

Formation of Amorphous Calcium Carbonate Thin Films and Their Role in Biomineralization

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A new and simple method for preparing large-area and continuous calcium carbonate films under mild conditions is described. Amorphous calcium carbonate (ACC) films have been formed both in the presence and absence of a poly(acrylic acid) inhibitor. The transformation from ACC to crystalline vaterite/calcite has been observed by optical microscopy and confirmed by external reflection infrared spectroscopy. We have shown that the inhibiting effects of substrates and inhibitors on the transformation of ACC result in the formation of good CaCO₃ films. From our results, we suggested that ACC precipitates are initially formed from highly supersaturated solutions, which then deposit as films through the cooperation between an insoluble matrix and a soluble inhibitor. The matrix and inhibitor were also found to affect the growth, morphology, and structure of CaCO₃ crystal by influencing the phase transformation of ACC into crystalline forms. It has been shown that ACC plays an important role in the biomineralization and crystallization of calcium carbonate.

Introduction

Inorganic/organic hybrid films have attracted considerable attention because they offer the possibility of combining the distinct properties of organic and inorganic components. Biominerals, natural inorganic/organic hybrid materials, are formed through a cooperative interaction of inorganic materials with organic macromolecules, where the macromolecules control the nucleation, growth, morphology, structure, and crystal orientation of the inorganic component.¹ Moreover, biominerals with controlled structure are generally formed by self-organization and under mild solution conditions. Recently biomineralization has achieved much attention from biologists, chemists, and material scientists.²

CaCO₃ is one of the most extensively researched biominerals. CaCO₃ exists as three anhydrous crystalline polymorphs: vaterite (the least stable), aragonite, and calcite (the most stable). Additionally, three metastable forms—amorphous CaCO₃ (ACC), and monohydrate and hexahydrate CaCO₃—are also known. However, unlike the well-documented crystalline polymorphs, the metastable ACC has often been overlooked because of its instability and higher solubility. In particular, its coexistence with crystalline forms is difficult to detect.³

ACC has been widely found in biological organisms,^{4–8} where it is used as temporary storage deposits which, in time, transform into the more stable crystalline forms. Some ACC morphs are quite stable and have been utilized as mechanical stiffeners.³ The formation of ACC as a transient precursor phase from highly supersaturated solution has been known for a long time.^{9–13}

Certain additives such as phosphorus-containing compounds (phosphonate, polyphosphonate),^{14,15} magnesium ions,^{6,16,17} and certain organic macromolecules⁶ have been used to inhibit the transformation of ACC, making it possible to understand the role ACC plays in the crystallization and biomineralization of CaCO₃. The role of metastable phases in the spontaneous precipitation of calcium carbonate was investigated in the presence of triphosphate as an inhibitor.¹⁴ Sawada¹⁵ studied the mechanisms of the formation and transfor-

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(1) Mann, S.; Webb, J.; Williams, R. J. P. *Biomineralization: Chemical and Biochemical Perspectives*; VCH Publishers: New York, 1989.

(2) (a) Mann, S. *Nature* **1988**, *332*, 119. (b) Addadi, L.; Weiner, S. *Angew. Chem., Int. Ed.* **1992**, *31*, 153. (c) Aksay, I. A.; Trau, M.; Manne, S.; Honma, I.; Yao, N.; Zhou, L.; Fenter, P.; Eisenberger, P. M.; Gruner, S. M. *Science* **1996**, *273*, 892. (d) Bunker, B. C.; Rieke, P. C.; Tarasevich, B. J.; Campbell, A. A.; Fryxell, G. E.; Graff, G. L.; Song, L.; Liu, J.; Virden, J. W.; McVay, G. L. *Science* **1995**, *264*, 48. (e) Calvert, P.; Rieke, P. *Chem. Mater.* **1996**, *8*, 1715.

(3) Addadi, L.; Raz, S.; Weiner, S. *Adv. Mater.* **2003**, *15*, 959.

(4) Beniash, E.; Aizenberg, J.; Addadi, L.; Weiner, S. *Proc. R. Soc. London, Ser. B* **1997**, *264*, 461.

(5) Weiss, I. M.; Tuross, N.; Addadi, L.; Weiner, S. *J. Exp. Zool.* **2002**, *193*, 178.

(6) Aizenberg, J.; Lambert, G.; Weiner, S.; Addadi, L. *J. Am. Chem. Soc.* **2002**, *124*, 32.

(7) Levi-Kalishman, Y.; Raz, S.; Weiner, S.; Addadi, L.; Sagi, I. *Adv. Funct. Mater.* **2002**, *12*, 43.

(8) Raz, S.; Testeniere, O.; Hecker, A.; Weiner, S.; Luquet, G. *Biol. Bull.* **2002**, *203*, 269.

(9) Brečević, L.; Škrčić, D.; Garside, J. *J. Cryst. Growth* **1986**, *74*, 399.

(10) Söhnel, O.; Mullin, J. W. *J. Cryst. Growth* **1982**, *60*, 239.

(11) Brečević, L.; Nielsen A. E. *J. Cryst. Growth* **1989**, *98*, 504.

(12) Kojima, Y.; Kawanobe, A.; Yasue, T.; Arai, Y. *J. Ceram. Soc. Jpn.* **1993**, *101*, 1145.

(13) Koga, N.; Nakagoe, Y.; Tanaka, H. *Thermochim. Acta* **1998**, *318*, 239.

(14) Clarkson, J. R.; Price T. J.; Adams, C. J. *J. Chem. Soc., Faraday Trans.* **1992**, *88*, 243.

(15) Sawada, K. *Pure Appl. Chem.* **1997**, *69*, 921.

