Self-Organized Hybrids

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Macroscopically Ordered Polymer/$CaCO_3$ Hybrids Prepared by Using a Liquid-Crystalline Template**

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Biominerals are organic/inorganic hybrids formed by living organisms under mild conditions.[1–3] Biomacromolecules such as polysaccharides, proteins, and glycoproteins serve as templates that control the crystal growth of biominerals and thus result in the formation of highly organized hybrid structures.[1–3] Recently, intensive work has been focused on the development of biomineralization-inspired hybrid materials based on $CaCO_3$.[1–14] For the preparation of these novel hybrid materials exhibiting versatile properties such as mechanical stability, optical properties, and biofunctionality,[11] it is essential to control the orientation and structure of both inorganic and organic components on the macroscopic scale. However, such control over orientation and morphology of the components has not yet been effectively achieved. Our intention was to obtain highly organized hybrid materials using macroscopically ordered templates based on liquid-crystalline (LC) macromolecules. Herein we report on the preparation of ordered polymer/$CaCO_3$ hybrids by using the macroscopically ordered LC states of a semisynthetic chitin derivative as template. Only a couple of studies on the use of ordered matrices have been reported, and these matrices were derived from biosystems.[12,15,16] Mann and co-workers demonstrated the formation of ordered porous chitin/silica composites using a macroscopically ordered β-chitin matrix obtained from cuttlebone.[15] Falini and co-workers used the aligned β-chitin obtained from the pen of a squid as a matrix for calcium salts, which resulted in the formation of oriented crystals.[12] If we could use and control the ordering processes of synthetic or semisynthetic polymer matrices, a variety of self-organized hybrid macroscale structures could be obtained more easily. In the formation of thin-film $CaCO_3$ crystals, the minerals are deposited on solid polymer matrices in the presence of acidic soluble macromolecules.[4,10] Cooperation of solid matrices such as polysaccharides and poly(vinyl alcohol) with acidic macromolecules is essential to form hybrid thin-film structures.[10]

We previously reported on the formation of unidirectionally oriented thin-film crystals of $CaCO_3$ on a randomly oriented chitin matrix in the presence of an acidic natural peptide isolated from the exoskeleton of a crayfish.[13] However, the size of these thin films was only $10 \times 10\ \mu m$ and the direction of orientation of each film was random on the macroscopic scale because of the random arrangement of the chitin matrix. Furthermore, acidic peptides having specific sequences of amino acids were required.

Our approach here is to obtain highly ordered hybrids using LC templates. Liquid crystals can form large domains of oriented molecules on the macroscopic scale.[17] We expected that oriented polymeric solid matrices processed through their LC states should serve as templates to induce unidirectional crystal growth of $CaCO_3$. The ordered chitin matrix was prepared by processing and deprotection of a corresponding LC chitin derivative[18] obtained by carbamation of chitin (Figure 1). The chitin phenylcarbamate formed a lyotropic nematic LC phase in DMSO/DMF solution at concentrations higher than 15 wt% (Figure 2a). This chitin derivative forms LC phases due to its rigidity and appropriate intermolecular interactions. The concentrated LC solution (15 wt%) was converted to a free-standing gel film by soaking in methanol, and then the gel film was stretched by 200% in methanol. The stretched film was treated with a refluxing methanolic solution of NaOH for 2 h to cleave the carbamoyl groups. Quantitative cleavage of the carbamate moieties was confirmed by FTIR measurements (see Supporting Information). Then the film was dried in vacuo. Macroscopic orientation of the polymer chains in the dried elongated film was confirmed by polarizing optical microscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) observations (Figure 2b–d). Under crossed polarizers, optical microscopic images of the film change from bright to dark on each rotation by 45° (Figure 2b). Small-angle X-ray diffraction

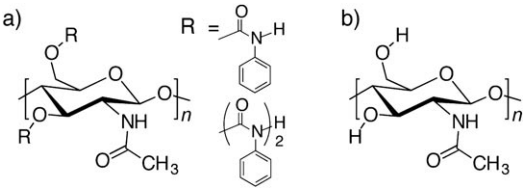

Figure 1. Chemical structures of a) chitin phenylcarbamate,[18] which shows a lyotropic nematic liquid-crystalline state and b) chitin before phenylcarbamation and after deprotection of chitin phenylcarbamate for the use as an ordered template for $CaCO_3$ crystallization.

