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# Patterned crystallization of calcite in vivo and in vitro

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**Abstract**

Biologically formed calcite crystals have unique, sculptured shapes that are believed to be regulated locally by specific macromolecules and by directional flux of ions into the microenvironment of crystal growth. This paper describes a biologically inspired synthesis of patterned calcitic films using micropatterned self-assembled monolayers (SAMs) which served as spatially constrained microenvironments for crystallization. The approach is based on the ability to govern mass transport to different regions of the surface at a micron scale, by patterning rapidly nucleating regions (carboxylate-terminated SAMs) in regions that are slowly nucleating (methyl-terminated SAMs) and by regulating their sizes, geometry and concentration of the crystallizing solution. The ion flux into the regions of crystal growth keeps the crystallizing solution over slowly nucleating regions undersaturated. Crystallization is then entirely restricted to the carboxylate-terminated regions of SAMs and results in the formation of large-area, high-resolution inorganic replicas of the underlying organic patterns. 

*Keywords:* Biomineralization; Patterned crystallization; Overgrowth; Diffusion-limited nucleation

## 1. Introduction

Modern technologies require innovative methods for controlled fabrication of crystalline materials with complex form [1–4]. Precise localization of particles, nucleation density, size and morphology are important, but not easily regulated parameters that affect the performance of inorganic materials. Recently, a range of strategies has been explored to control nucleation and growth of crystals based on molecular recognition at interfaces [4–6]. These methods include template-directed crystallization under compressed Langmuir monolayers [5,6], on self-assembled monolayers (SAMs) [7,8], on oriented polymer films [9,10], and in surfactant aggregates [4,11]. These studies have shown the great potential of using supramolecular templates to regulate crystallization, but it remains a challenge to control the patterns and architectures of the growing crystals.

At the same time, nature produces a wide variety of exquisite mineralized tissues fulfilling diverse functions, and often from simple inorganic salts [12]. This reflects directly or indirectly the controlling activity of the *organic macromolecules* that are involved in the formation of these materials [13]. Another important feature of biological crystallization is almost perfectly orchestrated control over the *microenvironment* of crystal growth [14]. Our approach to artificial crystallization is based on the combination of these concepts: that is, the use of organized organic surfaces patterned with specific initiation domains on a submicron scale

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(micropatterned SAMs) to control the microenvironment and structure of the nucleation site [15]. Here we explore the possibility to use these organic substrates for a high-resolution, patterned deposition of calcitic films. We show that crystallization is confined to well-defined, spatially delineated sites, and results in the formation of mineral patterns that replicate the templating surface with the edge resolution of $<50$ nm. The ability to construct periodic arrays of discrete single crystals or patterned crystalline films could find important applications in advanced inorganic materials and composites.

## 2. Experimental procedure

*Substrates*: Silicon wafers were coated with 2 nm of Ti, to promote adhesion, and then typically with 50 nm of metal (Ag or Au) using an electron beam evaporator (base pressure $10^{-7}$ Torr). Patterned SAMs were formed on metal films using microcontact printing [15–17]. The stamps were prepared by casting and curing poly(dimethylsiloxane) (PDMS) against rigid masters bearing a photoresist pattern formed using conventional lithographic techniques. PDMS stamps with various relief structures and periodicities ranging from a submicron to millimetre scale were "inked" with a 10 mM solution of $HS(CH_2)_{15}CO_2H$ in ethanol and brought into conformal contact with metal surfaces for 5 s. The stamps were then removed, and the substrates were washed with ethanol. The non-contact areas were derivatized with a 10 mM solution of $HS(CH_2)_{15}CH_3$ in ethanol by immersion for 1 min, washed with ethanol and dried.

*Crystallization*: The substrates (patterned SAMs or cleaned biogenic calcite crystals) were placed upside-down on supports in 10–100 mM calcium chloride solution in a closed desiccator containing vials of ammonium carbonate [10,15,18]. All experiments were carried out at room temperature for 30 min. Precipitation of calcite results from the diffusion of carbon dioxide vapor into the $CaCl_2$ solution. The overgrown specimens were then lightly rinsed in DDW and dried. The patterns of calcite crystals were examined using optical and scanning electron microscopy.