(16) Lose, E.; Wilson, R. M.; Seshadri, R.; Maldrum, F. C. *J. Cryst. Growth* **2003**, *254*, 206.

(17) Raz, S.; Weiner, S.; Addadi, L. *Adv. Mater.* **2000**, *12*, 38.

mation of calcium carbonate, and its inhibition by phosphorus-containing compounds. Essentially, supersaturated solutions of CaCO_3 yield ACC precipitates, which are transformed into the metastable crystalline polymorph, and then finally into the more stable calcite polymorph, and then finally into the more stable calcite phase.¹⁵ Xu et al.¹⁸ prepared a macroscopic CaCO_3 film on an amphiphilic porphyrin template in the presence of a poly(acrylic acid) (PAA) inhibitor. Micropatterned self-assembled monolayers (SAMs) on Au or Ag have been employed to induce the formation of metastable ACC films. In this case, crystallization was initiated on the SAM surface using a calcite nucleation center, which formed a mm-sized single calcite crystal.¹⁹ Gower and Tirrell^{20,21} reported that the addition of charged polypeptides, in particular poly(aspartate), to supersaturated solutions of calcium carbonate resulted in the deposition of CaCO_3 films. Recently, powerful synchrotron radiation has been used to determine the nucleation and growth of ACC,^{22–25} and to characterize its fine structure.^{26–28} The role of ACC in the crystallization and biomineralization of CaCO_3 has long been underestimated. We believe that ACC plays a significant part in these activities, and that extending the knowledge base of this metaphase will provide valuable insight into the fields of biomineralization and material science in general.

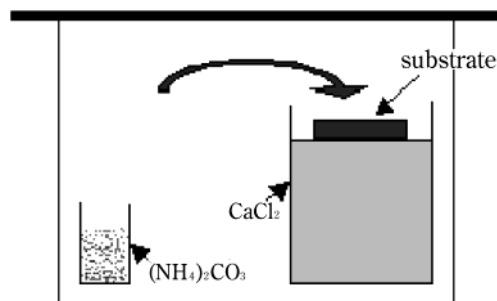
Here, we describe a new and simple method to prepare large-area and continuous ACC thin films. Good ACC films can be formed both in the presence and absence of inhibitor. Transformation from ACC to crystalline forms was directly observed. The inhibiting effects of the substrate with different surface energy and soluble additives on the transformation of ACC are examined.

Experimental Section

Substrates. Octadecyltrichlorosilane and chloropropyltriethoxysilane were purchased from Aldrich and used without further purification. Silicon wafers were cleaned by immersion in freshly prepared piranha solution (concentrated $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 7:3, w/w) and heated for 1 h at 100 °C, then rinsed thoroughly with distilled water.

Cleaned silicon wafers were immersed in anhydrous toluene solutions of the appropriate silanizing agent (10 mM) for 2 h under argon. The modified wafers were rinsed with both toluene and ethanol, blown dry with argon, and then baked on a hot plate at 120 °C for 20 min.²⁹ The modified substrates were sonicated twice in toluene for 1 min, rinsed with ethanol, and then dried in a vacuum. Additionally, cleaned silicon

Scheme 1. Illustration of the Experimental Setup



wafers were employed as OH modified surfaces. Static contact angles on the monolayer films were measured using ultrapure water as the probe liquid (Face CA-A contact-angle goniometer). At least five replicate measurements were performed for each specimen, where the measurement error was below 2°.

Film Preparation. The experimental setup for preparing CaCO_3 thin films is illustrated in Scheme 1.

Two vials, one containing a 20 mM calcium chloride (CaCl_2) solution in the presence of 10 $\mu\text{g}/\text{mL}$ poly(acrylic acid) (M_w 2000, Aldrich), and the second, containing ammonium carbonate powder, were placed in a desiccator, and a cleaned silicon wafer was inverted and placed above the CaCl_2 solution. A CaCO_3 thin film was then deposited on the cleaned substrate via slow diffusion of CO_2 , produced by decomposition of ammonium carbonate at room temperature (4 h). The film was rinsed with ultrapure water for 1 min after deposition, and then dried in a stream of nitrogen. The resulting film was kept in air at different time intervals and observed using optical microscopy (OM), and characterized by field emission scanning electron microscopy (FESEM), IR spectroscopy, and X-ray diffraction (XRD).

A reference experiment was performed using a 20 mM CaCl_2 solution in the absence of PAA, deposited over a period of 4 h. In general, however, experiments were conducted without inhibitors, using 40 mM CaCl_2 solutions and deposition times of 1 h.

Film Characterization. CaCO_3 thin films formed on Si wafers were observed using an optical microscope (Zeiss) equipped with cross polarizers in the reflective mode. High-resolution images of the deposited films (Pt-coated prior to examination) were obtained using a field emission scanning electron microscope (FESEM, Hitachi S-4200), at an operating voltage of 8 kV. For external reflection IR spectroscopy (ERS) analysis, the films on silicon wafers were scanned from 4000 to 400 cm^{-1} using p-polarized light with an incident angle of 80° and a resolution of 4 cm^{-1} (FTIR, Bruker IFS 66v). A cleaned silicon wafer was used as a reference. X-ray diffraction (XRD) was performed using a synchrotron X-ray radiation source (3C2 beamline, wavelength 1.598 Å) at the Pohang Accelerator Laboratory, Pohang, Korea.

Results

Formation of Calcium Carbonate Films. Although the biomineralization and crystallization processes associated with CaCO_3 have been extensively studied, there have been relatively few reports concerning the preparation of CaCO_3 films.^{18–21,30–34,44} The formation of self-organized hybrid ceramic films in mild condition is very interesting to material scientists. Previous reports have shown that ACC is readily

(18) Xu, G. F.; Yao N.; Aksay, I. A.; Groves, J. T. *J. Am. Chem. Soc.* **1998**, *120*, 11977.