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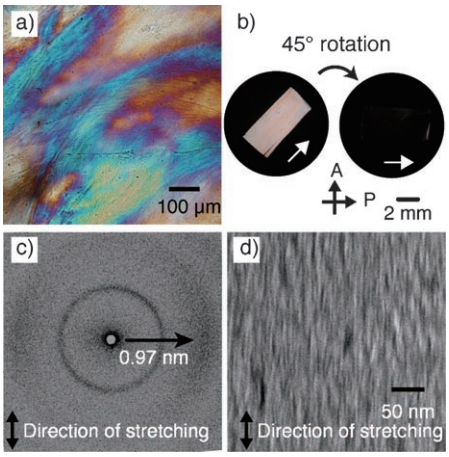

Figure 2. a) Polarized optical micrograph of chitin phenylcarbamate in DMF/DMSO solution (30 wt%, 1/1 v/v). b) Polarized optical micrographs of the oriented chitin film. In the right-hand image the sample has been rotated by 45° from its position in the left-hand image. Directions of A: analyzer and P: polarizer. c) Small-angle X-ray diffraction pattern of the oriented chitin film with the X-ray beam perpendicular to the film. d) SEM image of the oriented chitin surface.

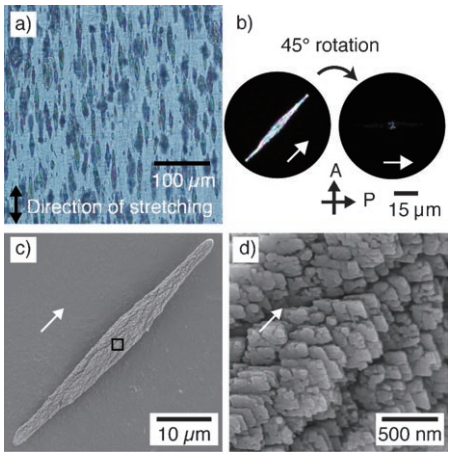

Figure 3. a) Optical micrograph of calcium carbonate grown in the oriented chitin film. The arrow indicates the direction of elongation of the film matrix. $CaCO_3$ crystals grown in the oriented chitin film: b) Polarized optical micrograph of the crystal. In the right-hand image the sample has been rotated by 45° from its position in the left-hand image. c) SEM image of the rod shape of the isolated crystal. d) Magnified image of the square area on the crystal surface in c).

indicated that the chitin backbone is aligned parallel to the direction of film elongation (Figure 2c). The diffraction peaks at 0.97 nm are due to chitin fibers aligned along the direction of elongation. Furthermore, the alignment of bundles of chitin fibers, which contain tens of chains of chitin molecules, was directly observed in a high-resolution SEM image (Figure 2d).

The oriented chitin films were used as templates for $CaCO_3$ crystallization. They were immersed in an aqueous solution of calcium chloride ($[Ca^{2+}] = 25$ mM) in the presence of poly(acrylic acid), PAA. Ammonium carbonate vapor was slowly diffused into the calcium chloride solution.[14]

