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BIOMIMETIC PROMOTION AND INHIBITION OF CRYSTAL GROWTH IN CALCIUM CARBONATE

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Abstract

Living organisms have evolved effective mechanisms to control the growth of inorganic crystalline materials as structural elements. Previous research has shown that proteins rich in aspartic acid play a pivotal role in driving crystalline orientation, structure, and morphology of calcium carbonate biominerals in the formation of shells in marine bivalves (mussels, clams, abalone). In our research, we examine the effect of aspartic acid on the growth of calcium carbonate by a novel vapor diffusion-based growth technique. The vapor diffusion approach uses a gas-permeable membrane as a barrier to control the physical location of crystal growth. In this way, we are able to directly observe the crystal nucleation and growth using optical microscopy and record changes in growth rates and crystal orientation as we add peptide or polyelectrolyte-based modifiers to the growing crystals. Raman spectroscopy was employed to characterize the resulting crystals and provide insight into the actions of the growth modifiers. Our work is directed towards understanding how molecular modifiers interact with inorganic materials. Developing controls for the growth of calcium carbonate materials could have impacts in many industries which rely on the materials (including pharmaceutical development, household products, and paper, rubber, and paint manufacturing).

Introduction

Biological organisms have developed specialized techniques to incorporate inorganic and organic components into well structured hybridized materials (Ludwigs et al. 2006; Naka and Chujo 2001). This is observed with some biological organisms that produce protective structures that have been shown to increase in strength up to three thousand times when formed as

biominerals complexed with proteins, as compared to the same materials produced in purely mineralized form (Naka and Chuja 2001). This is a commonly found motif in mineralized frameworks found in organisms such as mollusks and other organisms (Addadi et al. 2006; Orme et al. 2001; Sarikaya et al. 2003; Sugawara et al. 2003). The formation of these hybrid materials in living organisms is part of a process called biomineralization (Miura et al. 2005). Recently, efforts to harness such processes to produce inorganic materials has lead to the development of the field known as biomimetics. This approach seeks to emulate approaches that biological organisms employ but apply it to synthetic materials for potential use in a wide range of applications, such as semiconductor devices, pharmaceuticals, and household products (Boncheva et al. 2002).

A common material found in many organisms as a biomineral is calcium carbonate ($CaCO_3$). This compound is found in the eggshells of birds, as well as in teeth and shells of mollusks (Thompson et al. 2000). Calcium carbonate exists in three typical structures in nature: vaterite (generally spherical), aragonite (spicules), and calcite (rhombohedra), in order of increasing thermodynamic stability (Sarikaya et al. 2003). Although calcite has the lowest energy structure, the presence of other polymorphs depends on nucleation and other kinetic factors.

The investigation into CaCO3 structure and formation in biomineralization has been extensive. Kim et al. (2006) incorporated proteins AP7-N, AP24-N, and n16-N, rich in aspartic acid and all found as constituents of abalone nacre and oyster, to promote elongation and inhibition of different surface region domains in calcite (Kim et al. 2006). Butler et al. (2006) investigated the incorporation of carboxylic acids and sulfated polysaccharides into calcite and found that carboxylic acid promoted formation of stacked rosettes, while sulfated polysaccharides inhibited calcite crystallization (Butler et al. 2006). Some studies have investigated the control of surface modification of calcite by the use of self-assembled monolayers on gold, while others have investigated growth control by titration and filtration through glass wool to monitor orientation in calcium carbonate (Addadi and Weiner 1985; Lee et al. 2001).

Our research utilizes similar approaches but mimics the membrane scaffolding and integration of solution modifiers of invertebrate systems. This paper examines a novel approach for crystal modification at the gas-liquid-solid interface via a semi-permeable polymer membrane composed of polydimethylsiloxane (PDMS). This is a novel application of PDMS as a gas permeable membrane to investigate the growth modification of calcium carbonate in solution. Our biomimetic methods have successfully demonstrated that in the presence of L-aspartic acid vaterite is the dominant form and that this crystal is stable with time. Previous studies have found that vaterite will thermodynamically reorganize into calcite through an amorphous $CaCO_3$ phase (Vagenas et al. 2003). In the absence of solution modifiers, calcite is the dominant polymorph produced. One goal of this research is to understand how the amino acid interacts with the calcium carbonate and drives the nucleation and growth of specific polymorphs.