(19) Aizenberg, J.; Muller, D. A.; Graul, J. L.; Hamann, D. R. *Science* **2003**, *299*, 1205.

(20) Gower, L. A.; Tirrell, D. A. *J. Cryst. Growth* **1998**, *191*, 153.

(21) Gower, L. B.; Odem, D. J. *J. Cryst. Growth* **2000**, *210*, 719.

(22) Rieger, J.; Thieme, J.; Schmidt, C. *Langmuir* **2000**, *16*, 8300.

(23) Bolze, J.; Peng, B.; Dingenouts, N.; Panine, P.; Narayanan, T.; Ballauff, M. *Langmuir* **2002**, *18*, 8364.

(24) Dimasi, E.; Patel, V. M.; Sivakumar, M.; Olszta, M. J.; Yang, Y. P.; Gower, L. B. *Langmuir* **2002**, *18*, 8902.

(25) DiMasi, E.; Olszta, M. J.; Patel, V. M.; Gower, L. B. *CrystEngComm* **2003**, *5*, 346.

(26) Pontoni, D.; Bolze, J.; Dingenouts, N.; Narayanan, T.; Ballauff, M. *J. Phys. Chem. B* **2003**, *107*, 5123.

(27) Levi-Kalishman, Y.; Raz, S.; Weiner, S.; Addadi, L.; Sagi, I. *J. Chem. Soc., Dalton Trans.* **2000**, *21*, 3977.

(28) Hasse, B.; Ehrenberg, H.; Marxen, J.; Becker, W.; Epple, M. *Chem. Eur. J.* **2000**, *6*, 3679.

(29) Archibald, D. D.; Qadri, S. B.; Gaber, B. P. *Langmuir* **1996**, *12*, 538.

(30) Kato, T.; Suzuki, T.; Amamiya, T.; Irie, T.; Komiyama, M.; Yui, H. *Supramol. Sci.* **1998**, *5*, 411.

(31) (a) Kato, T.; Amamiya, T. *Chem. Lett.* **1999**, 199. (b) Sugawara, A.; Kato, T. *Chem. Commun.* **2000**, 487. (c) Kato, T. *Adv. Mater.* **2000**, *12*, 1543.

(32) Hosoda, N.; Kato, T. *Chem. Mater.* **2001**, *13*, 688.

(33) Kato, T.; Sugawara, A.; Hosoda, N. *Adv. Mater.* **2002**, *14*, 869.

(34) Zhang, S.; Gonsalves, K. E. *Langmuir* **1998**, *14*, 6761.

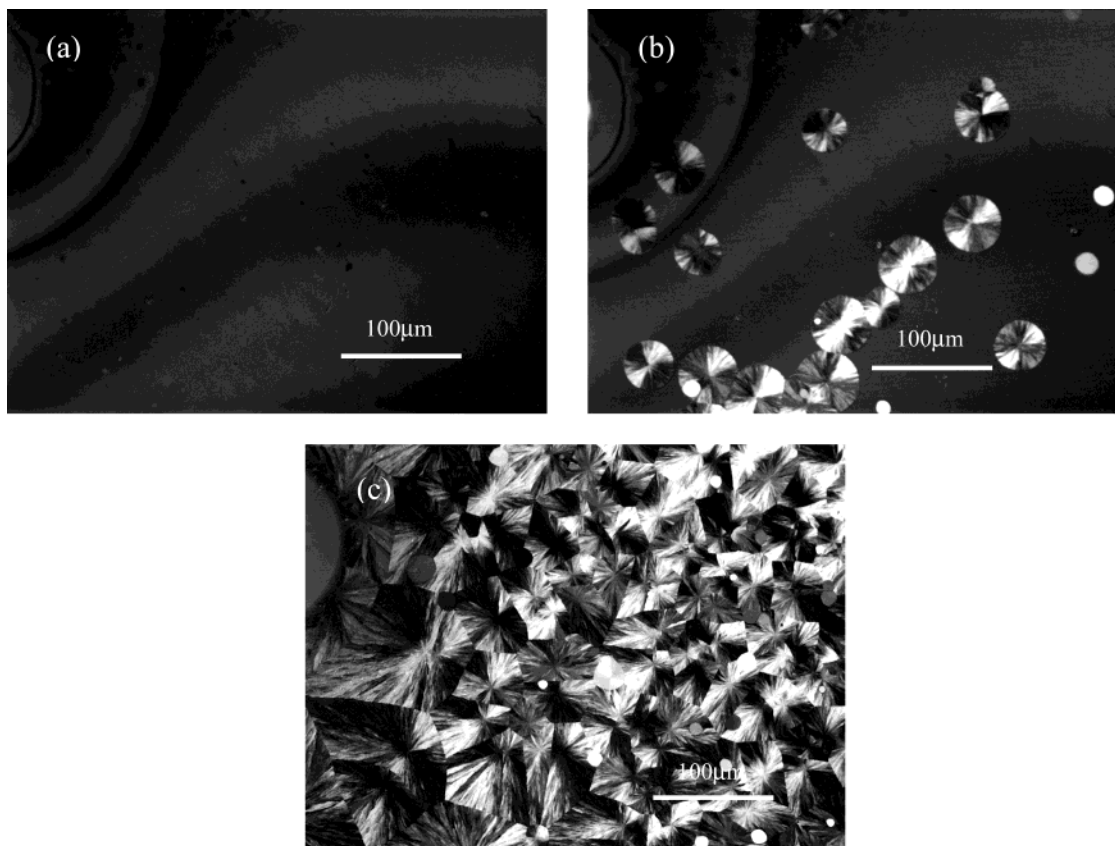


Figure 1. Optical micrographs of a large-area and continuous calcium carbonate film deposited on a silicon wafer under crossed-polarized light in reflective mode: (a) as-deposited; (b) 2 h later; (c) 16 h later.