## 3. Results and discussion

Biologically formed calcite crystals often exhibit convoluted architectures with a remarkable, microscopic design (Fig. 1A) [12,13]. How they develop is still a mystery. We can only guess that their formation is controlled *locally* by specialized macromolecules, which implies the stimulation of crystal formation at certain sites and relative inhibition of the process at all other sites [12–14,18,19]. In order to illustrate the localized function of biological macromolecules that are involved in the formation of biominerals, we performed the slow epitaxial overgrowth of new calcite crystals on the

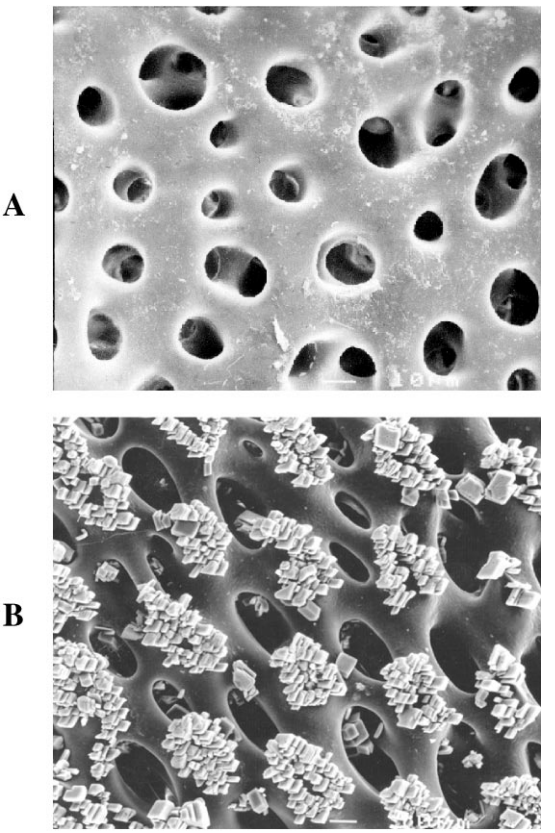


Fig. 1. Biogenic calcite crystals. (A) Convoluted shape of the spine of the brittle star *Ophiocoma wendti*. (B) Epitaxial overgrowth of synthetic calcite crystals on the spine surface. Note that the nucleation of the newly formed calcite crystals occurred locally at specific sites on the surface.

surfaces of biogenic calcite crystals [18] (Fig. 1B). It has been shown [18] that this technique can be used to map the distribution and availability of macromolecules within skeletal elements on the submicron scale. Crystallization occurred only at well-defined locations, due to the release of intracrystalline (conceivably nucleating) macromolecules into the microenvironment of the surface of biogenic calcite.

Our attempt to control patterned crystallization originated from the above concept of using specific macromolecules to build the microenvironment for the localized nucleation of crystals. We formed patterned SAMs of $\omega$-terminated alkanethiols with a controlled distribution of active nucleation sites within an inert background [15], using microcontact printing [16,17] (Fig. 2). We will focus on the crystallization of calcite on thus patterned organic substrates, although the experimental conditions and the mechanism discussed are applicable to the patterned precipitation of a wide range of inorganic salts. We chose SAMs terminated with $CO_2H$ and $CH_3$ groups to study the influence of surface structure on patterned crystallization based on previous studies demonstrating that these groups showed the largest difference in their ability to induce crystallization of calcium carbonate, with nucleation occurring most readily on the $CO_2H$-terminated surface [7,8].

In initial experiments, we compared the nucleating activity of the surfaces prepared by printing a pattern of one thiol and filling in the bare space not occupied by this pattern by dipping into a solution of a second thiol; the thiols used in these two steps could be different or the same (Table 1). The geometry of the control pattern consisted of circles with different diameters $d$ and periodicities $p$ (Fig. 2). The important conclusions from these studies are: (i) for different thiols, the induction time was shorter, and the density of nucleation was much higher, on the carboxylate-terminated surface than on the methyl-terminated surface; (ii) for the same thiols used in both steps, nucleation occurred more rapidly and with higher density, on printed regions of SAMs than on regions derivatized from solution; (iii) for large $p$, crystallization occurred in a characteristic "halo" pattern with no detectable nucleation within a certain distance from the more active