Experimental Section

Our experimental design was implemented to understand the nucleation and synthesis of the generated crystals. To develop a system that mimicked biological activity, we employed a gas permeable membrane formed from PDMS. This was made by mixing a 1:10 ratio of precursor polymer to curing reagent (Dow Coming 187). The PDMS membrane was formed in Petri-dishes of two different thicknesses, a thin membrane of approximately 57μm and a thick membrane of approximately 1cm thick. The thick PDMS was cut into 0.5 x 1.0inch blocks. Two holes were then punched into the block with a metal rod generating a 0.3cm in diameter hole. The thin membrane was cut into small 0.6 x 0.6cm squares which were placed over the holes. The membranes were cleaned with liquid soap and rinsed extensively in de-ionized water and placed on Corning cover glass slips of 24mm x 50mm (Fig. 1).

This experiment utilized two amino acids: D and L aspartic acid. Solutions of 20 mM $CaCl_2$ and 1mM aspartic acid (Fluka), in 50 μl increments were used to exhibit $CaCO_3$ formation. Calcium carbonate crystals were generated using a slow nucleation process. We used ammonium carbonate (Fisher), which decomposes to give off CO_2 and NH_3 gas. To induce vaterite formation 1mM D or L-aspartic acid with 20mM $CaCl_2$ solutions were made. The following equations detail calcium carbonate crystal growth:

A. $(NH_4)_2CO_3 \Rightarrow NH_3g + H^2CO_3$
B. $H_2CO_3 \Rightarrow H_2O + CO_2g$
C. $CaCl_2 \Rightarrow Ca^{+2} + Cl^-$
D. $CO2g + H_2O \Rightarrow H_2CO_3 \Rightarrow H+ + CO_3^{2-}$
E. $CO_3^{2-} + Ca^{+2} \Rightarrow CaCO_3$

As the $(NH_4)_2CO_3$ decomposes into carbon dioxide and ammonia gas, the CO_2 will diffuse from the external environment and into the thin membrane. The CO_2 is then converted into bicarbonate when reacted with water, which then decomposes into aqueous carbonate ion. The carbonate ion binds to the calcium ion in solution and nucleates calcium carbonate crystals. Note that reactions A and B take place outside the thick PDMS segment, while C, D, and E take place inside the thick PDMS segment (Fig. 1, Fig. 2).

The setup designed in Figure 1 was placed into a 100mm diameter Wheaton desiccator. The pH of the $CaCl_2$ solution was found to be 10.3 and the pH in the desiccator environment was 8.5. Ammonium carbonate, crushed and weighed at 0.7g was placed into a small cap on the bottom of the desiccator. This allowed the slow diffusion of CO_2 gas to penetrate the thin membrane. Since CO_2 diffuses through the PDMS membrane, it will pass through the thin 57μm thick membrane before diffusing through the 1cm monolith. Thus nucleation occurs on the thin membrane (Fig. 2).