precipitated from highly supersaturated solutions, and rapidly transforms into more stable crystalline forms.¹⁵ We propose that if the crystallization process is intercepted during ACC formation, an ACC film may be achieved. Here we describe a new and simple method to prepare ACC films by intercepting the crystallization process. Figure 1 shows the polarized optical micrographs of a good CaCO_3 film prepared from a 20 mM CaCl_2 solution containing a 10 $\mu\text{g/mL}$ PAA inhibitor, deposited over a period of 4 h. Following removal from solution, the CaCO_3 film was dried; the resulting film exhibited a dark, homogeneous appearance, with a few sporadic crystals on the surface (Figure 1a). This reflects the amorphous character of the CaCO_3 film, which is further confirmed by X-ray diffraction (not shown here). No crystal peaks appear in the XRD spectrum of the film immediately after its removal from the solution. However, after 2 h in ambient conditions, crystal nucleation and growth were observed by polarized microscopy on the surface of the CaCO_3 film (Figure 1b), and after a further 16 h, the entire film was composed of spherulites (Figure 1c). The observed Maltese-cross texture suggests that these films are composed of polycrystalline spherulites.

SEM was used to characterize the morphology of the final CaCO_3 film following complete transformation from ACC. In the presence of a PAA inhibitor, a CaCO_3 film was readily achieved on bare silicon wafers. By the naked eye several square centimeters of film can be seen on the substrate, Figure 2a is shown as a part of the film. Although some patches and pores exist within these films, the image clearly demonstrates that large-area and continuous CaCO_3 films can be formed.

Inspection of the film at higher magnification using FE-SEM (Figure 2b) revealed the existence of several nanopores on the surface. The film thickness is approximately 400 nm (inset of Figure 2b). Additionally, X-ray diffraction data (Figure 3) of the ACC film indicates it has undergone complete transformation to give a polymorphic phase composed of calcite and vaterite crystallites.

The transformation from ACC to the CaCO_3 crystalline phase was monitored using external reflection infrared spectroscopy (ERSIR) (Figure 4). Infrared spectra were taken from the same CaCO_3 film at various times during phase transformation, representative of the amorphous, partially transformed, and completely transformed stages, respectively. The slightly broad adsorption bands in the IR spectra of the as-deposited ACC film are characteristic of amorphous and poorly crystalline materials (Figure 4). Additional adsorption bands such as the carbonate out-of-plane bending adsorption at 866 cm^{-1} (ν_2), the symmetric stretch in noncentrosymmetric structure at 1070 cm^{-1} (ν_1), and a split peak at 1472 and 1384 cm^{-1} (ν_3) are all peaks attributable to ACC.^{3,11} It is well-known that ACC is particularly unstable in ambient conditions, where it rapidly transforms into crystalline forms. After the CaCO_3 film was removed from the ERSIR vacuum chamber and stored in ambient conditions for approximately 30 min, the IR spectrum (partial transformation in Figure 4) revealed a slight shift of the peak at 866 cm^{-1} due to crystallization of ACC (inset of Figure 4). Additional IR adsorption bands (874 , 745 , and 712 cm^{-1}) characteristic of the crystalline polymorphs (vaterite and calcite) are clearly visible in the ERSIR

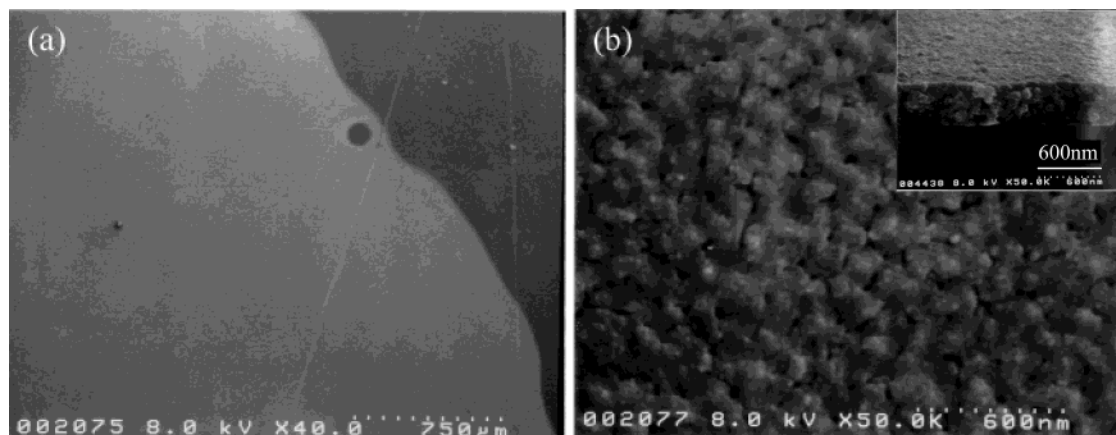


Figure 2. SEM photographs of a large-area and continuous calcium carbonate film deposited on a silicon wafer after complete transformation: (a) low magnification; (b) high magnification. Inset: cross-sectional view of the film.

