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Hydrogels Coupled with Self-Assembled Monolayers: An in Vitro Matrix To Study Calcite Biomineralization

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Abstract: This paper describes the control of the nucleation and growth of calcite crystals by a matrix composed of an agarose hydrogel on top of a carboxylate-terminated self-assembled monolayer (SAM). The design of this matrix is based upon examples from biomineralization in which hydrogels are coupled with functionalized, organic surfaces to control, simultaneously, crystal morphology and orientation. In the synthetic system, calcite crystals nucleate from the (012) plane (the same plane that is observed in solution growth). The aspect ratio (length/width) of the crystals decreases from 2.1 ± 0.22 in solution to 1.2 ± 0.04 in a 3 w/v % agarose gel. One possible explanation for the change in morphology is the incorporation of gel fibers inside of the crystals during the growth process. Etching of the gel-grown crystals with deionized water reveals an interpenetrating network of gel fibers and crystalline material. This work begins to provide insight into why organisms use hydrogels to control the growth of crystals.

Introduction

Bio-inspired, mineralizing systems usually have focused on controlling either growth (crystal morphology) or nucleation (crystal orientation and density). In biomineralization, however, these two processes are controlled simultaneously. This paper presents an experimental setup, which combines hydrogels and self-assembled monolayers, to control both the nucleation and the growth of calcite ($CaCO_3$) crystals. This approach provides the ability to tune the orientation, morphology, and materials properties of the resulting crystals.

Crystal Growth in Hydrogels. Recently, researchers have described protein-based hydrogels as the primary component of the organic matrices associated with a variety of biominerals, including tooth enamel,[1] the nacreous layer in mollusk shells,[2] and trout otoliths.[3] In both tooth enamel and nacre, there is a defined, and possibly patterned, nucleating surface at which crystal growth is initiated.[4,5] Synthetically, crystal growth in hydrogels[6−10] is an alternative to solution-based strategies[11−16] for controlling the morphology (growth) of crystals. One advantage of using gels as a media for crystal growth is that they provide a stable mass transport mechanism that is dominated by diffusion.[17−20] A disadvantage of gels is that this diffusion-limited mass transport among isolated pores makes it more difficult than in solution for a homogeneous nucleus to reach the critical size required for growth. In other words, gel media suppress homogeneous nucleation by isolating the solutes in small pores.[17] Therefore, growth does not begin until the ions accumulate to a fairly high threshold supersaturation.

Crystal Growth on SAMs. In this work, to promote uniform nucleation in a hydrogel environment, we provide a defined, heterogeneous nucleating surface: a self-assembled monolayer (SAM) of ω-functionalized alkanethiols on gold (Figure 1). Functionalized surfaces, such as SAMs, can control the nucleation of minerals.[21−23] Several researchers have demonstrated that the identity and orientation of the terminal functional group on the SAM determine the crystallographic plane on which the

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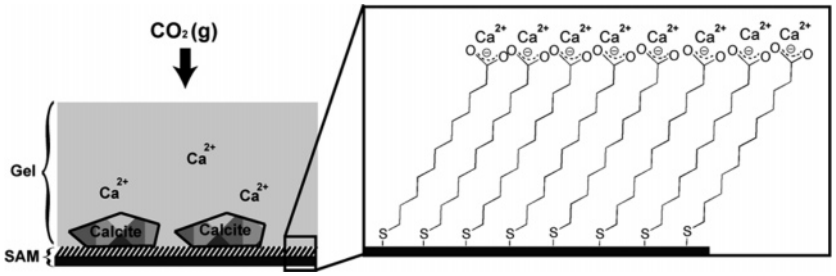

Figure 1. A schematic representation of the synthetic, organic matrix used to grow calcite crystals. An agarose gel containing $CaCl_2$ (7 mM) is formed on top of a carboxylate-terminated SAM (16-MHDA) on a gold-coated silicon wafer. The entire construct is then placed in a desiccator with $(NH_4)_2CO_3$(s). The slow diffusion of CO_2(g) into the gel results in the precipitation of calcite crystals. (The drawing is not to scale.)

crystals nucleate.[22−24] When SAMs are combined with soluble additives such as Mg^{2+}, it is also possible to control the morphology of the crystals as a function of the nucleating surface.[25]

Results and Discussion

Role of the SAM in Controlling Nucleation. Calcite crystals grown in the SAM-gel matrix (Figure 2c−h) are different from control crystals grown on a carboxylate-terminated SAM either in solution (Figure 2a,b) or in bulk agarose gel, in the absence of a SAM (Figure 2i). As compared to the crystals grown in bulk agarose gel, the polymorph distribution changes when the SAM is introduced. In bulk gel, a small number of vaterite spherulites form in addition to the star-shaped calcite crystals (Figure S1).[26] In contrast, when a carboxylate-terminated SAM (16-mercaptohexadecanoic acid (16-MHDA) on gold) is introduced into agarose gels of different concentrations (1−3 w/v %), calcite is the only polymorph observed, and the crystals grow primarily on the functionalized surface (as opposed to in bulk gel, Figure S2).