Growth Chamber

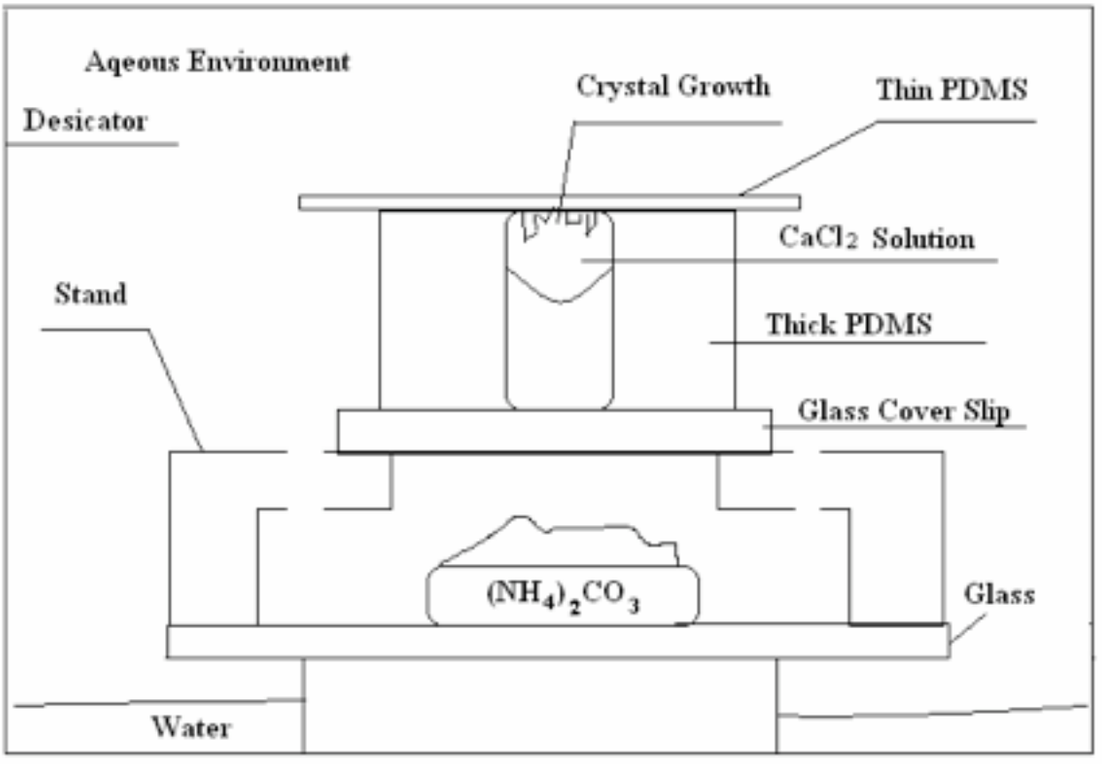

Figure 1: Experimental set up for production of calcite crystals.

Reactions Occurring in PDMS Membranes

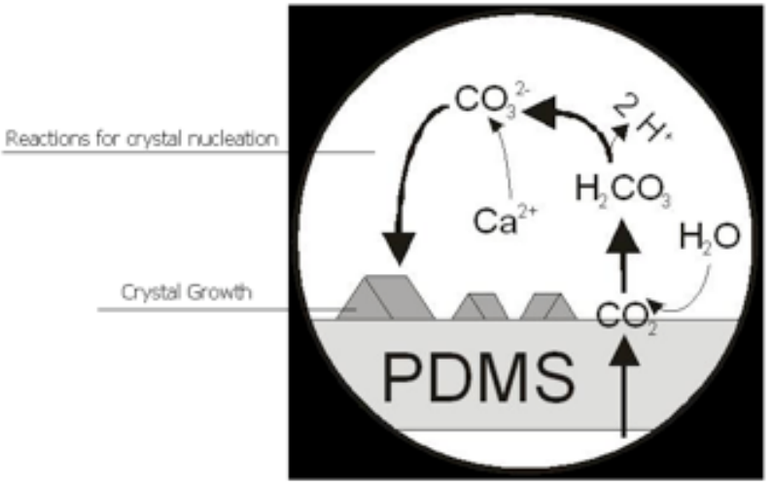

Figure 2, above: Illustrates how the ions of Ca^{+2} and CO_3^{2-} combine to form calcium carbonate crystals.

Calcium carbonate crystals were allowed to grow for 24 hours. The 57μm PDMS membrane bearing crystals was removed, briefly washed in de-ionized water, dried in a stream of filtered air, and stored for further investigation. Light microscopy and Raman anaylsis were used to identify specific polymorphs of $CaCO_3$.

Results/Discussion

Calcite and vaterite may be distinguished by shape, as they are characteristically either rhombohedral or spherical, respectively. To identify specific polymorphs of calcium carbonate, inspection by optical microscopy and identification by Raman microscopy and spectroscopy were used. To ensure that the crystals forming were calcium carbonate, we used simple polarization microscopy. $CaCO_3$ is a birefrigent material. Thus, by using crossed polarizers at 90 degrees, we were able to distinguish between precipitated starting material, $CaCl_2$ (which is not birefringent), and the material of interest, $CaCO_3$.