We observed that small rods of $CaCO_3$ about 8 µm in length started to form after immersing the film in the crystallization solution for about 10 h in the presence of 3.0×10^{-3} wt% of PAA. These rods became longer (40 µm after 15 h and 80 µm after 50 h) as crystallization proceeded. The rodlike crystals formed in the films at 5 °C after 50 h are shown in Figure 3a. The diameter is between 10 and 30 µm. These $CaCO_3$ rods were aligned parallel to the direction of film elongation. The polymorph of the crystals formed in the ordered chitin template was calcite, which was confirmed by FTIR and Raman spectroscopy. Similar crystallization behavior was observed at 30 °C. When unstretched LC chitin films were used as the matrix, no rodlike crystals were obtained (see Supporting Information). A crystal isolated from the chitin matrix[19] was observed by polarizing optical microscopy to examine the crystallographic *c* axis of the crystal. The direction of the *c* axis of the rodlike calcite corresponds to the direction of the longitudinal axis of the rods, which is confirmed by the periodic change in the pattern with rotation of the sample under the polarizing microscope (Figure 3b). No strong birefringence is observed when the longitudinal axis of the rod is along the polarizer or analyzer axis. The highest brightness is seen when the longitudinal axis is at an angle of 45°. Scanning electron micrographs of the crystals reveal that they are assemblies of rhombohedral calcite crystals with fine (104) facets about 500 nm in size (Figure 3c and d). These nanocrystals are also aligned in the direction of the rod axis (Figure 3d). Recently, such crystalline assemblies on the nanometer scale have been described as mesocrystals.[20]

Transmission electron microscopy (TEM) was performed on a calcite rod to examine the crystallographic orientation (Figure 4). For this measurement, a thin specimen (thickness ca. 200 nm) of the rod-shaped calcite was prepared by the focused ion beam (FIB) technique, as shown schematically in Figure 4a. The bright-field TEM image (Figure 4b) and the

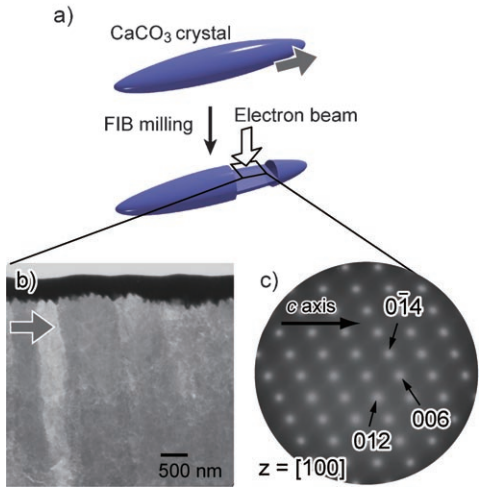

Figure 4. a) Schematic illustration of sample preparation by the FIB technique and cross-sectional measurement on a calcite rod. b) Bright-field TEM image of a thin section of the calcite rod. The opaque material over the specimen is a tungsten coating deposited in the FIB process. c) Selected-area electron diffraction pattern corresponding to the thin section.

corresponding selected-area electron diffraction pattern (Figure 4c) of the sample show that the *c* axis of calcite is unidirectionally orientated along the rod axis. These results suggest that the calcite rods formed in the oriented chitin films are oriented with the *c* axis along the backbone of chitin, which aligned parallel to the direction of film elongation.

The macroscopically oriented organic template strongly influenced the morphology and crystallographic orientation of the $CaCO_3$ crystals. The key to the formation of oriented crystals on the macroscopic scale is the use of LC processing for preparation of the matrix. We assume that the carboxyl groups of the adsorbed PAA are aligned along the oriented chitin backbone and serve as template for unidirectionally oriented nucleation and growth of $CaCO_3$ crystal.

We previously reported on the selective formation of aragonite $CaCO_3$ by means of the template effects of crystalline matrices of poly(vinyl alcohol).[10c] A local arrangement of carboxyl groups of PAA adsorbed on the crystalline polymer matrix was considered to play a critical role only in the nucleation of aragonite. However, in this case, the orientation was radial because the crystal grew on the polymer matrix consisting of randomly oriented polydomains.