The 2-D ordered, anionic SAM serves as an active interface for heterogeneous nucleation so that the energetic barrier to nucleation and, thus, the threshold supersaturation required for nucleation of calcite are reduced.[27] The nucleation activity of the SAM has been discussed by several authors and may result from a small interfacial energy between the SAM and calcite due to an attractive interaction between the carboxylates on the SAM and the cationic (012) layer in calcite.[23,27] In addition, molecular dynamics simulations have indicated that the presence of a carboxylate monolayer enhances the nucleation rate of calcite as compared to the rate of homogeneous nucleation.[28]

Morphology of Gel-Grown Calcite. The morphology of the calcite crystals also changes upon introduction of the SAM to the agarose gel. The formation of the star-shaped calcite crystals in bulk agarose gel, as reported in the literature,[29] is attributed to the roughly spherical concentration contours of calcium and carbonate ions surrounding each rhombohedral crystal.[30] Above a certain critical size of the crystal, the difference in concentration between the vertices and the centers of the rhombohedral faces becomes significant enough that the vertices grow more rapidly, via 2-D nucleation, than the centers. This type of growth

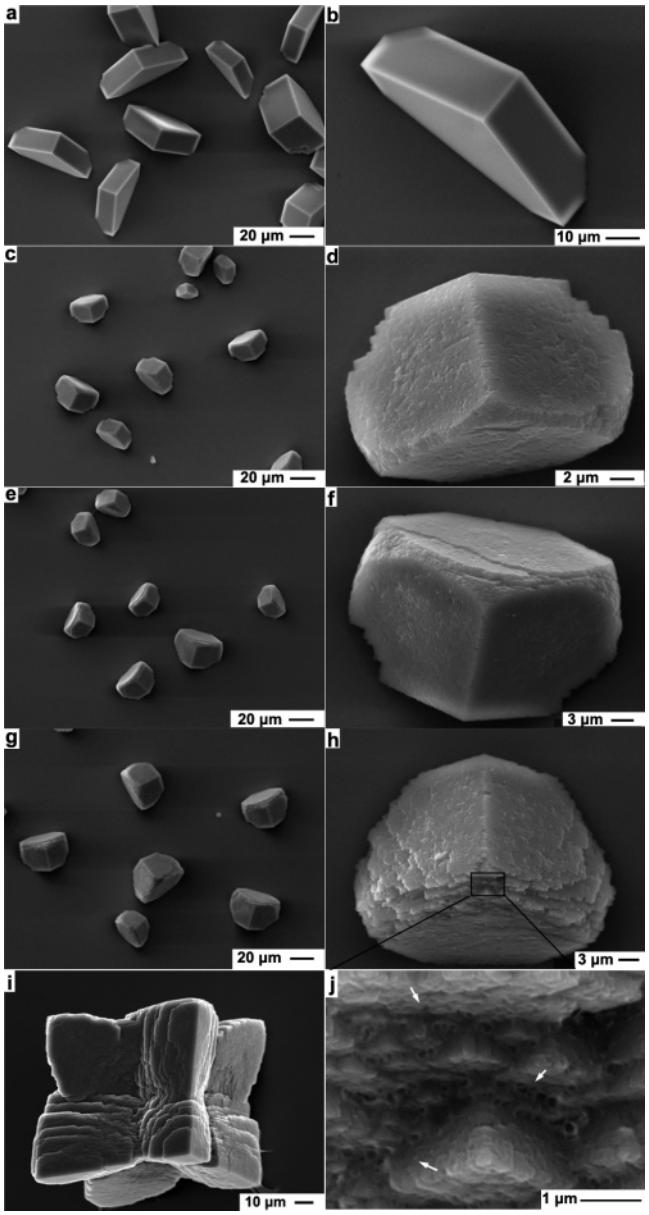

Figure 2. (a−h,j) SEM images of calcite crystals grown on carboxylate-terminated SAMs in (a,b) solution (7 mM $CaCl_2$); (c,d) agarose gel (1 w/v %; 7 mM $CaCl_2$); (e,f) agarose gel (2 w/v %; 7 mM $CaCl_2$); and (g,h,j) agarose gel (3 w/v %; 7 mM $CaCl_2$). (i) An SEM image of a calcite crystal grown in bulk agarose gel (2 w/v %; 7 mM $CaCl_2$).