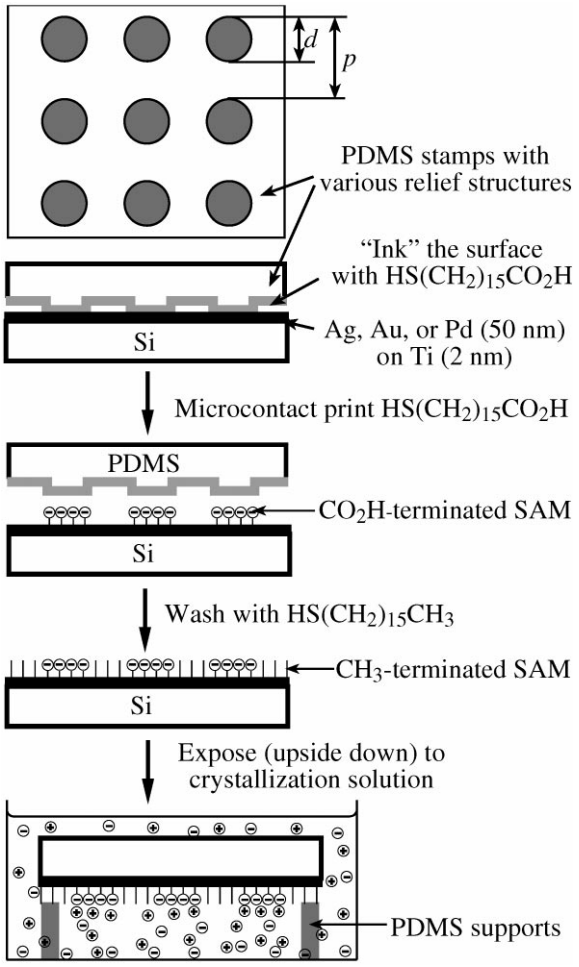


Fig. 2. Schematic presentation of the experimental design for patterned crystallization.

region (depletion zone); (iv) the most precise inorganic replication of the underlying organic film was achieved on patterned SAMs that were formed by stamping with the acid-terminated thiol and filling with the methyl-terminated thiol from solution, with features in the pattern smaller than the size of the depletion zone. Such conditions induce the highest nucleation density and unimodal size distribution of particles on acid-terminated regions and *totally* inhibit nucleation on the methyl-terminated regions; (v) for the latter experimental setup, the number of crystals nucleated within each active site

Table 1
Nucleation activity of patterned and nonpatterned SAMs of $HS(CH_2)_{15}CO_2H$ and $HS(CH_2)_{15}CH_3$ on silver

| Type of the surface | Induction time (min) | Nucleation density (crystals/mm$^2$)[a] | Crystal sizes (μm)[a] |
|---|---|---|---|
| Bare metal | ~ 2.2 | 900 | 0.1–40 |
| $CO_2H$-terminated SAM formed from solution | ~ 1.5 | 3000 | 4–18 |
| $CO_2H$-terminated SAM printed with a flat stamp | ~ 1.3 | 4000 | 6–15 |
| $CH_3$-terminated SAM formed from solution | ~ 3.4 | 250 | 1–33 |
| $CH_3$-terminated SAM printed with a flat stamp | ~ 2.9 | 450 | 3–27 |
| $CO_2H/CO_2H$[b] | ~ 1.1/1.4 | 5000/2000 | 9–11/3–19 |
| $CH_3/CH_3$[b] | ~ 2.7/3.5 | 500/200 | 5–19/1–31 |
| $CH_3/CO_2H$[b] | ~ 3.2/1.4 | 350/4000 | 3–23/8–14 |
| $CO_2H/CH_3$[b] | ~ 0.8/– | 6000/none | 9–10/– |

[a] After 30 min.
[b] $R_1/R_2$ surface corresponds to a micropatterned SAM with printed $R_1$-terminated thiol and filled with $R_2$-terminated thiol from solution.

