

Control of Crystal Nucleation and Growth of Calcium Carbonate by Synthetic Substrates

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Construction of organic–inorganic hybrid materials with controlled mineralization analogous to those produced by nature is now of current interest for both organic and inorganic chemists to understand the mechanism of the natural biomineralization process as well as to seek industrial and technological applications. This review provides a general survey of recent research on control of crystal nucleation and growth of calcium carbonate by synthetic substrates from the viewpoint of organic and polymer chemistry. Model systems in which low-molecular-weight, linear polymeric, and dendritic organic additives are used to study the effect of molecular properties such as charge and functionality on inorganic crystallization are providing insights into the possible mechanisms operating in biology. To probe the organic–inorganic interface and resolve some of the molecular events mediating template-directed crystal nucleation and growth, a simple model surface, that is, compressed monolayers at the air/water interface and self-assembled monolayers on a solid surface, was used for the nucleation of oriented inorganic materials. The template-inhibition strategy for the synthesis of CaCO₃ thin film has also been reported. Microscale phase separation in reaction media has importance in the synthesis of inorganic materials with unusual form and hierarchical structure. These interesting efforts have led to fundamental developments in areas relating to the biomineralization process, which is expected to be the impetus for the next industrial revolution.

Introduction

There has been a worldwide realization that nanotechnology is the impetus for the next industrial revolution. A key aspect of nanotechnology is that nanoscale materials offer different chemical and physical properties than the bulk materials and that these properties could form the basis of new technologies. Nanomaterials will be assembled from simpler components such as molecules, polymers, and other nanostructures. This approach is similar to the one nature uses to construct complex biological architecture. Among various research fields aimed for constructing nanostructures, organic–inorganic hybrid materials have opened a new horizon in the field of material science. The sol–gel reaction of a metal alkoxide is a widely used technique for the preparation of the hybrid materials.¹ However, the obtained metal oxide has an irregular structure and the morphology of the gel is dependent on the reaction conditions.

In nature, biological organisms produce polymer–inorganic hybrid materials such as bone, teeth, diatoms, and shells. These hybrids have superior mechanical properties as compared to synthetic hybrids. For example, the abalone shell, a composite of calcium carbonate with a few percent of the organic component (Figure 1), is 3000 times more fracture resistant than a single crystal of the pure mineral.² The core of the organic template is composed of a layer of β -chitin layered between “silk-like” glycine- and alanine-rich proteins. The outer surfaces of the template are coated with

hydrophilic acidic macromolecules. Natural inorganic–organic hybrid materials are formed through mineralization of inorganic materials on self-assembled organic materials. Biopolymers and low-molecular-weight organic molecules are organized as nanostructures and used as frameworks for specifically oriented shaped inorganic crystals such as calcium carbonate, hydroxyapatite, iron oxide, and silica. These processes are using aqueous solutions at temperatures below 100 °C. Moreover, no toxic intermediates are produced in these systems. Therefore, the processes and materials that control such crystal growth are of great interest to materials scientists who seek to make composite materials and crystalline forms analogous to those produced by nature.

In these mineralized tissues, crystal morphology, size, and orientation are determined by local conditions and, in particular, the presence of “matrix” proteins or other macromolecules.³ Morphological control can also be accomplished by adsorption of soluble additives onto specific faces of growing crystals, altering the relative growth rates of the different crystallographic faces and leading to different crystal habits. These processes take place usually at an organic–inorganic interface, the organic portion providing the initial structural information for the inorganic part to nucleate on and grow outward in the desired manner. However, there remain many unknowns as to how the matrix affects the crystallization process. The final crystalline phase could arise through a series of steps, initiated by the formation

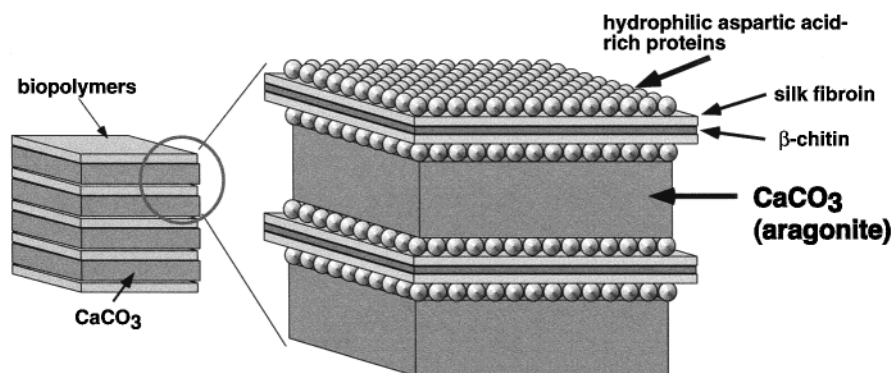


Figure 1. Schematic illustration of the nacre of the abalone shell.

of an amorphous phase that undergoes subsequent phase transformations. The existence of several phases would enable organisms to control mineralization through intervention with the kinetics. By selectively interacting with the mineral at different stages during the crystal-forming process, the organisms could choose to manipulate both the polymorph and the orientation of the mineral to meet specific biological requirements.

Most of the researcher's interest lies in understanding how organized inorganic materials with complex morphological form can be produced by biomineralization processes and how such complexity can be reproducibly synthesized in biomimetic systems. Because of the complexity of the natural biomineralization systems, mineralization research has been studied on model organic interfaces. In the beginning, these studies focused on the main fundamental question of how inorganic crystallization can be controlled in an aqueous solution. Construction of organic–inorganic hybrid materials with controlled mineralization is now of current interest for both organic and inorganic chemists to understand the mechanism of the natural biomineralization process as well as to seek industrial and technological applications. Although many researchers have realized that design of organic templates is important for controlled mineralization of inorganic materials, many biomineralization studies have been approached from the viewpoint of inorganic materials chemistry. The effect of pH, temperature, foreign inorganic ions, and the degree of supersaturation in aqueous solution have been extensively studied.^{4–6} Several excellent review papers have already been devoted to biomineralization, usually focusing mainly on inorganic materials by biomimetic processes.^{7–12} Recently, several organic and polymer research scientists have been interested in the research area of biomineralization. Various types of organic matrixes as a structural template for crystallization of inorganic materials should be easily designed and synthesized. Current significant development of so-called “supramolecular chemistry” also provided a highly ordered template for biomineralization. In this short review, we make a general survey of recent research on the control of crystal nucleation and growth of minerals by synthetic substrates from the viewpoint of organic and polymer chemistry.

Here, we focus on calcium carbonate as the inorganic phase. Calcium carbonate makes an attractive model mineral for studies in the laboratory because its crystals are easily characterized and the morphology of CaCO_3

has been the subject to control in biomineralization processes. The precipitation of calcium carbonate in aqueous solution is also of great interest for industrial and technological applications. The particular interest in this system is due to the polymorphism of calcium carbonate, which has three anhydrous crystalline forms, that is, vaterite, aragonite, and calcite in order of decreasing solubility and increasing stability. The three polymorphs have markedly different physicochemical characteristics, and it is often found that less stable forms are stabilized kinetically. Vaterite transforms into thermodynamically the most stable calcite via a solvent-mediated process.¹³ Vaterite, however, is expected to be used for various purposes because it has some features such as high specific surface area, high solubility, high dispersion, and small specific gravity compared with the other two crystal phases. Organizations of calcium carbonate crystals in biological systems in the three polymorphs, calcite, vaterite, and aragonite, are well-known illustrations of the biomineralization processes. Calcite and aragonite are widespread in marine organisms, and vaterite, monohydrocalcite, and amorphous calcium carbonate are formed and stabilized by some organisms.¹⁴ The manner in which organisms control polymorph formation is not well understood.

In Vitro Study

An in vitro study of biomineralization provides useful information for the design of organic templates. Falini and co-workers assembled in vitro a complex containing the major matrix components present in a mollusk shell, namely, β -chitin, silk-fibroin-like protein, and water-soluble acidic macromolecules.¹⁵ When this assemblage was placed in a saturated solution of calcium carbonate, multicrystalline spherulites formed within the complex. They extracted aspartic-acid-rich glycoproteins from an aragonitic mollusk shell layer or a calcitic layer. These were aragonite if the added macromolecules were from an aragonitic shell layer or calcite if they were derived from a calcitic shell layer. In the absence of the acidic glycoproteins, no mineral formed within the complex.

