kato, A. Sugawara, N. Hosoda, *Adv. Mater.* **2002**, *14*, 869–877; c) S. I. Stupp, P. V. Braun, *Science* **1997**, *277*, 1242–1248; d) K. J. C. van Bommel, A. Friggeri, S. Shinkai, *Angew. Chem.* **2003**, *115*, 1010–1030; *Angew. Chem. Int. Ed.* **2003**, *42*, 980–999.

[12] a) K. Akiyoshi, S. Deguchi, N. Moriguchi, S. Yamaguchi, J. Sunamoto, *Macromolecules* **1993**, *26*, 3062–3068; b) K. Akiyoshi, S. Deguchi, H. Tajima, T. Nishikawa, J. Sunamoto, *Macromolecules* **1997**, *30*, 857–861; c) K. Kuroda, K. Fujimoto, J. Sunamoto, K. Akiyoshi, *Langmuir* **2002**, *18*, 3780–3786.

[13] G. Falini, S. Albeck, S. Weiner, L. Addadi, *Science* **1996**, *271*, 67–69.

[14] The Fourier transform infrared spectrum of the patterned crystals shows sharp bands at 875 and 714 cm^{-1}, which are characteristic of calcite. No other bands for aragonite, vaterite, and amorphous calcium carbonate are detected.

[15] The sublimation rates of ammonium carbonate in our experimental system at 10 °C, 20 °C, and 50 °C are about $6.3 \times 10^{-2}\,\text{mmol}\,h^{-1}$, $7.1 \times 10^{-2}\,\text{mmol}\,h^{-1}$, and $7.8 \times 10^{-1}\,\text{mmol}\,h^{-1}$, respectively.

[16] a) G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295*, 2418–2421; b) K. Iwamoto, S. Mitomo, M. Seno, *J. Colloid Interface Sci.* **1984**, *102*, 477–482; c) M. Tsapatsis, D. G. Vlachos, S. Kim, H. Ramanan, G. R. Gavalas, *J. Am. Chem. Soc.* **2000**, *122*, 12864–12865.

[17] a) H. K. Henisch in *Crystals in Gels and Liesegang Rings*, Cambridge University Press, Cambridge, **1988**; b) H.-J. Krug, H. Brandtstädter, *J. Phys. Chem. A* **1999**, *103*, 7811–7820.

[18] a) Y. Levi-Kalisman, S. Raz, S. Weiner, L. Addadi, I. Sagi, *Adv. Funct. Mater.* **2002**, *12*, 43–48; b) L. Addadi, S. Raz, S. Weiner, *Adv. Mater.* **2003**, *15*, 959–970.