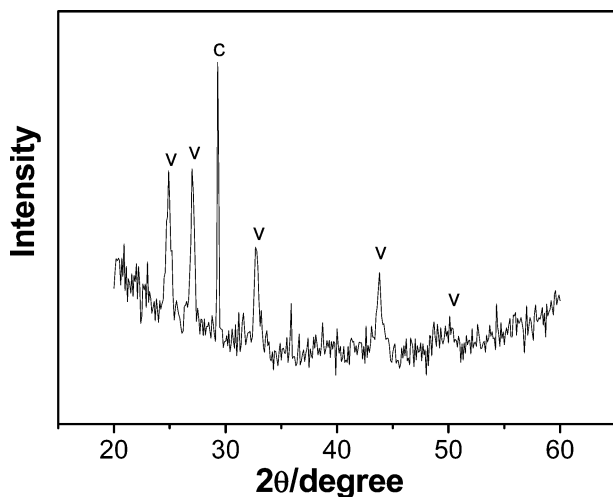


Figure 3. X-ray diffraction spectrum of the CaCO_3 film on silicon wafer after complete transformation (c, calcite; v, vaterite).

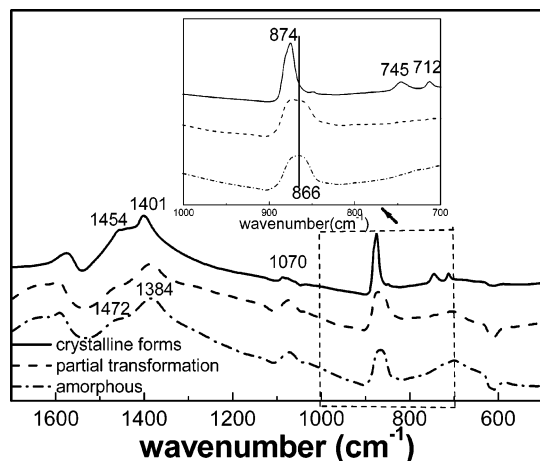


Figure 4. IR spectra of the CaCO_3 film prepared by using 40 mM CaCl_2 solution in the absence of PAA on a silicon wafer (three curves were obtained from the same film at different time intervals). The inset is the enlargement of spectra from 1000 to 700 cm^{-1} .

spectrum following complete transformation (Figure 4). It shows the process of transformation from metastable ACC into crystalline vaterite and calcite. The results are in accordance with the X-ray diffraction result (Figure 3), which confirms the presence of both vaterite

and calcite phases within the final CaCO_3 film. The concurrent shifts in the asymmetric and symmetric carbonate peaks (ν_1 and ν_3) provide additional confirmation for the phase transformation. The characterization of IR is in accordance with the observation of optical microscopy (Figure 1).

Inhibiting Effect of Inhibitors. It is well-known that amorphous CaCO_3 (ACC) is less stable and more soluble than the anhydrous crystalline forms of CaCO_3 . Its coexistence with crystalline forms makes it difficult to detect. The strategy forming crystalline aragonite or calcite via a transient ACC phase is widespread in nature.³ It has been found that magnesium ions, phosphate, and certain organic macromolecules are involved in the inhibiting effect on the transformation of the ACC phase in biological systems.^{6,17} In the past, PAA was widely used as a soluble additive to control the crystallization of CaCO_3 because of its similarity with soluble proteins which are known to control mineralization in biological organisms. On one hand, PAA adsorbed on the surface of a functional template promotes the nucleation and growth of CaCO_3 crystals, but on the other hand, PAA adsorbed on CaCO_3 nuclei or the surface of a growing crystal in solution inhibits the crystallization process, and modifies the overall crystal morphology.^{1, 34}

An ACC film is easily prepared on silicon substrates from a 20 mM CaCl_2 solution in the presence of a PAA inhibitor (4 h). This is in stark contrast to the deposition behavior observed under the same conditions but in the absence of PAA, where only separate or aggregated crystals were observed to form on the silicon substrate, indicating that the inhibiting effect of PAA on crystallization of amorphous CaCO_3 should also be considered. The inhibiting effect of PAA on CaCO_3 crystallization, and its effect on crystal morphology, may derive from its inhibiting effect on the transformation of amorphous CaCO_3 .

In our system, the deposition time plays an important role in the formation of ACC films. If the deposition time is too short, then poor quality films are produced. If the deposition time is too long, then ongoing film formation will become disrupted and formation of aggregated crystals will occur on the substrate. It must be noted that the diffusion of NH_3 and CO_2 gases into the solution, which we assume occurs at a similar rate for every sample run, sets a time scale for the rate of change