In conclusion, we have demonstrated that macroscopically oriented polymer/$CaCO_3$ hybrid materials are obtained by using LC chitin derivatives. The nanocrystals of calcite align along the ordering direction of the polymer template. In nature, macroscopically ordered structures such as the silkworm cocoon and the exoskeleton of the jewel beetle are considered to form via LC structures.[21] Our present results suggest that to obtain highly ordered hybrid synthetic materials, use of LC structures could be one of the most promising approaches. Moreover, this method involving derivatization of matrix polymers to induce LC order followed by deprotection of the functional groups of the ordered matrix may be widely applicable to the syntheses of novel organic/inorganic hybrids with ordered structure on the macroscopic scale not only for $CaCO_3$ but also for other inorganic crystals.

Experimental Section

Chitin phenylcarbamate was prepared according to literature procedures.[18] Details can be found in the Supporting Information.

Purified water from an Auto pure WT100 purification system (Yamato, maximum relative resistivity $1.8 \times 10^7\ \Omega$ cm) was employed for crystallization of calcium carbonate. Poly(acrylic acid) (M_w = 2000) was added to calcium chloride aqueous solution ($[Ca^{2+}]$ = 25 mM). The solution was transferred to vessels containing the ordered chitin film. The vessels were then placed in a closed desiccator together with a vial of ammonium carbonate.[14] An incubator (Fukushima) was used to maintain a constant crystallization temperature (5 or 30 °C). The pH value of the solution increased from 5.2 to 9.0 during the crystallization experiment.

Polarizing optical micrographs were taken with an Olympus BX51 polarizing optical microscope. SEM images were obtained with a Hitachi S-900S field-emission SEM operated at 6 kV. Samples were coated with platinum by using a Hitachi E-1030 ion sputterer. TEM images were taken with a JEOL JEM-4000FXII at 400 kV. ^{1}H (400 MHz) NMR spectra were measured on a JEOL 400 spectrometer. Laser Raman spectra were taken on a JASCO NR-1800. IR spectra were recorded with JASCO Fourier transform IR-660 Plus. Attenuated total reflection (ATR) IR spectra were recorded on the ZnSe element of a Jasco ATR Pro410s ATR instrument, fitted with a detector. X-ray diffraction measurements were performed on a Rigaku RINT2400 X-ray diffractometer with $Cu_{K\alpha}$ radiation.