Addai and co-workers purified the soluble macromolecules by ion-exchange chromatography from an aragonitic shell layer of the mollusk *Atrina serrata* to better understand their individual functions of the fractionated proteins in an in vitro system.¹⁶ A small fraction within the entire macromolecular ensemble, consisting of only 6 mol % of the total amino acids, has

a remarkable ability to nucleate aragonite. They also tested a series of aspartic acid (Asp) and leucine (Leu) or glutamic acid (Glu) and Leu-containing synthetic peptides in the *in vitro* assay system. They found that only poly(Asp-Leu) was capable of specifically inducing aragonite formation. Furthermore, the aragonitic crystallites were in the shape of elongated needles, similar to those obtained from the protein fraction described above. The presence of the silk is essential for the absorption of the acidic macromolecules. Indeed, in the absence of the silk, no aragonite was formed with any of the proteins and the polypeptides. Circular dichroism measurements of poly(Asp-Leu) indicated that in a 1:2 of Ca:–COOH solution, this polypeptide assumes a β -sheet conformation. The carboxylate groups of the alternating Asp residues are thus exposed on one side of the sheet. Poly-L-asparatate, with a predominantly β -sheet conformation, produces more aragonite than poly-L-glutamate, which has a random conformation. Although poly(Glu-Leu) assumes mainly a β -sheet conformation in solution, crystallization of CaCO₃ was inhibited. All these observations demonstrate that polymorph specificity is dependent upon the amino acid sequence. The conformation and microenvironment of the macromolecules might be important for the specific nucleation of inorganics. Studies of the relations between them and the crystals are necessary to better understand the nature of the nucleation site *in vitro*.

Regulation of nucleation, growth, morphology, and aggregation does not always require condensed, pre-organized organic arrays as described above. Belcher et al. demonstrated *in vitro* studies of the crystallization of calcium carbonate in the presence of soluble polyanionic proteins extracted from abalone shell.¹⁷ Crystals grown in the absence of soluble protein exhibited the characteristic rhombohedral morphology of calcite. In contrast, crystals grown from a solution containing the polyanionic proteins extracted from the calcitic composite of the shell exhibit the characteristic spherulitic morphology of calcite. Crystals grown in the presence of the polyanionic proteins solubilized from the aragonitic composite displayed needles of aragonite in the plane of the nucleation layer. These results showed that these proteins alone are sufficient to control the crystal phase. Subsequent addition of the polyanionic proteins extracted from the aragonitic composite resulted in the growth of new aragonite needles on the rhombohedral calcite face. Sequential transition of calcite to aragonite and back once again to calcite occurred by these proteins. Interestingly, addition of soluble shell proteins was sufficient to promote the formation of stacked thin plates of crystallographically oriented aragonite crystals resembling those in an abalone nacre.

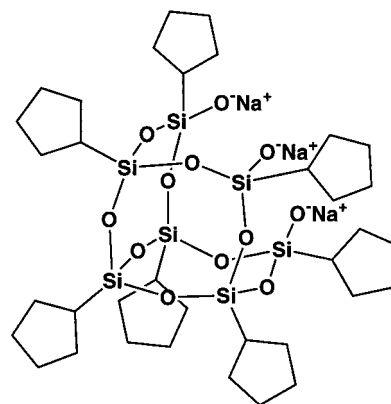
Because the high-dimensional structures of these proteins were unknown, a detail mechanism of the effect of nucleation at the molecular level remains unknown. However, these *in vitro* studies motivate us to design an artificial template for controlled nucleation of minerals.

Low-Molecular-Weight Organic Additives

Model systems in which low-molecular-weight organic additives are used to study the effect of molecular properties such as charge and functionality on inorganic

crystallization are providing insights into the possible mechanisms operating in biology. These additives were chosen to mimic the active protein ligands. The influence of organic molecules on the nucleation and crystal growth of calcium carbonate has been studied by a number of authors. The polymorphs and morphologies of CaCO₃ crystals were influenced in the presence of phosphorus- or sulfate-containing anions.¹⁸ The influence of α,ω -dicarboxylates on the morphology of calcite crystals grown from supersaturated bicarbonate solutions was systematically studied by Mann and co-workers.¹⁹ A rigid, unsaturated molecule such as maleate, with a short linkage between terminal carboxylates, was a more effective inhibitor than the homologous saturated molecule succinate. The surface complexation of calcite by small molecules appears to follow the general rules that apply to complexation in solution.²⁰ Ogino et al. stated that ethylenediamine-tetrakis-*N,N,N,N*-(methylenephosphonic acid) (EDTMPA) completely inhibits phase transitions of metastable calcium carbonate phases.²¹ In the presence of EDTMPA, vaterite modification agglomerates are formed, with primary vaterite spheres strongly bound by an amorphous mass.

On the basis of specific interactions, various organic molecules with coordination groups have potent inhibitors or habit modifiers of crystal morphology by absorption onto the surface of the growing crystal. Thus, the physical characteristics of mineral particles might show a marked dependence on the habit modifiers. Surface modification of calcium carbonate with a hydrophobic species would lead to great expansion for industrial applications because mineral particles are hardly dispersed in organic solvents. We have prepared hydrophobic calcium carbonate particles via crystallization of calcium carbonate with a sodium trisilanolate [(*c*-C₅H₉)₇-Si₇O₉(ONa)₃] in methanol.²² The last few decades have



Sodium trisilanolate

seen further developments in a chemistry of incompletely condensed polyhedral oligosilsesquioxanes. Of particular interest have been reactions of trisilanols with ligand-deficient trivalent metal complexes because the inability of these trisilanols to support trigonal planar coordination environments is expected to produce more complex structures. A strong coordination ability of the oligosilsesquioxanes may enable the design and synthesis of composites in which the CaCO₃ surface is coordinated to a group of the trisilanol. Aqueous solu-

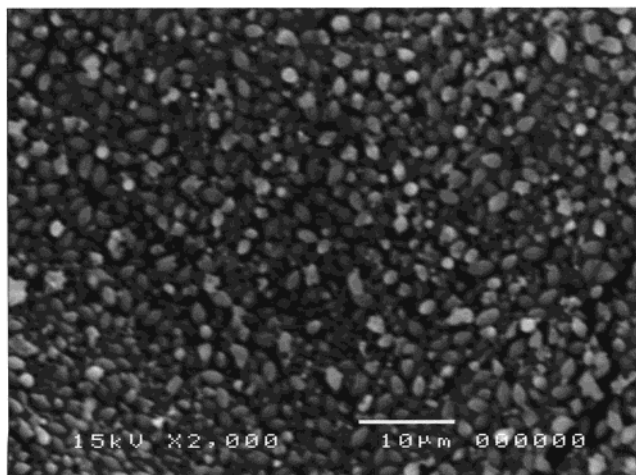


Figure 2. Scanning electron micrographs of the crystalline CaCO_3 products obtained in the presence of a sodium trisilanolate, $[(\text{c-C}_5\text{H}_9)_7\text{Si}_7\text{O}_9(\text{ONa})_3]$. The molar ratio of calcium ion to the sodium trisilanolate was 0.1. (Reproduced from ref 22b.)

tions of CaCl_2 and $(\text{NH}_4)_2\text{CO}_3$ were added into a methanol solution of the sodium trisilanolate. The molar ratio of calcium ion to the trisilanol was varied from 1.0 to 0.1. FT-IR and TGA analysis of the obtained products indicated that the trisilanol was bound to the crystalline CaCO_3 . When the concentration of the trisilanol was changed, the composite particles with different shapes and polymorphisms were obtained. Low concentration of the trisilanol gave stable vaterite crystals of which the crystal polymorph did not change when this particle was kept in an aqueous solution at room temperature for more than 1 week (Figure 2). Dispersibility of the composites in organic solvents was significantly improved compared with that of a bare CaCO_3 . These observations indicated that the surface of the CaCO_3 particles was modified with hydrophobic cyclopentyl groups.

When the precipitation of CaCO_3 in the absence of the trisilanol was carried out in methanol, the obtained crystal was spherical vaterite. It was already reported that the presence of ethanol, 2-propanol, and diethylene glycol affects the rates of spontaneously precipitated vaterite and stabilizes this polymorph, preventing the transformation to thermodynamically stable calcite.²³ Therefore, vaterite formations are favorable in non-aqueous solvents. Low concentrations of the trisilanol gave vaterite. This means such concentration had no influence on the nucleation and growth of vaterite formation in methanol. However, the amount of the trisilanol may be enough to block all active surface sites of the vaterite particles. A higher concentration of the trisilanol showed a marked acceleration of the crystallization. The trisilanol captured calcium ions and increased the local concentration of the calcium reactant to promote the nucleation of CaCO_3 . Aragonite was induced by the higher amount of the trisilanol in methanol.