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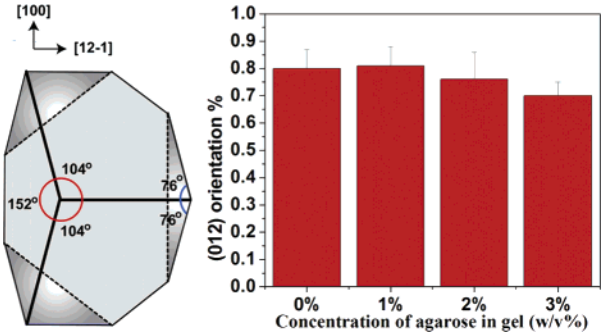

Figure 3. Left: Top view of a computer simulation of a calcite crystal nucleated on the (012) plane (SHAPE 7.1). The three angles (in red) between the crystal edges meeting at the upper corner of the crystal were measured to determine the crystallographic orientation direction. The crystallographic orientation of a regular {104} calcite rhombohedron unequivocally relates to these three angles, and for a (012) oriented crystal, the values of the three angles are 104°, 104°, and 152°, respectively.[31] When the top corner was not clearly defined, the angles in blue were measured to calculate the angles in red. Right: The results of the morphological analysis for the calcite crystals grown on carboxylate-terminated SAMs in solution (7 mM $CaCl_2$) or agarose gel (1−3 w/v %; 7 mM $CaCl_2$). Each crystal growth experiment was repeated three times, and 50 crystals from each experiment were measured.

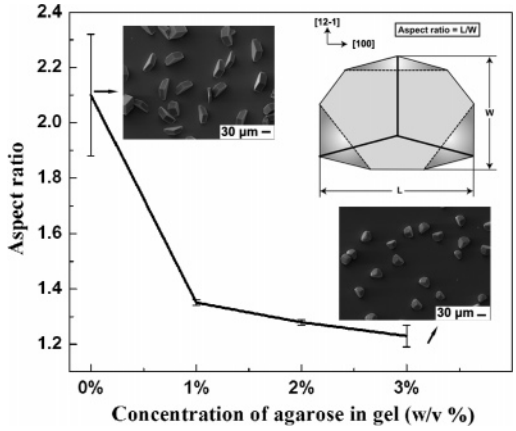

Figure 4. The aspect ratio (see inset) of calcite crystals nucleated on carboxylate-terminated SAMs changes as a function of the concentration of the agarose gel. Inset: Top view of a computer simulation of a calcite crystal nucleated on the (012) plane. The aspect ratio is defined as the length divided by the width.[33]

results in "skeletal" or "hopper-like" crystals.[30] Similar morphologies are not observed for the crystals grown on the anionic SAM, most likely because of the high nucleation density on the SAM (Figure S2). Typically, the star-shaped crystals are 50−100 μm along an edge, as compared to the crystals grown on the SAM, which are 20−40 μm in length (Figure 4, inset). On the basis of our limited data set, we speculate that the short distances between the crystals on the SAM prevent them from reaching the critical size to begin the "hopper-like" growth observed in bulk gel.

Orientation of Crystals on the SAMs. The quality of the SAM after introduction of the gel can be evaluated indirectly by determining the orientation of the calcite crystals grown on the SAM. The nucleating plane of calcite is very sensitive to the structure of the SAM surface and the conformation of the carboxylate headgroup.[22,24] The orientations of the calcite crystals grown on the SAMs were determined by powder X-ray diffraction and morphological analysis (Figures 3 and S3).[31] Statistical analysis of the data (Figure 3, right) shows that, in all experimental conditions (gel and solution), greater than 70% of the crystals have a (012) orientation. This orientation is in agreement with the literature on growth from solution on SAMs and thus indicates that the agarose gel has not (significantly) perturbed the structure of the SAM.[32] The slight reduction in orientation at the highest gel concentration (3 w/v %) may indicate some level of disruption of the SAM structure by the gel.