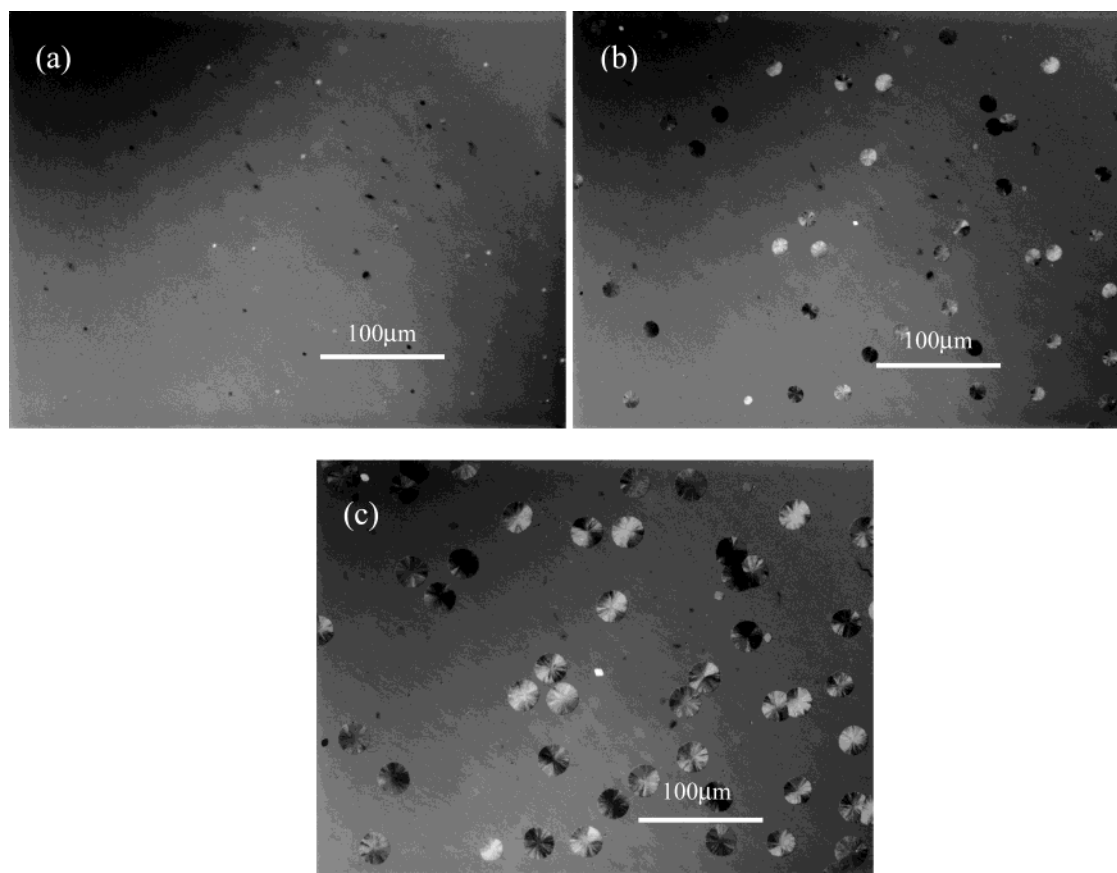


Figure 5. Optical micrographs of the CaCO_3 film prepared by using 40 mM CaCl_2 solution in the absence of PAA on a silicon wafer under crossed-polarized light in reflective mode: (a) as-deposited film; (b) 25 min later; (c) 50 min later.

of supersaturation, along with the chemical concentrations chosen. This time scale evidently has a marked effect on the films obtained. Consequently, there appears to be a specific set of kinetic conditions allowing good quality, reproducible ACC films to form on silicon substrates. It is well established that the presence of a PAA inhibitor promotes the formation of ACC films; however, the present results additionally emphasize that some kinetic conditions allow for the formation of uniform ACC films.

This is clearly evident in the image shown in Figure 5, where an ACC film was successfully deposited on a silicon wafer in the absence of a PAA inhibitor. The gradual transformation from ACC into a crystalline form is observed in the air over a period of 50 min. It is interesting to note that in the absence of PAA, both crystal nucleation and growth in the ACC film are decidedly more rapid, indicating that the deposition period favoring the formation of ACC films is narrower in the absence of the inhibitor. Additionally, higher-concentration CaCl_2 solutions are required to prepare good ACC films in the absence of an inhibitor. This was highlighted by the failure to prepare ACC films from 5 mM and 10 mM CaCl_2 solutions in the absence of PAA. Yet, in the presence of just a small quantity of PAA (5 $\mu\text{g}/\text{mL}$), both solutions yielded ACC films, underlining the importance of the inhibitor's role. Indeed, we have also observed that magnesium ions behave as excellent inhibitors in the preparation of ACC films, exhibiting a better inhibiting effect on the transformation of the unstable ACC phase than PAA.

Inhibiting Effect of Substrates. Biominerals (natural inorganic/organic hybrid materials) are formed through the cooperative interaction between inorganic materials and organic macromolecules, where the latter control the nucleation, growth, morphology, structure, and crystal orientation of the inorganic component. Generally, organic macromolecules can be divided into two categories: soluble additives and insoluble matrixes. The promoting effect imparted by templates on crystal nucleation and growth has been explored using various types of insoluble matrixes such as Langmuir monolayers,^{18,35–39} insoluble polymers,^{40–42} self-assembled monolayers (SAMs),^{43–47} and biopolymers.^{48,49}

(35) Mann, S.; Heywood, B. R.; Rajam, S.; Birchall, J. D. *Nature* **1988**, *334*, 692.

(36) Heywood, B. R.; Rajam, S.; Mann, S. *J. Chem. Soc., Faraday Trans.* **1991**, *87*, 735.

(37) Heywood, B. R.; Mann, S. *Chem. Mater.* **1994**, *6*, 311.

(38) Heywood, B. R.; Mann, S. *Adv. Mater.* **1994**, *6*, 9.

(39) Champ, S.; Dickinson, J. A.; Fallon, P. S.; Heywood, B. R.; Mascal, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 2716.

(40) Berman, A.; Ahn, D. J.; Lio, A.; Salmeron, M.; Reichert, A.; Charych, D. *Science* **1995**, *269*, 515.

(41) Ueyama, N.; Kozuki, H.; Doi, M.; Yamada, Y.; Takahashi, K.; Onoda, A.; Okamira, T.; Yamamoto, H. *Macromolecules* **2001**, *34*, 2607.

(42) Manoli, F.; Dalas, E. *J. Cryst. Growth* **1999**, *204*, 369.

(43) Aizenberg, J.; Black, A. J.; Whitesides, G. M. *Nature* **1999**, *398*, 495.

(44) Aizenberg, J.; Black, A. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1999**, *121*, 4500.

(45) Lee, I.; Han, S. W.; Lee, S. J.; Choi, H. J.; Kim, K. *Adv. Mater.* **2002**, *14*, 1640.

(46) Küther, J.; Tremel, W. *Chem. Commun.* **1997**, 2029.

(47) Küther, J.; Seshadri, R.; Knoll, W.; Tremel, W. *J. Mater. Chem.* **1998**, *8*, 641.