Raman spectroscopy revealed characteristic peaks for each polymorph crystal examined. This quantitative analysis is used to strictly identify which polymorph is being formed. This was conducted by selecting crystals through optical microscopy then collecting Raman spectra of specific crystals, which allowed us to characterize and identify individual crystals on the PDMS membrane. Raman spectroscopy was employed to demonstrate quantitative analysis of distinct peaks for each polymorph, calcite at 154, 280 and 711cm^{-1} and vaterite at 288, 301, and 750 cm^{-1} (Fig. 3) (Kontoyannis and Vagenas 2000). The Raman spectra were also used for categorizing data since the less stable vaterite has been observed to thermodynamically morph to the more stable calcite morphology over time (Vagenas et al. 2003).

We were also interested in the mechanism between the amino acid aspartic acid and calcium chloride to induce the specific crystal orientation of vaterite. Based on electrostatic charges, it is thought that the calcium ions on the crystal during growth are interacting with the negatively charged acidic carboxyl groups. This would cause an impeding interaction of ions to form on the crystal faces. Therefore the crystal cannot grow in a certain direction. This has been seen with similar acidic polypeptides, where the control of crystal faces has been hindered in the presence of acidic peptides (Volkmer et al. 2004). Much about this interaction is still unknown; however some potential explanations can be suggested.

One mechanism behind this electrostatic relationship suggests that the Ca^{+2} from $CaCl_2$ will interact with the COO^- from aspartic acid and thereby induce crystal modeling, causing adsorption of layers of amino acid on the crystal faces and lowering the surface energy, which

should induce a change in equilibrium (Teng et al. 1998). The binding of such ions is not random and has been shown to be part of an ordered array of calcium and carboxyl groups (Addadi et al. 1986). According to Gibbs free energy considerations, stable vaterite can be favored in the formation of supersaturated micro-environments in which the concentration of carboxyl groups increases with a corresponding increase in the electrostatic interactions between Ca^{+2} and COO^{-} groups on the aspartic acid (Tong et al. 2004).

Evidence for such an interaction can be seen from light microscopy growing crystals (Fig. 4). Some of the faces of crystal grown after several hours are clearly seen to be inhibited after the addition of aspartic acid. Some faces of the calcite crystals appear to be degenerate and become rounded at the edges after additions of aspartic acid, compared to the pictures before the acid additions (Fig. 4). This appearance indicates interactions between the calcium (on the growing calcite surface) and carboxylate funtional groups on the amino acid, which seem to be impeding growth on the edges of the calcite crystals. Similar affects have been observed in other studies (Teng et al. 1998).