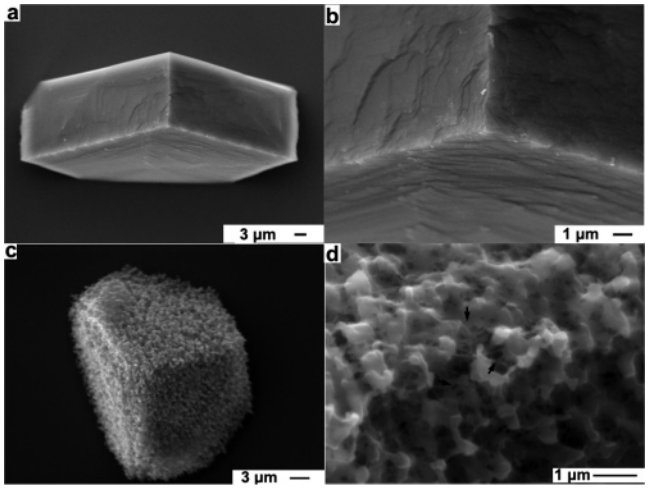

Figure 5. SEM images of: (a,b) a solution-grown crystal etched in DI water for 2 days and (c,d) a gel-grown (2 w/v % agarose) crystal etched in DI water for 2 days. Arrows in (d) highlight the locations of gel fibers.

Effect of the Gel on Crystal Growth. The introduction of the agarose gel to the SAM changes the crystal morphologies, as described by the aspect ratios (defined as the length/width, Figure 4 (inset)).[33] With increasing agarose concentration (from 1 to 3 w/v %), the aspect ratio decreases (Figure 4). The control crystals grown in solution have a high aspect ratio (2.1 ± 0.22), which has been attributed to a lattice match between calcite and the SAM in the [100] direction (length) but a mismatch in the [12-1] direction (width).[33] The aspect ratio drops to 1.4 ± 0.01 in 1 w/v % agarose gel and continues to decrease to 1.2 ± 0.04 for crystals grown in 3 w/v % agarose (Figure 4). From these results, it is clear that the anisotropic effect of the substrate is reduced for crystals grown in the agarose gel.

One explanation for the change in morphology is the incorporation of gel fibers inside of the calcite crystals.[10,17,19] Incorporated gel matrix can change the kinetics of growth, and thus the morphology, by altering the interface energy of the crystals.[19] There is evidence of incorporated organic material on the surfaces of crystals grown in the 3 w/v % agarose gels (Figure 2j, arrow). Gel fibers appear to bridge the macroscopic steps of the crystal, suggesting that they are inside of the crystal. To investigate further the presence of organic material inside of the crystals, we etched the gel-grown crystals in deionized (DI) water (pH ≈ 6.5) for 2 days. Etching is a common tech-

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nique used to detect the presence of organic material inside of biogenic minerals.[34−36] When synthetic calcite crystals are etched in DI water, shallow etch pits with {104} geometries appear on the surface (Figure 5a,b).[37] In contrast, after the gel-grown crystals are etched, the surfaces of the remaining crystals are covered completely by irregular etch pits (Figure 5c). A magnified image of the etched surface further reveals an interpenetrating network of gel fibers and crystalline material (Figure 5d). On the basis of these experiments, we suggest that the gel-grown crystals are composites that contain both agarose fibers and calcite.

Conclusion

The SAM/gel platform has the potential to allow us to control, independently, the nucleation and growth of inorganic crystals at surfaces. In this study of calcite crystals grown on carboxylate-terminated SAMs in agarose gel, we find that, at the SAM/gel interface, the SAM regulates the orientation of the calcite crystals with high fidelity (>70% have a (012) orientation). The SAM also contributes to phase selection by providing a heterogeneous substrate, thereby reducing the threshold supersaturation required for nucleation of calcite at the interface. Under these conditions, the formation of the metastable polymorph, vaterite, is suppressed.[27] The hydrogel, on the other hand, changes the growth kinetics and thus the morphology of the calcite crystals, as expressed by a decrease in the aspect ratio with increasing gel concentration.

This in vitro crystal growth system was designed based upon models of the organic matrices in which biomineralization occurs.[38] Like many biominerals, crystals grown in the gel/SAM setup have incorporated organic material, as revealed by etching experiments. The incorporation of proteins in biominerals is hypothesized to enhance their biogenic function, such as improving the mechanical properties of the brittle minerals.[39] The structure and properties of these single-crystal composites are of great interest to biologists and materials scientists.[40−43] The combination of biologically relevant hydrogels with SAMs is a promising approach for studying multiple features of biomineralization, including the mechanisms governing protein incorporation, crystal orientation, and polymorph selectivity.

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Supporting Information Available: Complete experimental details, Raman spectra, optical micrographs, and X-ray diffraction patterns (Figures S1−S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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