Linear Polymeric Organic Additives

Because the proteins that have been found to be associated with biominerals are usually highly acidic macromolecules, simple water-soluble polyelectrolytes,

such as the sodium salts of poly(aspartic acid) and poly(glutamic acid), were examined for the model of biomineralization in aqueous solution. Studies of inorganic crystallization in the presence of soluble polymers, modeled on biogenic proteins, have shown that selectivity for certain crystal faces appears to be highly dependent on the secondary structure of the macromolecules. It was found that the addition of polyaspartate to supersaturated solutions of calcium carbonate led to unusual morphologies in the inorganic phase. Mann et al. reported that the addition of poly(α,β -aspartate) induced vaterite nucleation and the formation of helicoid morphologies.²⁴ They proposed that the adsorption of poly(α,β -aspartic acid) at high surface concentration on the crystal faces of vaterite resulted in an amorphous CaCO_3 -peptide gel which inhibits further crystal growth. Localized fragmentation of this membrane could be responsible for episodic outgrowth along a direction dictated by supersaturation and diffusion gradients in the surrounding medium. A screw dislocation along the vaterite *c* axis couples with the diffusion gradients in propagating the crystallization process. Unusual morphologies of calcium carbonate by the addition of polyaspartate to supersaturated solutions of calcium carbonate were also reported by Tirrell's group.²⁵ A crystalline vaterite aggregate containing a helical protrusion was produced when 5–30 mg/mL of the polymer was added to a calcium chloride solution with CO_2 vapor diffusion. It is not essential that the polypeptide be chiral to produce these helical structures. Even achiral poly(α,β -D,L-aspartate) produces helical protrusions.

Crystallization of CaCO_3 in the presence of various synthetic non-peptide polymers has been investigated as a model of biomineralization. Poly(acrylic acid) and poly(methacrylic acid) are used to inhibit the precipitation process.^{26–28} They affect crystallization by binding to the surface of the growing crystal. Poly(vinyl alcohol) is highly selective for vaterite nucleation.¹⁸ The influence of polyhydroxy groups on water structure and hydration/dehydration steps could be responsible for the kinetics of crystallization processes. Poly(vinyl alcohol) is considered to be a synthetic analogue of anti-freeze glycopeptide, which had the effect of inhibiting calcite in favor of vaterite.¹⁸ When sodium poly(styrene sulfonate) (PSS) was employed for crystallization of CaCO_3 , monodispersed spherical vaterite was formed.²⁹ An increase in the molecular weight and concentration of PSS gave smaller size of spherical crystals.

Ueyama et al. designed novel polyamide ligands containing the $\text{NH}\cdots\text{O}$ hydrogen bond.³⁰ These polymers, $\{\text{NHC}_6\text{H}_3(\text{COO})\text{NHCOC}(\text{CH}_3)_2\text{CO}\}_n$ (**1**), $\{\text{NHC}_6\text{H}_3(\text{COO})\text{NHCO-}m\text{-C}_6\text{H}_4\text{CO}\}_n$ (**2**), and $\{\text{NHC}_6\text{H}_3(\text{COO})\text{NHCO-trans-CH=CHCO}\}_n$ (**3**), were synthesized from 2,6-diaminobenzoic acid and dimethylmalonyl dichloride, isophthaloyl dichloride, or fumaryl dichloride. The $\text{NH}\cdots\text{H}$ hydrogen bond protects the metal-carboxylate bond from hydrolysis due to the lowering of the pK_a of the corresponding carboxylic acid. Crystalline CaCO_3 was prepared in the presence of the polyamides. The molar ratio of Ca ion to carboxylic acid group is 100. The crystalline CaCO_3 was washed thoroughly with DMSO or THF to remove any contaminating polymer ligand that is not involved in the crystals. The ^{13}C cross-

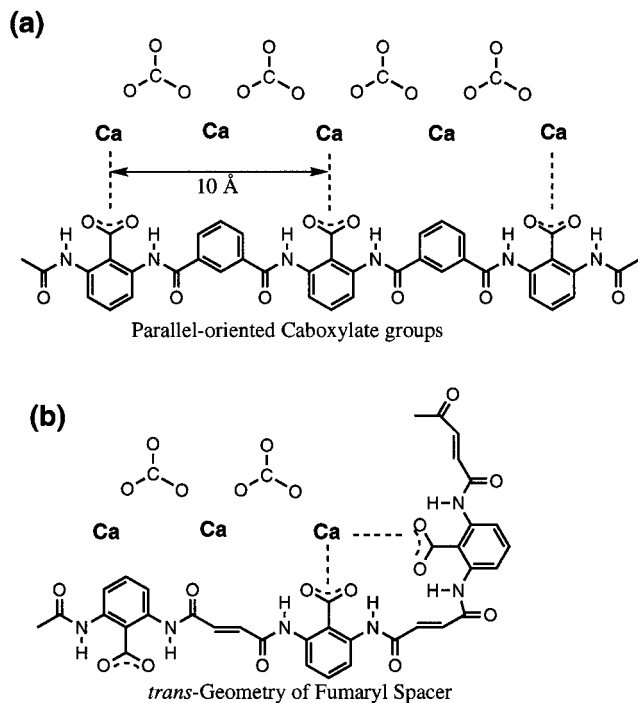


Figure 3. Schematic illustrations for interaction between the poly(amide) ligands and calcium ions in calcium carbonate. (a) $\{\text{NHC}_6\text{H}_3(\text{COO})\text{NHCOC}(\text{CH}_3)_2\text{CO}\}_n$ (**1**) and calcium ions and (b) $\{\text{NHC}_6\text{H}_3(\text{COO})\text{NHCO-*trans*-CH=CHCO}\}_n$ (**3**) and calcium ions (as in ref 30).

polarization/magic angle spinning solid-state (CP/MAS) NMR spectra show that the polyamides, **1** and **2**, bind to the CaCO_3 cluster depending on the polymer structure. **3** is not involved in the CaCO_3 cluster. The ^{13}C CP/MAS NMR is a powerful tool for detecting the participation of polymer ligands in CaCO_3 crystals. The relative position of the carboxylate group in the polymers primarily influences their ability to bind the CaCO_3 cluster. They proposed that the parallel-oriented carboxylate groups in the polymer structure are very important for making inorganic–organic complexes. The distances of adjacent carboxylate distances are almost 10 Å for **1** and 9 Å for **2**, which are almost twice the distances of calcium ion separation in calcium carbonate as shown in Figure 3a. On the other hand, **3** cannot have parallel oriented carboxylate groups because of the *trans* geometry of the fumaryl spacer (Figure 3b). When the Ca–O bonds of anionic polymers are easily dissociated by water, the polymers are thought not to be occluded in the crystals. Strong Ca–O bonds are thus required to control the polymorph of CaCO_3 and prepared inorganic–organic composite materials. Linear polyamides having various combinations of rigidity of the main chain and separation of carboxylate groups can be designed. The systematic studies combined with ^{13}C CP/MAS NMR and other spectroscopic techniques will give better understanding for the correlation between the structures of polymers and polymorphs of CaCO_3 .

Cölfen and co-workers reported the crystal design of calcium carbonate using a series of so-called “double-hydrophilic” block copolymers.^{31,32} Such polymers consist of one hydrophilic block which is designed to interact with inorganic salts and surfaces, whereas the other hydrophilic block just promotes dissolution in water, but does not interact (or just weakly interacts)

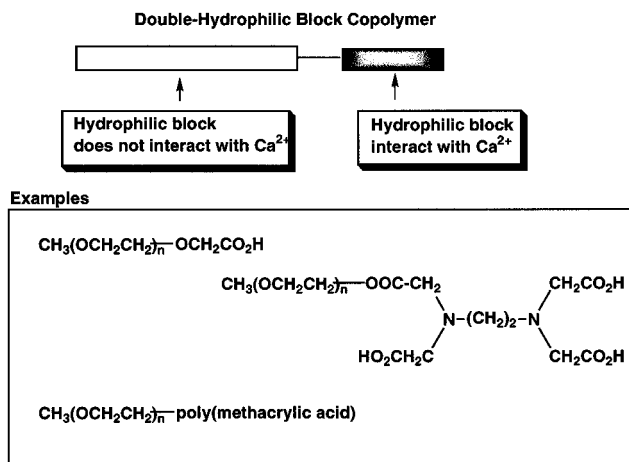


Figure 4. Double-hydrophilic block copolymers for crystallization of calcium carbonate. Adapted from refs 31 and 32.