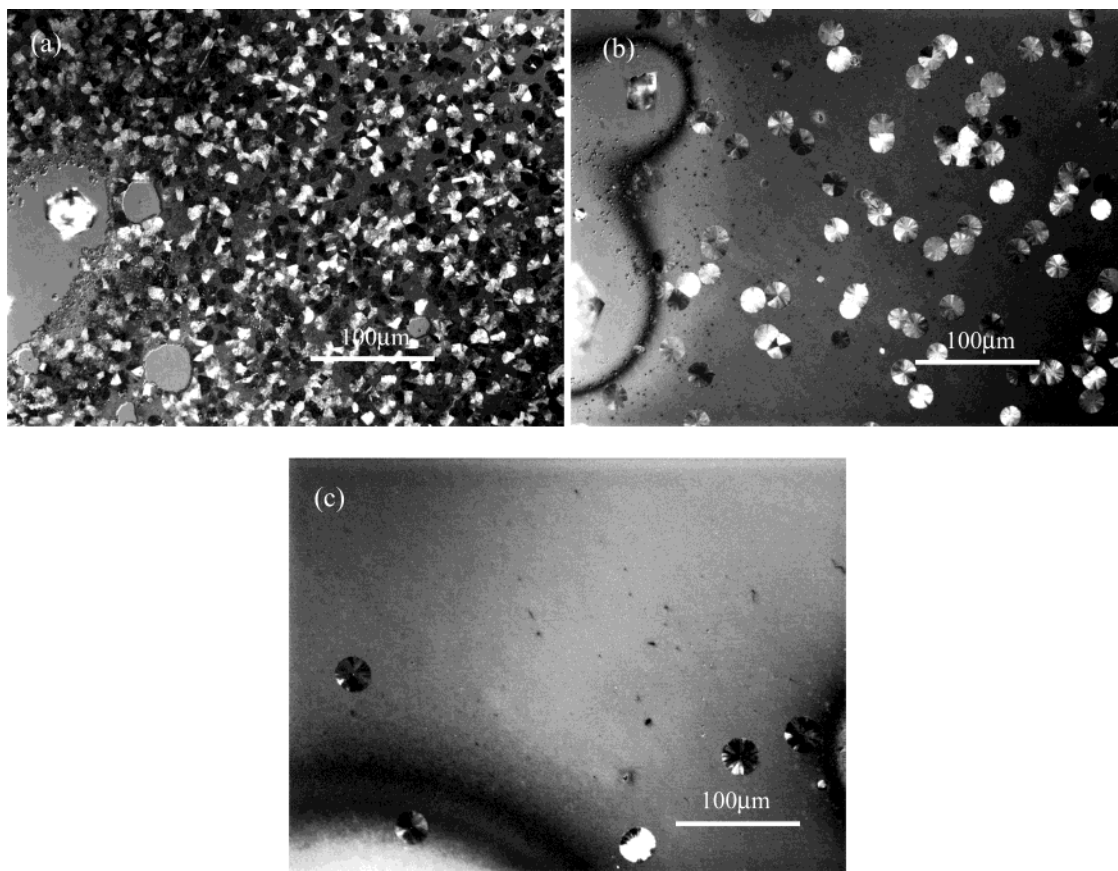


Figure 6. Crossed-polarized optical micrographs (reflective mode) of the CaCO_3 film on substrates with different surface energy prepared in the presence of PAA and at the same crystallization time (20 mM CaCl_2 , 10 $\mu\text{g/mL}$ PAA, 4 h): (a) surface with end group $-\text{CH}_3$; (b) surface with end group $-\text{Cl}$; (c) surface with end group $-\text{OH}$.

Table 1. Water Contact Angles of Substrates with Different End Groups

end group on surface	contact angle ($^\circ$)
$-\text{OH}$	25
$-\text{Cl}$	44
$-\text{CH}_3$	112

It was observed that the surface energy of the substrates had a significant effect on the formation of ACC films. To confirm this effect, we modified the surface of silicon wafers to yield surfaces with the following end groups (OH, Cl, and CH_3). The water contact angles for each surface were recorded (see Table 1).

Surfaces modified with OH end groups have a relatively small contact angle, expressing typical hydrophilic character, whereas the CH_3 -terminated SAMs present a typical hydrophobic surface. These surfaces, together with the Cl-terminated surface, were then used as substrates to deposit ACC films to gauge the effect of substrate on film formation. The photographs shown in Figure 6 represent the ACC deposited films grown on CH_3 - (a); Cl- (b); and OH- (c) terminated surfaces after 20 min crystallization.

The nucleation and growth of CaCO_3 crystals on CH_3 -terminated SAMs is typically faster than that on Cl- and OH-terminated surfaces. However, although a film

was observed, the quality of the produced film was decidedly poor. The hydroxy-terminated surface, on the other hand, gave rise to much slower nucleation and growth of CaCO_3 crystals on the surface, which yielded a better film. This implies that OH end groups show better inhibiting effects toward the transformation of ACC, and inhibit the nucleation and growth of CaCO_3 crystals. Furthermore, Cl-terminated SAMs also present a more compatible surface for the formation of ACC films. In the absence of a PAA inhibitor, CH_3 -terminated monolayers yield only the remnants of a film, coupled with the production of aggregated crystals on the surface. In contrast, ACC films are readily formed on OH- and Cl-terminated monolayers, despite the absence of PAA. This suggests that the more hydrophilic surfaces exhibit an inhibiting effect on the ACC metastable phase similar to that of the PAA inhibitor. We can therefore surmise that hydrophilic surfaces promote the formation of good ACC films. It is known that amorphous CaCO_3 usually contains a small amount of water, which is released following transformation from ACC to crystalline polymorphs.¹⁹ The more hydrophilic surfaces may interact with these water molecules within the ACC phase, preventing them from escaping, which results in formation of good ACC films.