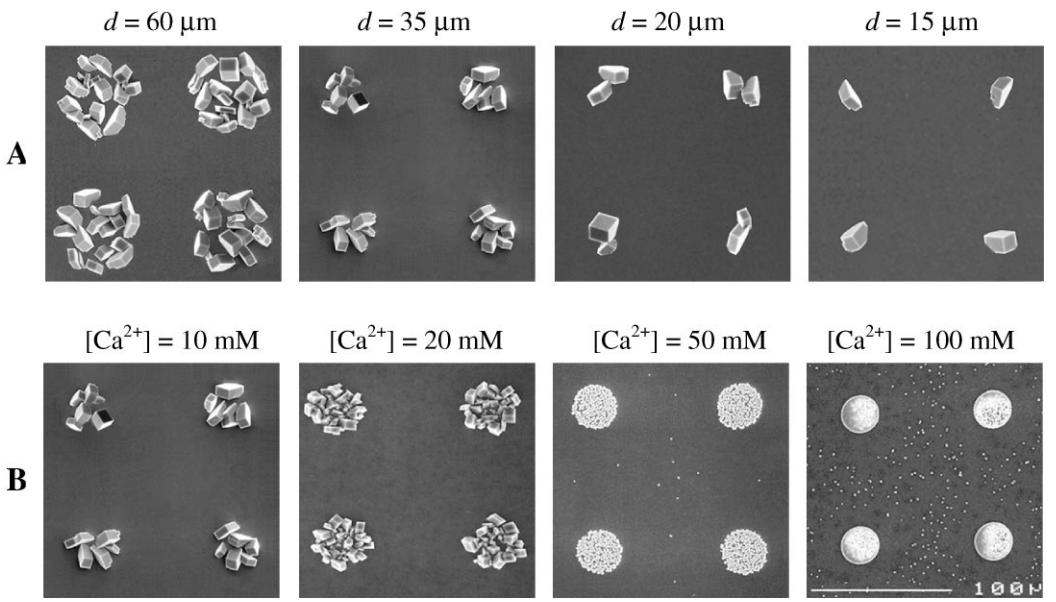


Fig. 3. Patterned crystallization of calcite on micropatterned SAMs consisting of a square array of printed, rapidly nucleating circles (SAMs of $HS(CH_2)_{15}CO_2H$) in a slowly nucleating background (SAMs of $HS(CH_2)_{15}CH_3$). (A) Number of crystals per active nucleation region, $n$, as a function of its size. The value, $n$, is proportional to the area, $S$, of the active region: $n \cong 16$, 6, 2 and 1 for $S \cong 2\,800$ ($d = 60$ μm), 960 ($d = 35$ μm), 320 ($d = 20$ μm), and 175 μm$^2$ ($d = 15$ μm). This relationship provides precise control over the nucleation density, by regulating the size of the features in the SAM. (B) Nucleation density and extent of area-selective nucleation as a function of concentration of crystallizing solution. The increase in concentration results in occasional crystallization on the methyl-terminated region, in agreement with the expected decrease in the size of the depletion zone [15,20].