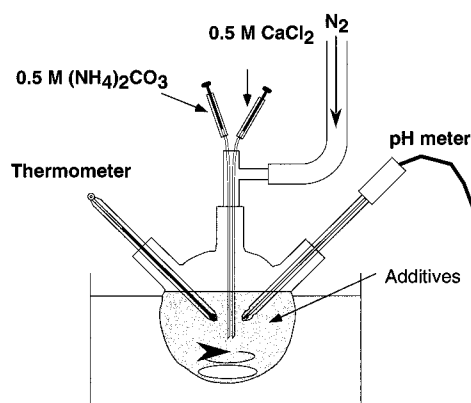


Figure 5. Experimental setup of a double-jet reactor for the precipitation of calcium carbonate. Adapted from ref 31.

with the dissolved ions (Figure 4).³³ The precipitation of CaCO_3 in the presence of various synthesized block copolymers was carried out in a double-jet reactor as shown in Figure 5. The two reactants (CaCl_2 and Na_2CO_3) are injected via capillaries into the reaction vessel under vigorous stirring to prevent heterogeneous nucleation at the glass wall. The two capillary ends are joined together so that a high local reactant concentration and thus extreme supersaturation is achieved at the moment when the two reactants leave the capillaries, which provides an immediate nucleation of CaCO_3 . The nuclei are then immediately transported to regions of lower CaCO_3 concentration and can grow further. The CaCO_3 crystal formation occurring after excess addition of reactants was easily observed as a sudden increase in the turbidity of the solution. The main idea behind this technique, which was set up for the controlled precipitation of silver halides in the photographic industry, is to maintain a rapid nucleation of a constant particle number in the beginning of the experiment to enable growth of monodispersed particles. Double-hydrophilic block copolymers, consisting of a hydrophilic poly(ethylene glycol) block and a second hydrophilic moiety which strongly interacts with alkaline earth ions, were employed to template the precipitation of calcium carbonate from aqueous solution. Depending on the type of polymeric functionalization pattern used, it was possible to control the crystal size, shape, and modification. The authors could precipitate purely crystalline

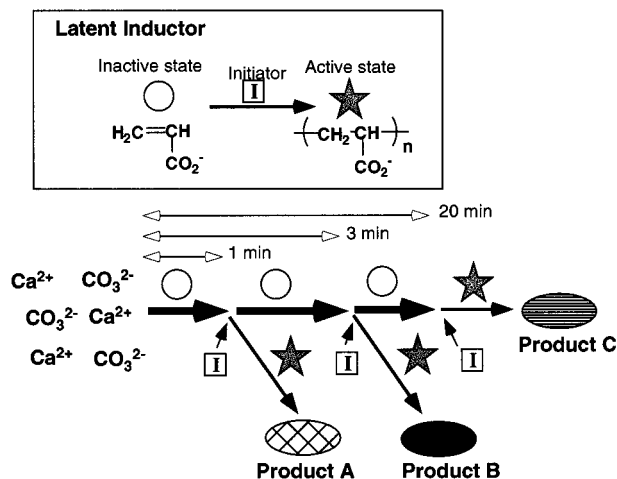


Figure 6. Schematic depiction for control of a crystal polymorph by a latent inductor. (Reproduced from ref 35.)

calcite and vaterite depending on the functionalized block and the pattern of functional groups. Vaterite could be stabilized for more than 1 year where it would normally transform into calcite within 80 h. Such an approach simplifies the optimization of the double-hydrophilic block copolymers with respect to crystal design of calcium carbonate and other inorganics.³⁴

In Situ Polymerization Method

A final crystalline phase might arise through a multistage crystallization process. The existence of several phases would enable organisms to control mineralization through intervention with the kinetics. By selectively interacting with the mineral at different stages during the crystal-forming process, the organisms may choose to manipulate both the polymorph and the orientation of the mineral to meet specific biological requirements. Although crystallization of minerals in the presence of various additives and synthetic polymers has been investigated as a model of biomineralization, selective interaction of synthetic additives with the minerals at different stages during the nucleation process has not been examined. Recently, we reported a new concept for controlling crystal polymorphs of CaCO_3 by a synthetic additive.³⁵ The key point of our method is using a "latent inductor" for crystal nucleation as seen in Figure 6. The latent inductor at its inactive state does not affect nucleation and growth of the crystal. After the inactive state is transferred to an active state by a stimulus, the active inductor can induce nucleation and growth of the crystal. We used sodium acrylate as a latent inductor for this purpose and a water-soluble radical initiator was used as a stimulus. Sodium acrylate may not affect nucleation and growth of the crystal. On the other hand, poly(acrylate) affects the crystal morphology by inhibiting the growth of particular crystal faces. Sodium acrylate can be transferred to poly(acrylate) by adding a radical initiator.

Aqueous solutions of CaCl_2 and $(\text{NH}_4)_2\text{CO}_3$ were initially added to an aqueous solution of sodium acrylate and then subjected to polymerization by adding the radical initiator at 30 °C after incubation for several minutes. After addition of the reactants into the aqueous solution of sodium acrylate was completed, an aqueous

solution of potassium persulfate as a water-soluble radical initiator was added to the reaction mixture after incubation at 30 °C for several minutes (1, 3, or 20 min). The yields of the crystalline products obtained when the radical initiators were added to the reaction mixture after incubation for 1 min (for product A), 3 min (for product B), and 20 min (for product C) were 33, 35, and 54%, respectively. Three different crystal polymorphs of CaCO_3 (aragonite, vaterite, and calcite) were selectively induced by changing the time of addition of the radical initiator to the calcium carbonate solution with sodium acrylate. Figure 7 shows the scanning electron micrographs (SEM) of the three crystalline products. Each SEM micrograph shows different crystal modifications. The aragonite crystal (product A) was efflorescent bundles of needles (Figure 7a), which is a typical aragonite crystal morphology. Product B consisted of two different crystal modifications, spherical vaterite and rhombs of calcite (Figure 7b). The fraction of vaterite in product B was 63% determined by powder X-ray diffraction (XRD) analysis. The crystal of product C was rhombohedral (Figure 7c). These results indicate that the three different polymorphs of CaCO_3 were controlled simply by changing the additional time of the radical initiator to the calcium carbonate supersaturated solution containing sodium acrylate at ambient temperature.

Calcite is thermodynamically stable compared to the other two crystalline structures, aragonite and vaterite. The crystal phases of CaCO_3 obtained without any additives and with sodium acrylate without radical initiators were both calcite under the same conditions described above. Sodium acrylate was regarded as an inactive form for induction of metastable CaCO_3 crystalline phases (vaterite or aragonite). In addition, the crystal phase of product C was also thermodynamically stable calcite. The final crystalline phase was not affected when the polymerization of sodium acrylate was started by the radical initiator after incubation of the reaction mixture containing calcium reactants and sodium acrylate for 20 min. Aragonite is usually achieved at a higher temperature than 50 °C using a solution method of preparation.³⁶ In our results, aragonite can be achieved at 30 °C when the radical initiator was added to the calcium solution with sodium acrylate after incubation for 1 min, in which the CaCO_3 crystal formation was not started. It is possible that aragonite is rapidly nucleated at the very beginning of the nucleation process, resulting in it being kinetically induced by the poly(acrylate). When the initiator was added to the reaction mixture after incubation for 3 min, a crystal consisting of calcite and vaterite was formed. Without any additives, it is well-known that vaterite transforms into stable calcite via a solvent-mediated process.¹³ Although the crystal polymorph of product B did not change when the solution was kept for 2 days, the vaterite crystal was transformed to calcite when the solution was incubated for 3 days. We speculate that the vaterite surfaces were stabilized by the resulting poly(acrylate) in aqueous solution to prevent phase transformation. These results indicate that the final crystalline phases are highly sensitive to the presence of the active additives at the very initial nucleation stage (within several minutes). During the phase trans-