Raman Spectra

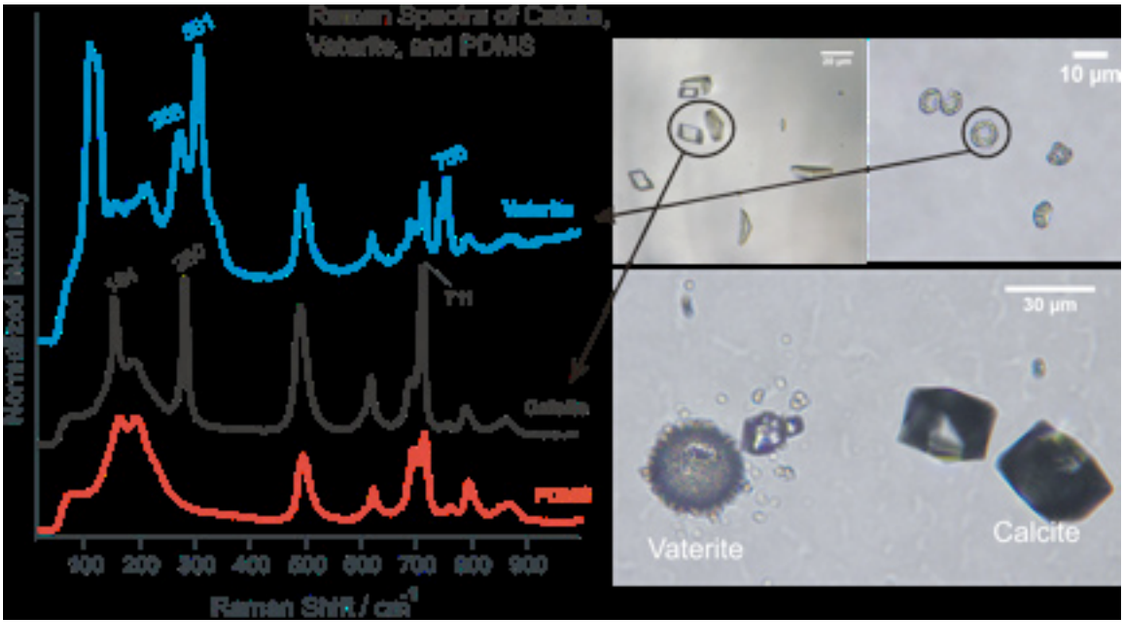

Figure 3: Raman spectra of calcite, vaterite, and PDMS. (a) Illustrates calcite crystals grown from $CaCl_2$. (b) Illustrates vaterite crystals grown from CaCl2 with L-aspartic acid. (c) Illustrates structures of calcite and vaterite crystals grown on the same PDMS membrane. The raman spectra to the left shows distinct peaks specific to crystalline calcium carbonate polymorphs, vaterite and calite, as well as those for the PDMS substrate.

Calcite Modification

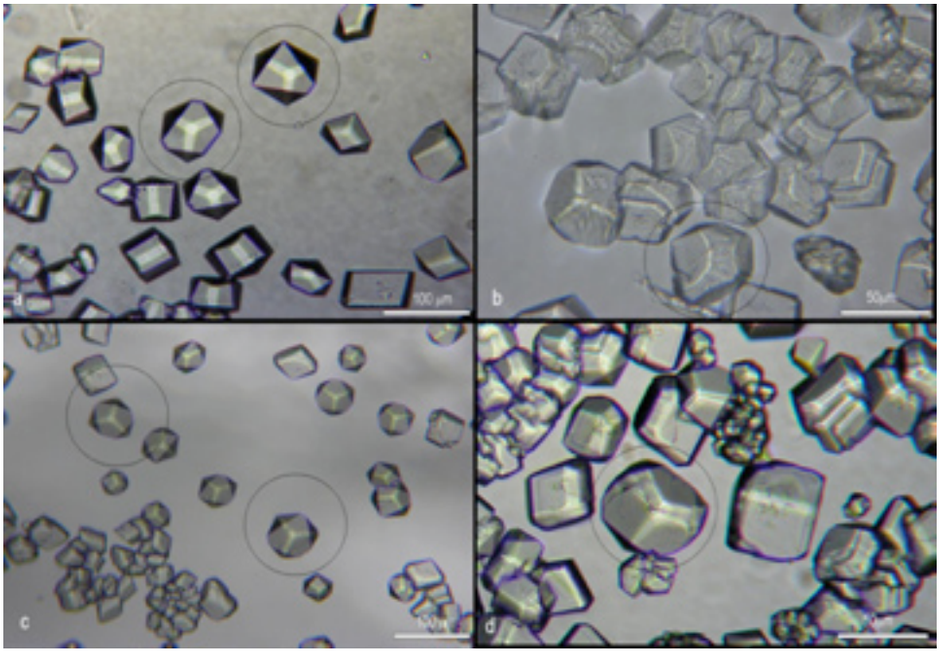

Figure 4: (a) Optical micrograph showing calcite growth before addition of L-aspartic acid. (b) Higher magnification micrograph of calcite growth after L-aspartic acid addition. (c) Optical micrograph of calcite growth before D-aspartic acid addition. (d) Higher magnification micrograph of calcite growth after the addition of D-aspartic acid.

Calcite was successfully modified using synthetic amino acids of D and L aspartic acid. More data is needed to understand these control effects and other modifiers which can be used to direct nucleation of calcite structure and orientation. We are currently investigating the use of optical and scanning electron microscopic examination to assign crystallographic orientations to understand which crystal faces are being selectively inhibited by amino acid interactions.

This research has many potential benefits. Understanding the mechanisms of biomimetic biomineralization gives insight into the fundamental approaches taken by living systems. Developing controls for the growth of calcium carbonate materials could have considerable effects on wide ranging industrial applications such as pharmaceuticals, cleaning products, and papermaking, which use calcium carbonate extensively. We also anticipate broader impacts in potential applications of non-biogenic material growth (such as metals and semiconductors) and applications in nanotechnology.

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