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## **Polymer Brushes as Ionotropic Matrices for the Directed Fabrication of Microstructured Calcite Thin Films\*\***

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Many biominerals are characterized by complex, hierarchically ordered structures.<sup>[1]</sup> In nature, the formation of biominerals is guided by matrix macromolecules. For example, acidic biomacromolecules play an important role in directing the mineralization of  $\text{CaCO}_3$ , one of the most abundant biominerals.<sup>[2]</sup> In addition to the three crystalline polymorphs, biogenic  $\text{CaCO}_3$  also occurs in two amorphous modifications: a stable hydrated form and a less stable, essentially anhydrous form, which can act as a transient precursor of the thermodynamically stable crystalline modifications.<sup>[3,4]</sup> The formation of various complex biogenic  $\text{CaCO}_3$  structures, such as sea urchin spines, has been proposed to proceed via the second, metastable amorphous- $\text{CaCO}_3$  (ACC) phase.<sup>[5,6]</sup> ACC has also been exploited as a

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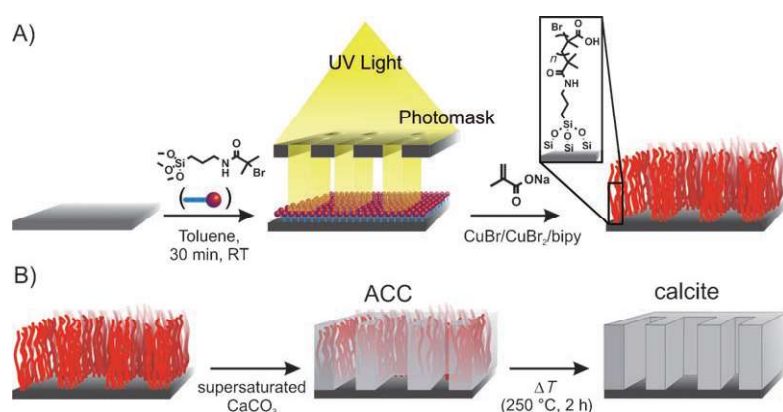
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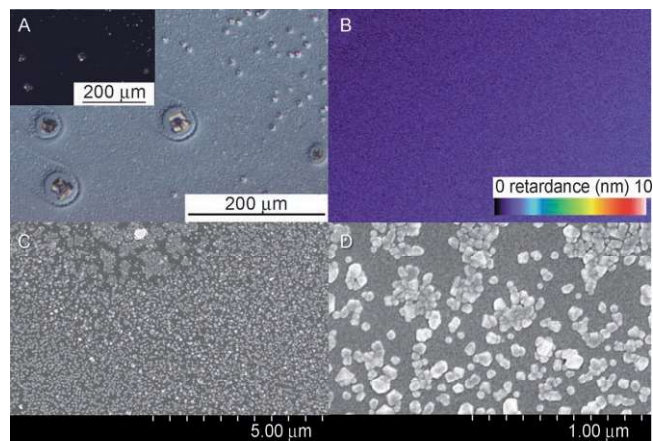
transient precursor *in vitro* to produce  $\text{CaCO}_3$  crystals in constrained geometries, to fabricate microstructured calcite single crystals, and to synthesize nacre-type laminated  $\text{CaCO}_3$  coatings.<sup>[7–9]</sup> The advantage of the transient ACC phase is that it can be more easily molded into complex structures than the crystalline  $\text{CaCO}_3$  polymorphs. The amorphous precursor route has also been used to prepare crystalline carbonate apatite films.<sup>[10]</sup> The application of such concepts to direct the size, shape, and microstructure of synthetic inorganic materials is of great technological importance and could lead to new materials with potential applications in, for example, optical information processing, catalysis, and regenerative medicine.<sup>[11–14]</sup> A convenient route for the fabrication of microstructured inorganic materials is the use of self-assembled monolayers (SAMs) to create 2D spatially constrained microenvironments for crystallization.<sup>[12,14]</sup> In this case, crystallization is limited to those regions of the substrate that present the appropriate surface chemistry. Instead of relying on specific chemical surface modification, micrometer scale patterned ceramic thin films can also be prepared by imposing physical boundaries on the crystallization.<sup>[13]</sup> These boundaries can be achieved using a variety of soft lithography techniques, including, microtransfer molding, micromolding in capillaries, and embossing.