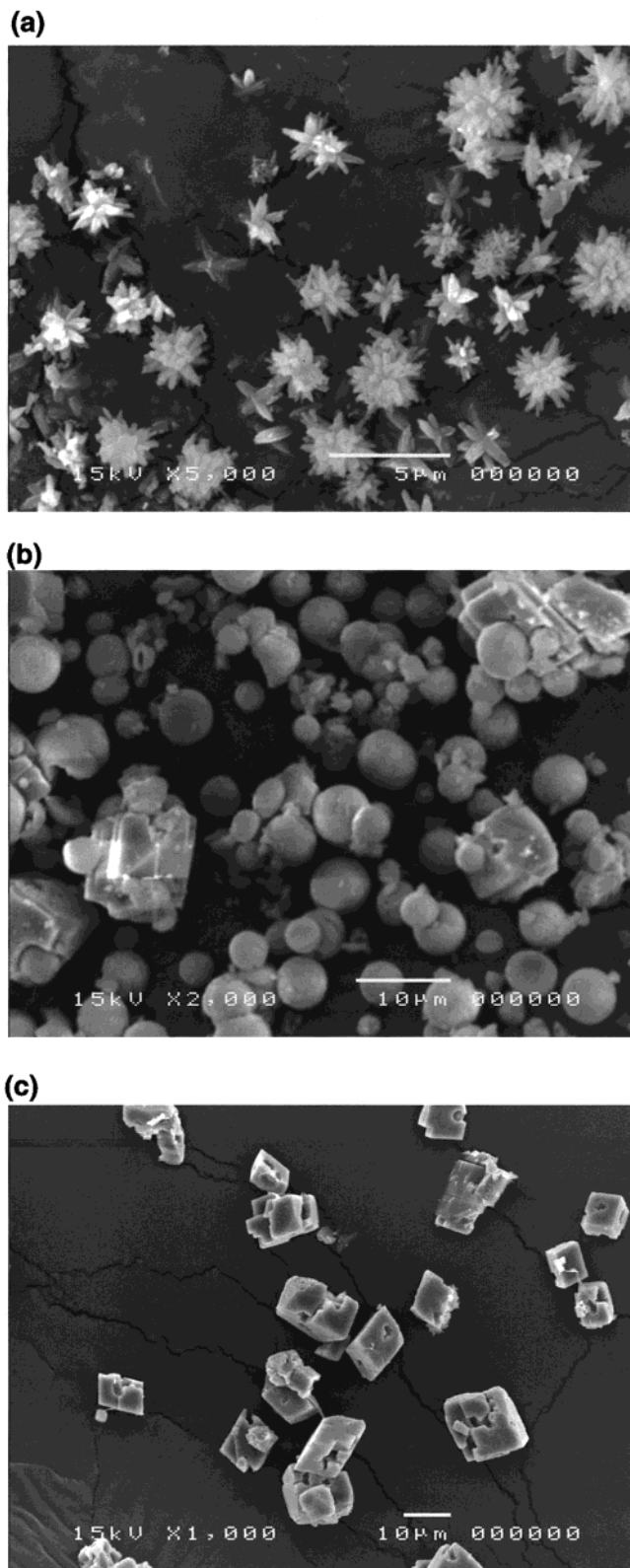


Figure 7. Scanning electron micrographs of the crystalline products obtained when the radical initiators were added to the reaction mixture after incubation for 1 min (for product A) (a), 3 min (for product B) (b), and 20 min (for product C) (c). (Reproduced from ref 35.)

formation, the poly(acrylate) may kinetically and thermodynamically induce crystal nucleation at each stage. Although we do not fully understand the mechanistic implication of the effect of in situ radical polymerization of sodium acrylate in aqueous solution for nucleation

and growth of calcium carbonate, our current data have provided a new concept for controlling the crystal polymorphs of calcium carbonate. Sodium acrylate was inactive for nucleation and growth of the crystal. After the radical initiator was added, acrylate was transferred to poly(acrylate), which could interact with nucleation and growth of CaCO_3 . Sodium acrylate was regarded as a latent active ligand for induction of the crystal phase. For controlling the polymorph of CaCO_3 , we believe that the initial nucleation processes play an important role in controlling the final crystal.

Dendrimers

Poly(amidoamine) (PAMAM) dendrimers with carboxylate groups at the external surface termed half-generation or $G = n.5$ dendrimers have been proposed as mimics of anionic micelles or proteins.³⁷ As shown in Figure 8, the starburst structures are disklike shapes in the early generations, whereas the surface branch cell becomes substantially more rigid and the structures are spheres.³⁸ Because of unique and well-defined secondary structures of the dendrimers, anionic starburst dendrimers should be good candidates for studying inorganic crystallization. The interaction of the negative surface of dendrimers with metal ions has been extensively examined.³⁹ PAMAM dendrimers were used as templates for the formation of metal nanoparticles.^{40–42} It was found that stable gold nanoparticles with a diameter < 1 nm are prepared in the presence of the later generation dendrimers, and the dendrimer concentrations required for obtaining stable gold nanoparticles are extremely low compared to those of other linear polymers.

Recently, we studied crystallization of CaCO_3 in the presence of anionic PAMAM dendrimers and found that an anionic starburst dendrimer was a habit modifier and affects the crystal morphology of inorganic crystallization.⁴³ The precipitation of CaCO_3 in the presence and the absence of PAMAM dendrimer ($G = 1.5$) was carried out under the same conditions of Cölfen³¹ to prevent heterogeneous nucleation at the glass walls as shown in Figure 5. The crystalline CaCO_3 was washed with water to remove contaminated dendrimers that were not involved in the crystal. The yields of the crystalline products in the absence and the presence of the dendrimer were 51 and 61%, respectively. The dendrimer content in the crystalline CaCO_3 was 33 wt % determined by elemental analysis. These results indicate that the anionic dendrimer was bound to the crystalline CaCO_3 . Crystallization of CaCO_3 in the presence of PAMAM dendrimer with carboxylate groups at the external surface resulted in the formation of spherical vaterite crystals (Figure 9a), whereas rhombohedral calcite crystal was formed without the additive (Figure 9b). In the presence of Na salt of poly(acrylic acid) (PAA), the formation of crystalline CaCO_3 was prevented. The crystalline CaCO_3 was hardly collected after incubation at 25 °C under N_2 for 4 days. This indicates that PAA acts as an inhibitor for crystal formation. We speculated that the vaterite surface was stabilized by the carboxylate-terminated dendrimer in aqueous solution to prevent phase transformation. In the presence of the dendrimer, further washing of the vaterite crystal with water did not change the crystal morphology.

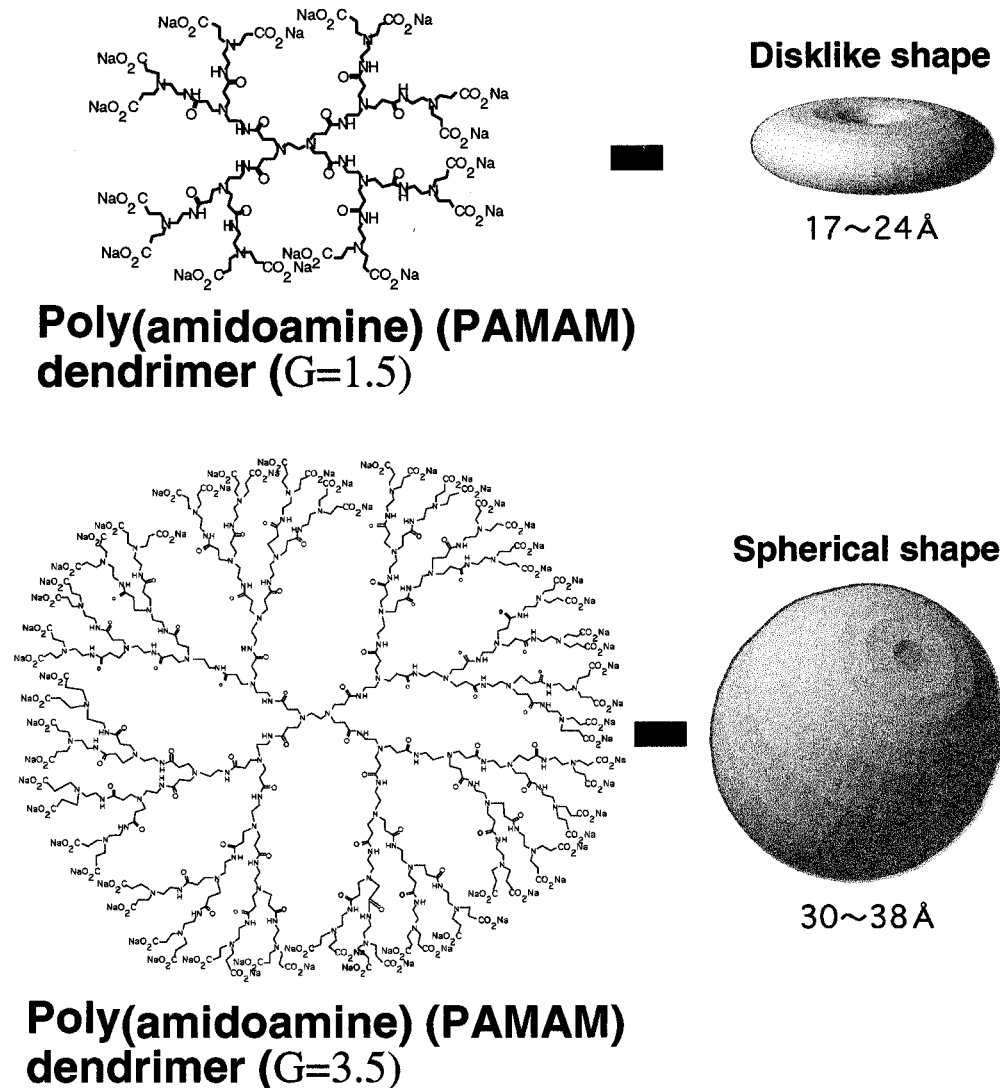


Figure 8. Poly(amidoamine) (PAMAM) dendrimers with carboxylate groups at the external surface.