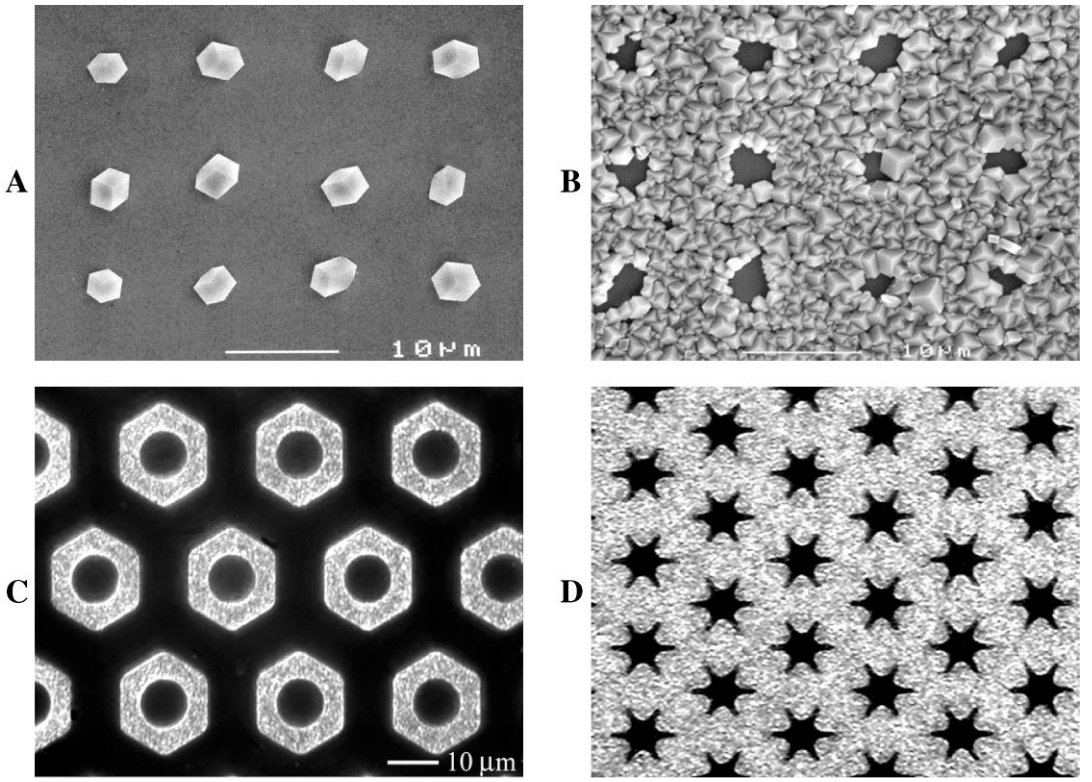


Fig. 4. Examples of high-resolution calcitic replicas of patterned SAMs (see text for details).

($n$) appeared to depend linearly upon its area ($n \sim d^2$) (Fig. 3A); (vi) variations in the concentration of calcium-containing solution affected the size of the "halo" pattern, induction time, density of nucleation and sizes of crystals (Fig. 3B).

We have suggested earlier that localized crystallization on patterned SAMs can be explained in terms of diffusion-limited nucleation [15,20]. By regulating the patterns of SAMs (size, geometry, functionality, supporting metal) and concentration of the crystallization solution, we can, therefore, control mass transport to different regions in the vicinity of the surface at a micron scale. As a result, a high-resolution, site-specific deposition of calcite occurs. The ability to pattern with SAMs of equivalent surface area, shape, distribution and functionality makes it possible to generate similar nucleation conditions that result in virtually simultaneous nucleation in different locations. The overgrown crystals are, therefore, of uniform size and nucleation density over large patterned areas.

Fig. 4 shows several examples of calcitic replicas of patterned SAMs with arbitrary sizes and complex shapes of active regions. Relationship (v) makes it possible to determine the size $d$ of the raised circles in the stamp to induce the formation of highly ordered two-dimensional arrays of single calcite crystals of uniform size and orientation [15] (Fig. 4A). We can reverse the crystallization pattern and fabricate the interconnected, oriented film of calcite crystals, by using the stamp with the opposite relief structure – recessed circles (Fig. 4B). The edge resolution of the calcitic replicas can be substantially increased with increasing the concentration of crystallizing solution: the complex patterns

of isolated calcitic islands (Fig. 4C) and interconnected calcitic films (Fig. 4D) follow the underlying organic pattern with the edge resolution of $< 50$ nm. The achieved resolution is two orders of magnitude higher than that reported in an earlier attempt to perform patterned crystallization on electron-beam damaged SAMs [21].

## 4. Conclusions

We conclude that the combination of two major ideas – (i) patterning SAMs in microregions having different nucleating activity and (ii) combining mass transport and the distances within the pattern so that the ion flux into the regions of crystal growth (the regions in which nucleation is rapid) limits the concentration of the solution over the slowly nucleating regions to values below saturation – provides a simple and convenient route to form crystalline materials with complex form. The power of this biologically inspired approach to patterned crystallization is its ability simultaneously to regulate the microenvironment and nanostructure of the nucleation site and to manipulate the near-surface gradients of concentrations of the crystallizing ions. Controlled fabrication of inorganic solids with microscale regularity and exquisite ornamentation could offer the exciting prospect of applications in the synthesis of new materials with optimized mechanical, optical, electric and catalytic performance.

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