Herein, we present a novel approach for the fabrication of microstructured, crystalline calcite thin films. Our strategy involves the use of photolithographically patterned poly(methacrylic acid) (PMAA) brushes grown by surface-initiated atom-transfer radical polymerization (SI-ATRP). These brushes function as ionotropic<sup>[15]</sup> matrices to produce crystalline calcite films that are an exact 3D replica of the PMAA brush. One of the attractive features of polymer brushes compared to SAMs is that film thickness and chemical composition can be precisely controlled using a variety of surface-initiated, controlled radical polymerization techniques, including ATRP.<sup>[16–18]</sup> Herein, we demonstrate for the first time the use of such brushes to act as ionotropic matrices for the directed synthesis of microstructured calcite thin films. The proposed strategy combines three key elements (Figure 1): 1) the use of photolithographic techniques to prepare microstructured PMAA brushes; 2) the ability of the PMAA brushes to stabilize the ACC; and 3) the possibility to convert the metastable ACC phase into a polycrystalline calcite film by thermal treatment.

A first series of mineralization experiments was carried out with 100-nm-thick, unstructured PMAA brushes grafted on glass substrates. The brushes were exposed to a continuous flow of a supersaturated calcium carbonate solution in a perfusion cell. After 30 min, the substrates were taken from the perfusion cell, washed, dried, and subsequently analyzed with various microscopic techniques. The differential interference contrast (DIC) micrograph in Figure 2A shows the formation of a homogeneously flat and unstructured  $\text{CaCO}_3$  film. The insert in Figure 2A shows an optical micrograph that was taken between crossed polarizers. The absence of birefringence in most of the micrograph indicates that the  $\text{CaCO}_3$  film is predominantly amorphous. Figure 2A, how-



**Figure 1.** Fabrication of microstructured calcite films. A) Synthesis of PMAA brushes by SI-ATRP of sodium methacrylate from photolithographically patterned ATRP initiator functionalized substrates (bipy=2,2'-bipyridine). B) Directed deposition of a thin, metastable layer of amorphous calcium carbonate (ACC) and subsequent temperature-induced transformation of the metastable ACC layer into a microstructured, polycrystalline calcite film.

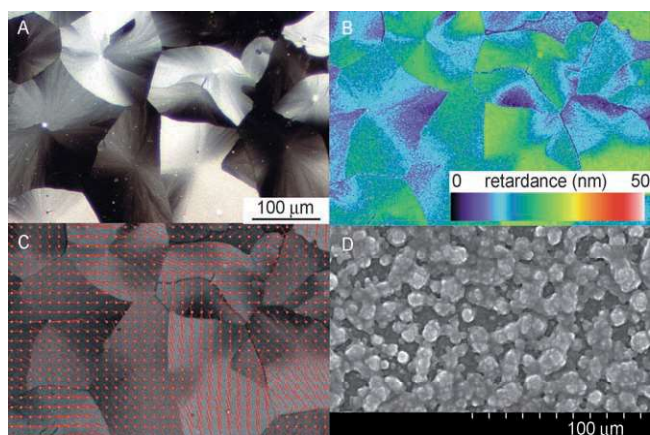


**Figure 2.** ACC film stabilized by a 100-nm-thick, unstructured PMAA brush. A) Differential interference contrast micrograph (inset: polarized optical micrograph); B) false-color LC-PolScope image; C) low- and D) high-magnification SEM images.

ever, also shows some isolated calcite crystals surrounded by circular features. These circular features can be attributed to the depletion of calcium carbonate around the forming crystal. Further confirmation of the amorphous nature of the  $\text{CaCO}_3$  film was obtained by determining the optical retardance of the film with a birefringence imaging system (LC-PolScope).<sup>[19,20]</sup> The optical retardance is a function of the orientation of the crystallographic *c* axis and the thickness of the crystalline film. As shown by the false color image in Figure 2B, the optical retardance of the ACC film varied by less than 1 nm throughout the whole micrograph, which can be attributed to the lack of crystallinity in the film. The small variations that can be observed are due to the residual stress in the film. The amorphous nature of the  $\text{CaCO}_3$  layer was also confirmed by attenuated total reflection (ATR)-FTIR experiments, which did not reveal the characteristic  $\nu_4$  absorption band of calcite at  $714\text{ cm}^{-1}$  (Supporting Information).<sup>[21]</sup> These experiments clearly indicate that the PMAA

brushes allow the deposition and stabilization of a thin, metastable ACC film. The scanning electron micrographs in Figure 2C and Figure 2D show that the ACC film is composed of spherical particles with diameters ranging from 20 to 100 nm.