The complexes of anionic starburst dendrimers with calcium ions are considerably stronger for higher generations than for lower generations. Figure 10 shows scanning electron micrographs of the obtained vaterite particles by changing the generation number of the dendrimers. In the presence of PAMAM dendrimer ($G = 1.5$) or PAMAM dendrimer ($G = 3.5$), average particle sizes of spherical crystals were 5.5 ± 1.1 or 2.3 ± 0.7 nm, respectively.⁴⁴ The concentrations of the two dendrimers were kept constant like the concentration of carboxylate units (corresponding to 0.26 mM of $-\text{CO}_2\text{-Na}$). These results indicated that an increase in the generation number of the dendrimers gave smaller size of spherical vaterite crystals. We also showed that an increase of the concentration of the dendrimers decreased the particle sizes of spherical vaterite crystals. Although the template mechanism of the dendrimers seems to be of a complex nature because simultaneous CaCO_3 nucleation and interaction with the polymer can be expected, complexation properties of PAMAM dendrimers may be a major role for the mineralization of calcium ions.

Poly(propyleneimine) dendrimers modified with long aliphatic chains are a new class of amphiphiles which display a variety of aggregation states due to their

conformational flexibility.⁴⁵ In the presence of octadecylamine, poly(propyleneimine) dendrimers modified with long alkyl chains self-assemble to form remarkably rigid and well-defined aggregates. When the aggregate dispersion was injected into a supersaturated solution of calcium carbonate, the aggregates stabilized the normally unstable amorphous calcium carbonate.⁴⁶ Over the course of 4 days a discrete population of rhombohedral calcite crystals was also identified, alongside large isolated amorphous calcium carbonate particles. This process might affect the gradual release of the aggregates. Although amorphous calcium carbonate has been observed as a short-lived intermediate in the presence of various scale prevention agents, only biological systems allow the coexistence of amorphous and crystalline calcium carbonate for extended periods of time. Stabilization of the amorphous phase against transformation to the thermodynamically stable form results in the formation of a unique inorganic-organic hybrid material.

Compressed Monolayers

Epitaxial mechanisms have been proposed for the nucleation of oriented inorganic minerals in biological

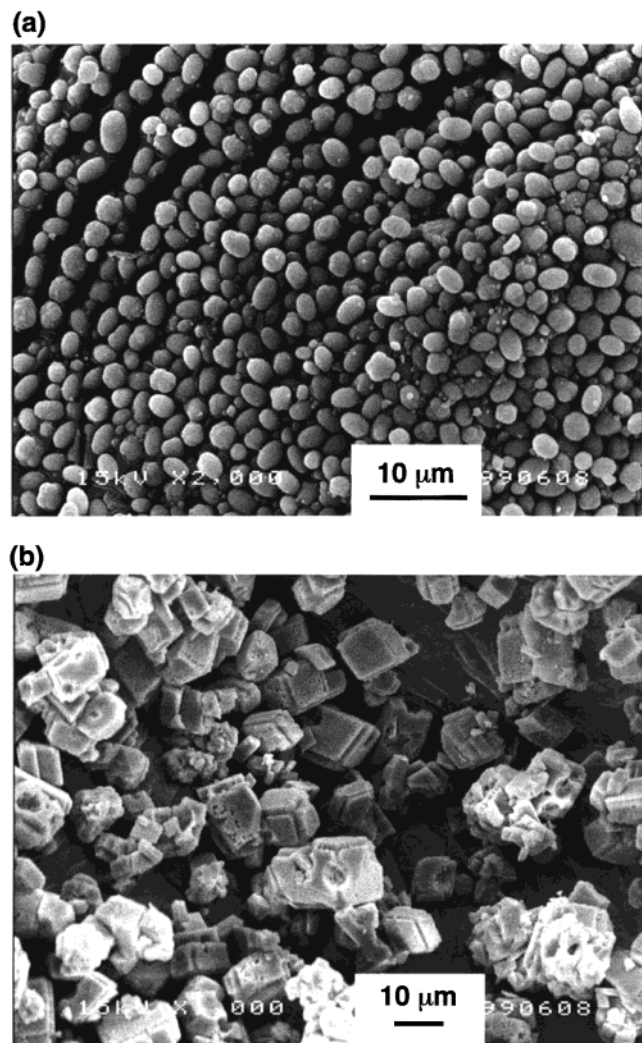


Figure 9. Scanning electron micrographs of the crystalline products in the presence of (a) and the absence of (b) PAMA dendrimer ($G = 1.5$). (Reproduced from ref 43.)

systems. The crystallographic relationships between the overgrowth and the substrates can be explained by the epitaxial matching of lattice spacing specific crystal planes at the crystal/substrate interface. However, in the biological systems the substrates are often an organic framework of complex composition. The possibility that organized organic surfaces are capable of mediating inorganic crystallization reactions has generic importance because these substrates can be extensively tailored by specific chemical modifications of functional groups. A number of different experimental strategies have been developed to elucidate the mechanisms of inorganic crystallization at organized organic templates. A biological approach as described above has been to isolate the matrix components of mineralized tissue. Earlier studies on mollusc shells highlighted the potential importance of epitaxial matching between Ca–Ca distances in the aragonite (001) crystal face and repeat distances of carboxylate functional groups on an anti-parallel β -pleated sheet protein backbone.⁴⁷ The difficulty with these studies is that the details of the surface structure cannot be assessed because of the complexity of the macromolecules used. To probe the organic–inorganic interface and to resolve some of the molecular events mediating template-directed crystal