Discussion

In biomineralization, it is not clear whether matrix molecules stimulate crystal nucleation or simply act to localize preformed crystals forming spontaneously in highly supersaturated solutions, i.e., direct epitaxy

(48) Falini, G.; Fermani, S.; Gazzano, M.; Ripamonti, A. *Chem. Eur. J.* **1997**, *3*, 1807.

(49) Grassmann, O.; Müller, G.; Löbmann, P. *Chem. Mater.* **2002**, *14*, 4530.

versus multistep crystallization. It has been reported that certain matrixes directly promote nucleation; however, there is a lack of strong and direct evidence supporting this claim.¹⁸ It has also been suggested that film assembly is a multistep process in which CaCO_3 is initially deposited in an amorphous state and then undergoes phase transformation into the final crystalline phase.¹⁸ Cölfen and Mann⁵⁰ recently proposed a model for matrix-mediated nucleation in biomineralization, which involves the phase transformation of amorphous primary particles, and Gower et al. suggested the use of an environmentally friendly, polymer-induced liquid-precursor (PILP) process for the deposition of calcium carbonate films. They observed that poly(aspartate) induces liquid-liquid phase separation of droplets of a mineral precursor, which deposit on the substrate and coalesce to form a coating, eventually solidifying into crystalline calcitic films.²¹ Recently, DiMasi et al.²⁵ have shown that there is no epitaxy relation to the structure of calcium carbonate crystal with fatty acid monolayer by in-situ synchrotron X-ray scattering.

Multistep crystallization typically involves a series of steps: initially forming an amorphous phase and then undergoing subsequent phase transformation to form a crystalline structure. Identification of the initial ACC phase is often precluded by its instability and high solubility. However, the metastable ACC phase can easily be captured on clean silicon wafers, by intercepting the crystallization process in its early stages as described above. In our experiment, large-area and continuous metastable ACC films have been prepared, both in the presence, and absence of an inhibitor. The subsequent transformation from ACC to more crystalline CaCO_3 phases was clearly observed by optical microscopy, and characterized by external reflection infrared spectroscopy (ERSIR), providing evidence to support the mechanism for a multistep crystallization process.

It is generally accepted that the biomineralization of CaCO_3 is dependent on soluble macromolecules and insoluble matrix templates. However, the mechanism by which they affect and control the crystallization of CaCO_3 has yet to be fully determined.^{1,51} Kato and co-workers³⁰⁻³³ obtained calcium carbonate thin films using a combination of a soluble macromolecular inhibitor and an insoluble macromolecular template. The authors declared that the cooperation between the template and the soluble, acidic, macromolecular inhibitor is essential for CaCO_3 thin film formation. In contrast, we investigated the phase transformation of ACC using PAA (inhibitor), without the use of a functional template. We observed the formation of a large-area and continuous ACC film, which then subsequently transformed into a crystalline CaCO_3 film. Interestingly, we found it was still possible to form an ACC film even in the absence of an inhibitor, providing that shorter deposition times and higher levels of supersaturation were used.

Several classes of organic surfaces, such as Langmuir monolayers,³⁵⁻³⁹ SAMs,⁴³⁻⁴⁷ biological macromole-

cules,^{48,49} and functionalized polymers,⁴⁰⁻⁴² have been used to control both nucleation and crystal growth. Those studies focused mainly on the dependence of crystal orientation on molecular complementarity (e.g., symmetry requirement and stereochemical requirement) between the crystal and the template.¹ However, in a multistep crystallization process, the insoluble substrates are thought to possess a certain degree of control over the morphology, structure, and crystal orientation of CaCO_3 , by ultimately affecting the stability and transformation of the ACC phase. The interaction between functional surfaces and the unstable ACC phase is an important factor that controls both nucleation and crystal growth.

From our results, it is surmised that in the initial stages, ACC precipitates are spontaneously formed in highly supersaturated solutions. The inhibitor in solution is adsorbed onto the ACC surface, inhibiting the crystallization of the metastable phase. The presence of adsorbed water^{11,13} within the ACC phase shows relatively high flow and favors film formation.²¹ ACC films are typically formed on substrates selected for their surface properties that promote interaction with the metastable CaCO_3 phase. Because of its greater instability and solubility, metastable ACC readily transforms into crystalline forms in supersaturated solution by dissolving and subsequently recrystallizing. When a crystal nucleus forms in an ACC film immersed in a supersaturated solution, the surrounding ACC dissolves and CaCO_3 crystal growth is promoted in the direction normal to the template. Consequently, the ACC film becomes destroyed and large crystals and crystal aggregates are formed on the template surface. By removing the films from solution, this redissolution-recrystallization process cannot occur, thus limiting crystallization to only occur within the films.

Conclusion

We have prepared large-area and continuous ACC films by intercepting the crystallization process at the point where ACC formation occurs and ACC transformation is inhibited. This strategy provides a pathway for the preparation of CaCO_3 thin films under mild conditions. This new route provides an excellent opportunity to elucidate the mechanism involved in the crystallization and biomineralization of CaCO_3 . Valuable evidence regarding the multistep crystallization of CaCO_3 can be derived from the observed formation of ACC films and their subsequent transformation into crystalline structures. Insoluble matrixes and soluble macromolecules are found to affect the growth, morphology, and structure of CaCO_3 by influencing the phase transformation of ACC.

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(50) Cölfen, H.; Mann, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 2350.

(51) Weiner, S.; Addadi, L. *J. Mater. Chem.* **1997**, *7*, 689.