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[1] S. Mann, *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry* (Eds.: R. G. Compton, S. G. Davies, J. Evans), Oxford University Press, Oxford, **2001**.
[2] a) *Biomineralization* (Ed.: E. Bäuerlein), Wiley-VCH, Weinheim, **2000**; b) *Handbook of Biomineralization* (Eds.: E. Bäuerlein, P. Behrens, M. Epple), Wiley-VCH, Weinheim, **2007**.
[3] a) L. Addadi, S. Weiner, *Angew. Chem.* **1992**, *104*, 159–176; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 153–169; b) P. Calvert, S. Mann, *J. Mater. Sci.* **1988**, *23*, 3801–3815.
[4] a) T. Kato, A. Sugawara, N. Hosoda, *Adv. Mater.* **2002**, *14*, 869–877; b) T. Kato, *Adv. Mater.* **2000**, *12*, 1543–1546.
[5] A.-W. Xu, Y. Ma, H. Cölfen, *J. Mater. Chem.* **2007**, *17*, 415–449.
[6] F. C. Meldrum, *Int. Mater. Rev.* **2003**, *48*, 187–224.
[7] a) S. I. Stupp, P. V. Braun, *Science* **1997**, *277*, 1242–1248; b) J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684–1688.
[8] H. Imai, Y. Oaki, A. Kotachi, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1834–1851.
[9] K. Naka, Y. Chujo, *Chem. Mater.* **2001**, *13*, 3245–3259.
[10] a) T. Kato, T. Suzuki, T. Amamiya, T. Irie, M. Komiyama, H. Yui, *Supramol. Sci.* **1998**, *5*, 411–415; b) N. Hosoda, T. Kato, *Chem. Mater.* **2001**, *13*, 688–693; c) N. Hosoda, A. Sugawara, T. Kato, *Macromolecules* **2003**, *36*, 6449–6452; d) A. Kotachi, T. Miura, H. Imai, *Chem. Lett.* **2003**, *32*, 820–821.
[11] a) A. Berman, D. J. Ahn, A. Lio, M. Salmeron, A. Reichert, D. Charych, *Science* **1995**, *269*, 515–518; b) S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, A. M. Belcher, *Nature* **2000**, *405*, 665–668; c) S. Tugulu, M. Harms, M. Fricke, D. Volkmer, H.-A. Klok, *Angew. Chem.* **2006**, *118*, 7619–7623; *Angew. Chem. Int. Ed.* **2006**, *45*, 7458–7461; d) D. Ogomi, T. Serizawa, M. Akashi, *J. Biomed. Mater. Res.* **2003**, *67 A*, 1360–1366; e) D. C. Popescu, E. N. M. van Leeuwen, N. A. A. Rossi, S. J. Holder, J. A. Jansen, N. A. J. M. Sommerdijk, *Angew. Chem.* **2006**, *118*, 1794–1799; *Angew. Chem. Int. Ed.* **2006**, *45*, 1762–1767.
[12] G. Falini, S. Fermani, A. Ripamonti, *J. Inorg. Biochem.* **2002**, *91*, 475–480.
[13] A. Sugawara, T. Nishimura, Y. Yamamoto, H. Inoue, H. Nagasawa, T. Kato, *Angew. Chem.* **2006**, *118*, 2942–2945; *Angew. Chem. Int. Ed.* **2006**, *45*, 2876–2879.
[14] a) G. Falini, S. Albeck, S. Weiner, L. Addadi, *Science* **1996**, *271*, 67–69; b) A. Sugawara, T. Ishii, T. Kato, *Angew. Chem.* **2003**, *115*, 5457–5461; *Angew. Chem. Int. Ed.* **2003**, *42*, 5299–5303; c) T. Sakamoto, A. Oichi, A. Sugawara, T. Kato, *Chem. Lett.* **2006**, *35*, 310–311.
[15] W. Ogasawara, W. Shenton, S. A. Davis, S. Mann, *Chem. Mater.* **2000**, *12*, 2835–2837.
[16] A. Matsuda, M. Kasahara, H. Kobayashi, N. Ichinose, J. Tanaka, *Mater. Sci. Eng. C* **2004**, *24*, 717–722.
[17] a) *Handbook of Liquid Crystals* (Eds.: D. Demus, J. W. Goodby, G. W. Gray, H.-W. Spiess, V. Vill), Wiley-VCH, Weinheim, **1998**; b) *Liquid Crystal Polymers: From Structures to Applications* (Ed.: A. A. Collyer), Elsevier, London, **1992**; c) T. Kato, N.

Mizoshita, K. Kishimoto, *Angew. Chem.* **2006**, *118*, 44 – 74; *Angew. Chem. Int. Ed.* **2006**, *45*, 38 – 68.
[18] a) C. Yamamoto, T. Hayashi, Y. Okamoto, *J. Chromatogr. A* **2003**, *1021*, 83 – 91; b) Y. Nishio, Y. Miyashita (Jpn. Kokai Tokkyo Koho), JP2005-17174A, **2005**.
[19] The ordered chitin matrix was removed by immersion in NaClO solution. A. Berman, L. Addadi, S. Weiner, *Nature* **1988**, *331*, 546 – 548.
[20] a) H. Cölfen, M. Antonietti, *Angew. Chem.* **2005**, *117*, 5714 – 5730; *Angew. Chem. Int. Ed.* **2005**, *44*, 5576 – 5591; b) M. Niederberger, H. Cölfen, *Phys. Chem. Chem. Phys.* **2006**, *8*, 3271 – 3287.
[21] a) J. E. Lydon, *Liquid Cryst. Today* **2004**, *13*, 1 – 13; b) Y. Bouligand, *Tissue Cell* **1972**, *4*, 189 – 17.