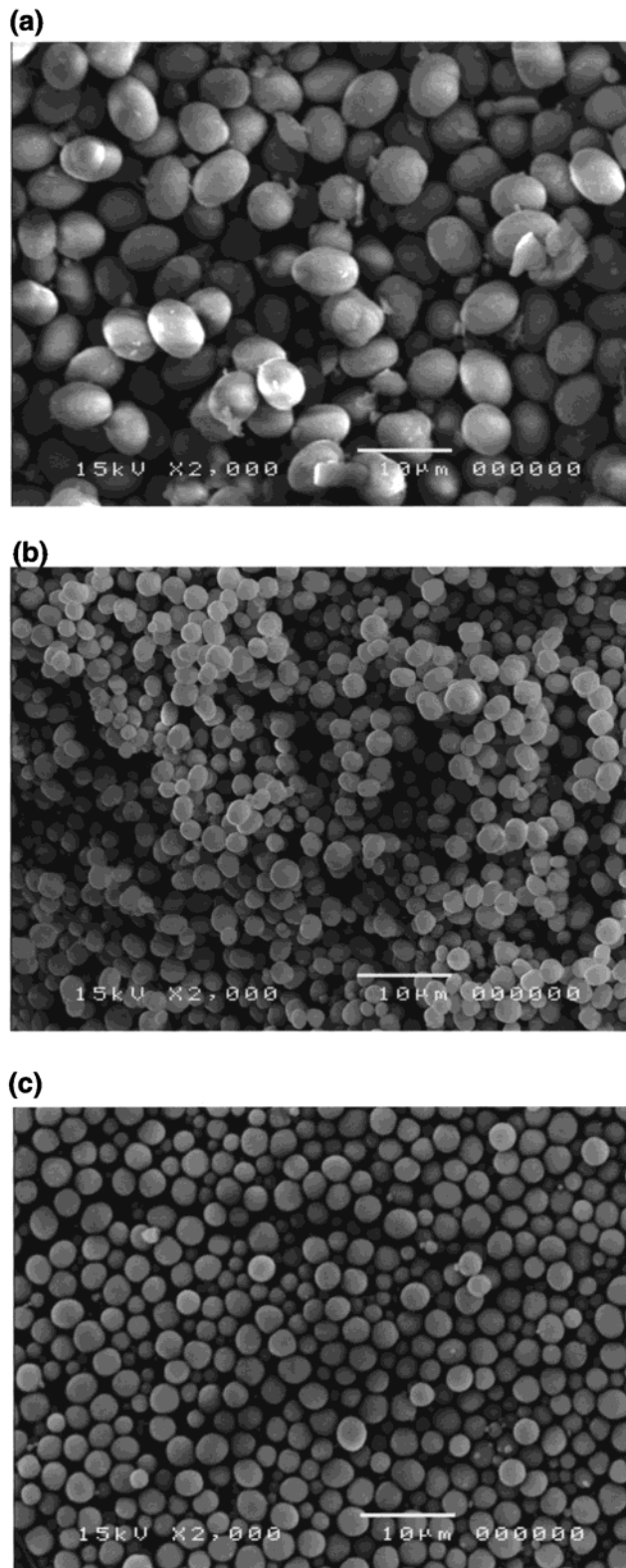


Figure 10. Scanning electron micrographs of vaterite particles in the presence of PAMAM dendrimer ($G = 1.5$) (a), ($G = 3.5$) (b), and ($G = 4.5$) (c) with constant concentration of carboxylate units (corresponding to 0.26 mM of $-\text{CO}_2\text{Na}$). (Reproduced from ref 44b.)

nucleation and growth, a simple model surface was required. Langmuir monolayers were adopted as planar two-dimensional substrates on the basis of the spreading of an insoluble amphiphile of the appropriate molecular design (headgroup identity, polarity, packing

conformation) at the gas–liquid interface. These interfaces could potentially mimic the surface of three-dimensional crystals with the result that nucleation would be favored at the monolayer–solution interface.

The crystallization of CaCO_3 under stearic acid monolayers at air/water interfaces resulted in dramatic changes in the crystallochemical properties of the crystalline product.⁴⁸ Crystallization in the absence of films gave rise to uncontrolled intergrowth of calcite of rhombohedral morphology at both the air/water interface and at the bottom of the reaction vessel. Crystallization under fully compressed monolayers at total $[\text{Ca}] = 9 \text{ mmol dm}^{-3}$ resulted in a white sheet of oriented calcite crystals. Reducing the Ca concentration to 4.5 mmol dm^{-3} promoted vaterite nucleation. The vaterite crystals were oriented with their c axis perpendicular to the monolayer surface, suggesting that stereochemical and electrostatic interactions were responsible for nucleation on this specific face. Disk-shaped single crystals of vaterite were prepared for the first time on an organized polymeric substrate. Stereochemical complementarity appears to be a critical factor determining the alignment of inorganic crystal nuclei under the monolayer surface. This work has been extended to the use of compressed monolayers of octadecylamine, octadecanol, and cholesterol at a range of supersaturation conditions.⁴⁹ Crystallization under fully compressed octadecylamine monolayers resulted in the orientated nucleation and growth of the metastable phase, vaterite, at the organic surface. The formation of vaterite was independent of the extent of monolayer compression and supersaturation. Neutral monolayers such as those formed from octadecanol and cholesterol inhibit crystallization and have minimal influence on the nucleation and growth processes. The nucleation of vaterite on films of positive and negative charge indicates that binding to the calcium ion is not a prerequisite for stabilization of this metastable phase. It is known that vaterite formation in aqueous solution is favored under conditions of high HCO_3^- to Ca^{2+} ratio, whereas calcite is favored at stoichiometric proportions.⁵⁰ Under such conditions, positively charged surfaces of CaCO_3 are covered with carbonate anions. Thus, a high charge density at the surface could kinetically favor vaterite nuclei through stabilization of disordered clusters of excess charge. For neutral monolayers, the absence of these interactions and the increased hydrophobic character of the headgroups serve to inhibit nucleation.

Self-assembled Monolayers of Alkylthiols on Gold

The discovery that thiols of long-chain organic molecules bind strongly and specifically to metal (usually mercury, silver, or gold) surfaces forming ordered monolayers, the self-assembled monolayers (SAM), has yielded an interesting class of molecular assemblies that permit the production of highly specific interfaces spread over relatively large areas. The SAM surfaces can be modified through the organic functionality at the end of the molecule, usually the ω functionality on a long-chain thiol. The results of model biomineralization studies using clean and thiol-modified gold surfaces as the templating substrate are reported in detail by Tremel's group.^{51–53} Calcium carbonate crystallization was per-

formed in a closed desiccator using solid $(\text{NH}_4)_2\text{CO}_3$ to the CO_3^{2-} concentration of CaCl_2 solution. The whole apparatus was placed in an oven to control the temperature. The parameters that could be controlled were the thiol chain length, the ω -substituent on the thiol, and the temperature. The slow crystallization of calcium carbonate at 22°C on SAM surfaces yielded in most cases the vaterite and calcite modifications. The results for crystal growth at 22°C largely match those obtained by Mann and co-workers, who used Langmuir monolayers at the air/water interface as templates. When the temperature at which the crystallization was carried out was elevated to 45°C , stabilization of aragonite modification was observed. The weight fractions of the calcite–aragonite ratio of the product obtained after crystallization on p -mercaptophenol SAM was 40:60 at 22°C . Under the same conditions but at 45°C , the calcite–aragonite ratio was 14:86. Aragonite was kinetically stabilized by elevating the temperature. They assumed that the effect of temperature is probably to increase the rate at which the ammonium carbonate is decomposed, thereby resulting in a greater degree of supersaturation. The long-chain alkyl-terminated thiol does not show any aragonite, even at 45°C . Aragonite has the propensity for ill-formed SAM surfaces such as those assembled from thiols with short alkyl chains. On such ill-formed surfaces, it is possible that aragonite is rapidly nucleated, resulting in it being kinetically stabilized. SAMs are also made on silicon surfaces by the specific binding of silyl functionalities, and these have been used in model biomineralization studies.⁵⁴

Aizenberg and co-workers reported a route to crystal formation, using micropatterned self-assembled monolayers, which affords control over all these parameters.⁵⁵ A metal surface was patterned with a self-assembled monolayer having areas of different nucleating activity in which an array of acid-terminated regions separated by methyl-terminated regions. When the patterned substrates were immersed in a calcium chloride solution and exposed to carbon dioxide, calcite crystals were formed in the polar regions. The nucleation density was controlled by varying the area and distribution of the polar regions.

The chemistry of thiol SAM on metal surfaces has been extended to metal colloids. Colloidal solutions of nanometer particles enable one to carry out "surface chemical studies in solution". Simple spectrophotometric methods can be used to investigate the optical changes that accompany the surface reactions since solutions of nanometer particles are transparent and the scattering of light can be neglected. Tremel and co-workers used p -mercaptophenol-protected gold colloids as templates for the growth of inorganic crystallization.⁵³ Going from flat surfaces to colloids in solution introduces some interesting new aspects. First, the crystallization is carried out heterogeneously (at an interface) in a homogeneous solution. Second, the templating substrate is changed from two-dimensional to the curved two-dimensional. When the crystallization experiments were carried out in the presence of p -mercaptophenol-protected gold colloids, the initial deep-pink solutions of protected gold colloids turned completely colorless at the end of the crystallization. This observation indicates that the precipitation of CaCO_3 in some way resulted

in an entrapment of the colloids. Comparing flat templates, they observed significant differences in the nature of the crystallization products. An important issue in using a small, round template is that the crystals mutually frustrate the growth of one another in the direction tangential to the growing spherical.