The ACC layer was subsequently crystallized by heating the sample to 250 °C for 2 h. The polarized optical micrograph of the resulting film in Figure 3 shows a mosaic structure of discrete domains, each consisting of a flat calcite single

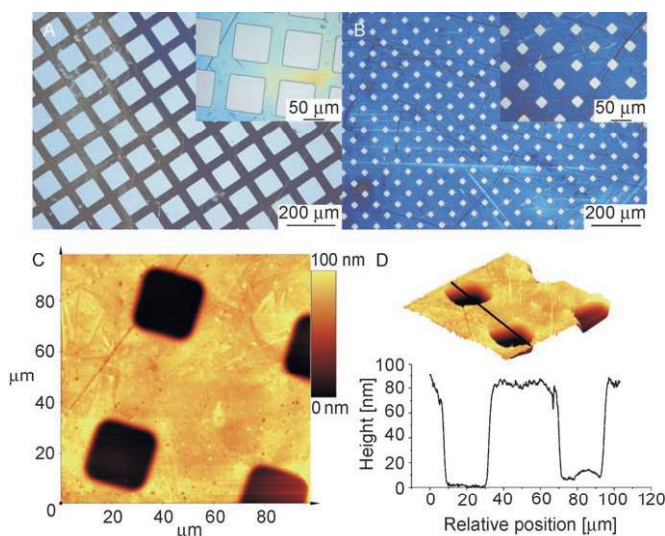


**Figure 3.** Thin calcite films obtained by heating a PMAA-brush-stabilized ACC film for 2 h at 250 °C. A) Polarized optical micrograph; B) LC-PolScope image (retardance values are indicated as false color); C) retardance gray-scale image (red vector overlay indicates the orientation of the slow birefringence axis); D) high-magnification SEM image.

crystal. ATR-FTIR spectra revealed a characteristic  $\nu_4$  absorption band at  $714\text{ cm}^{-1}$ , which supports the identification of the crystalline  $\text{CaCO}_3$  modification as calcite (Supporting Information).<sup>[21]</sup> To obtain insight into the crystallographic orientation of the crystalline domains in the calcite film, LC-PolScope experiments were performed. Figure 3B shows an LC-PolScope image, which illustrates that each domain in the polycrystalline film is characterized by a narrow range of optical retardance values. The small variations that can be observed within the individual domains can be ascribed to stress-induced birefringence, which might result from the drying and shrinking of the film during the heating process. Figure 3C is an image of the same area as shown in Figure 3B, however, with the direction of the slow axis of birefringence indicated at several points as small vectors. Within each domain in Figure 3C, the birefringence vectors point in the same direction, which suggests that the polycrystalline film is composed of flat single calcite crystals. Figure 3D shows a scanning electron micrograph, which illustrates that the calcite film consists of coalesced  $\text{CaCO}_3$  particles.

The experiments discussed so far indicate that PMAA brushes can act as ionotropic matrices for the stabilization of a metastable ACC phase and for the subsequent formation of thin, polycrystalline calcite films by a simple thermal treatment. A subsequent series of experiments addressed whether glass substrates covered with patterned PMAA brushes can

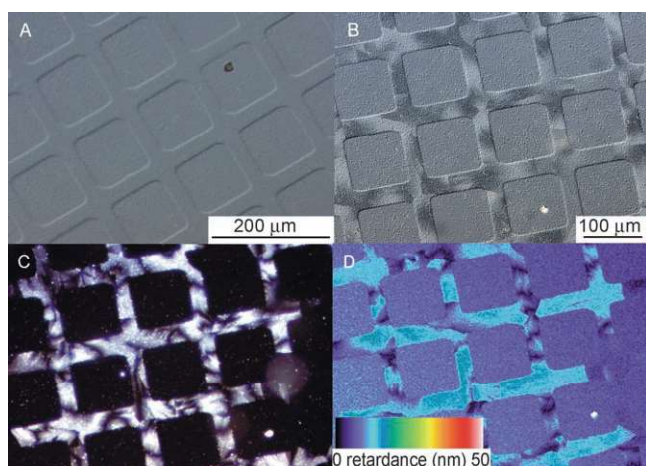
be used to fabricate microstructured calcite thin films. Patterned PMAA brushes were prepared by exposing substrates, which were functionalized with the ATRP initiator, to UV irradiation using a TEM grid as a photomask. Photo-irradiation leads to decomposition of the ATRP initiator in the exposed regions, and subsequent SI-ATRP of sodium methacrylate only takes place on regions on the substrates that are not irradiated.<sup>[22]</sup> This effect is illustrated in Figure 4, which shows optical and atomic force microscopy (AFM) images of 90-nm-thick PMAA brushes, which were prepared using photomasks of different mesh sizes.



**Figure 4.** A), B) Reflected light microscopy images of photolithographically patterned PMAA brushes supported on silicon substrates prepared using different photomasks (insets: enlargements); C) topographical AFM image; D) 3D projection of the AFM image and height profile of a photolithographically patterned PMAA brush.

Mineralization experiments with the micropatterned PMAA brushes were carried out as described above with subsequent thermal treatment (2 h, 250 °C) to induce the formation of a thin, polycrystalline calcite film. Figure 5 shows for comparison a DIC micrograph of a layer of ACC that has been formed using a 90-nm-thick, micropatterned PMAA brush with DIC, polarized optical, and LC-PolScope images of the same sample after heating to 250 °C for 2 h. Comparison of Figure 5A and C clearly indicates that deposition of ACC only occurs on regions of the substrate defined by the PMAA brush pattern. This feature is particularly evident from the absence of birefringence in Figure 5C in areas that are not covered with PMAA. Figure 5C also shows that after thermal treatment areas of the substrate that had been covered with PMAA reveal a mosaic-like texture, similar to that shown in Figure 3A, which indicates the formation of a crystalline calcite film. Using AFM, the thickness of the microstructured calcite film could be estimated at approximately 80–90 nm (Supporting Information). This result suggests that mineralization of  $\text{CaCO}_3$  occurs within the PMAA brush and indicates that the polycrystalline calcite thin film is an exact 3D replica of the





**Figure 5.** A) DIC micrograph of an ACC film deposited using a patterned 90-nm-thick PMAA brush on a glass substrate; B) DIC, C) polarized optical, and D) false-color LC-PolScope images of the same  $\text{CaCO}_3$  film after heating for 2 h at  $250^\circ\text{C}$ .

ionotropic PMAA matrix. Information about the crystallographic orientations of the calcite crystals in the microstructured film was obtained from LC-PolScope measurements. Comparison of Figure 3B and Figure 5D indicates a much narrower range of optical retardance values for the microstructured calcite film than for the unstructured sample. This result may be due to stress relaxation during crystallization which is facilitated in the microstructured sample.<sup>[8]</sup>

In conclusion, we have presented a novel strategy for the fabrication of microstructured calcite thin films. The process bears some resemblance to biological mineralization processes in that acidic macromolecules are used to form an ionotropic gel, which can host and stabilize a transient ACC phase and serve to direct crystal growth into geometries defined by the macromolecular matrix. We have used photolithographically patterned PMAA brushes as a biomimetic, acidic macromolecular matrix to fabricate microstructured calcite films that are an exact 3D replica of the PMAA brush. The success of the strategy relies on three key elements: 1) the use of photolithographic techniques to prepare microstructured PMAA brushes; 2) the ability of PMAA brushes to stabilize ACC and 3) the conversion of the metastable ACC phase into a polycrystalline calcite film by thermal treatment. In contrast to SAMs,  $\text{CaCO}_3$  mineralization does not occur on a 2D template, but within the ionotropic PMAA brush matrix. Consequently, the PMAA brushes act as a 3D matrix that is copied with high fidelity into the final calcite replica. While the lateral dimensions of the microstructures are defined by the lithographic techniques that are used to prepare the micropatterned PMAA brush, the thickness of the calcite film can be adjusted through the thickness of the PMAA brush, which can be easily controlled owing to the “living” nature of the SI-ATRP procedure. The feasibility of this strategy was demonstrated with the fabrication of relatively simple, microstructured calcite films. It is anticipated, however, that this technique may be readily extended to other inorganic materials and more complex structures by combining the flexibility of SI-ATRP with regards to the chemical compo-

sition and architecture of the polymer brush with the numerous lithographic and nonlithographic methods that are available to generate micropatterned surfaces. This approach could lead to the fabrication of a variety of novel microstructured inorganic materials with interesting optical, catalytic, or biological properties.

## Experimental Section

Details of the synthesis of the PMAA brushes and the mineralization experiments are given in the Supporting Information. Optical micrographs employing DIC and polarization contrast were recorded with a Hitachi HV-C20 A 3-CCD RGB camera at a resolution of  $3 \times 768 \times 576$  pixels mounted on an inverted stage microscope (Olympus IX70). Birefringence measurements were performed with a commercial LC-PolScope imaging system (CRI). Atomic force microscopy was carried out using a Nanoscope II microscope. Electron micrographs were taken from samples that were rotary shadowed with platinum (3 nm) in a Balzers BAF 300 freeze-fracturing device and examined in a Hitachi S-5200 field emission scanning electron microscope. ATR-FTIR spectroscopy was performed on a Nicolet Magna-IR 560 spectrometer equipped with a single-reflection diamond ATR system (Specac Golden Gate).

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