Calcium Carbonate Thin Films

Most of the techniques described above advances in the selective nucleation and growth of inorganic crystals with specific phase, orientation, and micropatterns. However, once the inorganic layer begins to grow away from the substrate–solution interface, there is no facility for organic material to adsorb onto or to become incorporated within the growing inorganic structure or to do both. An *in vitro* precipitation of a calcium carbonate in the form of a thin film has been attracting interest. Some features of these products resemble the morphologies found in biogenic minerals. In particular, the nacre of mollusc shells consists of lamellar sheets of aragonite tablets, separated by a thin organic matrix called the conchiolin membrane (Figure 1). Ceramic films are also in demand as active and passive components in microelectronic circuits as capacitors, memories, and insulating or passivating layers. Conventional ceramic processing, which involves high-temperature sintering, cannot be used for ceramic coatings on plastics. With biomimetic processing, high-quality, oriented, and patterned ceramic films can be deposited on plastics and other materials at temperature below 100 °C. Biomimetic film formation can produce materials of both industrial and scientific interest. The solution nucleation and growth of thin films of iron hydroxides onto sulfonated polystyrene substrates and sulfonated self-assembling monolayers attached to oxidized silicon was achieved.⁵⁶ Biomimetic processing of inorganic thin films except CaCO₃ was already reviewed.^{57,58}

Several factors could influence mineral nucleation and crystal growth on a polymer film such as the degree of saturation of a supersaturated solution and the surface charges on a polymer film. It is very crucial for successful biomimetic synthesis that only heterogeneous nucleation is promoted on the polymer film and that homogeneous nucleation is suppressed in the mother liquid. One key factor for successfully mimicking biomineralization to coat a dense ceramic film on organic polymer substrates is to increase the density of charges or polarity on the substrate surface. Poly(acrylic acid) (PAA) proved to be an effective additive, promoting calcium carbonate heterogeneous nucleation on chitosan-film surfaces and suppressing homogeneous nucleation in solution.⁵⁹ In the presence of poly(acrylic acid), calcium carbonate crystals heterogeneously nucleated and grew only on the chitosan-film surface and covered the whole film. In the absence of PAA, homogeneous nucleation occurred in the supersaturated calcium carbonate solution. Gower and Tirrell reported that a highly birefringent CaCO₃ film formed in streaks and patches on the glass substrate by addition of poly(aspartate).²⁵ The inorganic polycrystalline films form in a variety of textures and appear to control the type of particles produced. Mosaic films composed of interconnected single crystals tend to carry aggregates of rhombohedral calcite crystals, whereas films of spheru-

litic texture have spherulitic vaterite particles associated with them.

Kato et al. have independently introduced a series of CaCO₃ thin film formation on organic substrates. They initially reported that the effects of macromolecules as soluble additives and solid matrixes have been examined for the crystallization of CaCO₃.⁶⁰ Crystalline vaterite grows on a glass substrate in the presence of poly(glutamic acid). No crystal growth has been observed when poly(acrylic acid) exists as an additive. Thin film states of CaCO₃ crystals have been obtained as organic/inorganic composites with chitosan that acts as a solid matrix in the presence of poly(acrylic acid) or poly(glutamic acid) as a soluble additive. The cooperation of functional groups of these polymers may play a key role for the control of crystallization. They performed thin film coating of CaCO₃ crystals on a chitin fiber from CaCO₃ solution in the presence of an acid-rich macromolecule such as poly(acrylic acid), poly(aspartic acid), and poly(glutamic acid).⁶¹ The carboxylic acid of the polymer entrapped on the chitin surface through a hydrogen bond strongly binds the calcium ion. The local high concentration of the calcium ion on the surface and the inhibition of the growth to the direction vertical to the surface by the adsorbed acid polymers may result in the thin film formation of CaCO₃. Layered polymer/calcium carbonate composite films were obtained by alternate repetition of spin coating of polysaccharides and thin film crystallization of CaCO₃ in the presence of an acid polymer.⁶² Aragonite thin films of calcium carbonate were successfully deposited on chitosan matrixes by cooperation of chitosan, poly(aspartate), and MgCl₂ in CaCO₃ solution.⁶³ By alternate operations of chitosan spin coating and aragonite crystallization, double-layered composite films of aragonite and chitosan can be produced. Such multilayered composite materials are similar to the nacreous structure. More precise control of the composite structure in the crystallization processes may lead to high-performance properties such as high mechanical strength.

The template/inhibition strategy for the synthesis of CaCO₃ thin film has also been employed independently by Groves's group.^{64,65} They reported the synthesis of macroscopic and continuous calcium carbonate thin films at a porphyrin template/subphase interface by employing poly(acrylic acid) as a soluble inhibitor to mimic the cooperative promotion–inhibition in biogenic thin film production. The strategy is to prevent crystal growth in the direction normal to the template so as to limit the space available for mineral growth to the very thin layer right below the promoting substrate. The forced two-dimensional growth then leads to the formation of continuous thin films. A semirigid template for crystallization was spontaneously formed via the self-organization of the amphiphilic tricarboxyphenylporphyrin iron(III) μ -oxo dimer at an air/water interface. The observation of an amorphous calcium carbonate precursor phase provides direct evidence that the multistep-stage crystallization process is possible in a biomimetic environment.

Anionic poly(amidoamine) (PAMAM) dendrimer was selected as a model of the soluble acidic-rich proteins to prepare CaCO₃ film on a poly(ethylenimine) film.⁶⁶ The CaCO₃/poly(ethylenimine) composite film was ob-

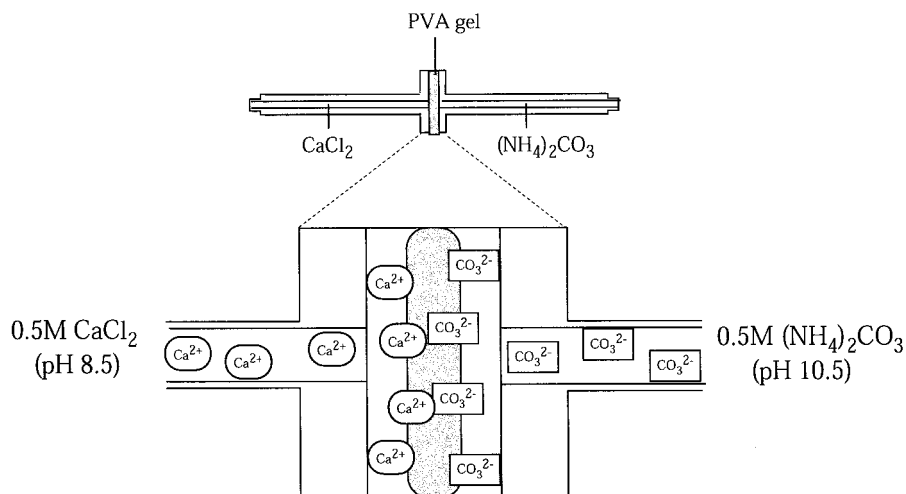


Figure 11. Experimental setup of a permeation supply method for the precipitation of calcium carbonate in the hydrogel.

tained in the presence of anionic PAMAM dendrimer ($G = 3.5$), whereas the formation of composite film was not observed without PAMAM dendrimer or with PAMAM dendrimer of lower generation ($G = 1.5$). PAMAM dendrimer with calcium ion was adsorbed on the poly(ethylenimine) surface through an insoluble polymer complex. In the absence of PAMAM dendrimer, poly(ethylenimine) was dissolved in an aqueous phase. The complexes of anionic dendrimers with calcium ions are considerably stronger for the higher generations than the lower generations. The amount of calcium ions entrapped in the internal coordination site of PAMAM dendrimer with higher generation might be higher than that with lower generation. Higher generation of PAMAM dendrimer ($G = 3.5$) inhibits the crystallization of CaCO_3 in the solution. The adsorption of PAMAM dendrimer on poly(ethylenimine) film caused high local concentration of calcium ion.

Insoluble Polymer Matrix

Biomimetic synthesis of inorganic crystals such as hydroxyapatite in the presence of an insoluble organic polymer matrix has been intensively investigated for the preparation of biomaterials.^{67,68} Apatite-polymer composites are expected to be useful for repairing not only hard human tissues but also soft ones since they can exhibit not only a high bone-bonding ability but also a high compatibility with soft tissues and mechanical properties analogous to those of both hard and soft tissues.

Crystallization of calcium carbonate in the presence of an insoluble template has been studied in recent years. Falini et al. induced the orientated crystallization of calcium carbonate by cross-linked gelatin films with entrapped poly-L-aspartate.^{69,70} No orientation of the mineral phase was observed with entrapped poly-L-glutamate at the same concentration. These results suggest that the oriented crystallization is controlled by the β -sheet structure of poly-L-aspartate. The local supersaturation in the microenvironment in which nucleation and growth occur plays an important role in controlling the deposition of aragonite or vaterite in cross-linked gelatin films. In addition, collagen bundles and the oriented polypeptide chains can contribute to

the control of polymorphism by inducing the formation of a specific phase by epitaxial crystallization.

Crystallization of CaCO_3 on insoluble polymer particles was studied. Carboxylate-group-containing polymer particles, poly(vinyl chloride-co-vinyl acetate-co-maleic acid), were suspended in an aqueous supersaturated calcium carbonate solution.⁷¹ SEM observation showed that vaterite crystals were overgrowths on the polymer particles. The kinetics of the overgrowth of the mineral phase on the polymer showed linear dependence of the rates on the solution supersaturation. The rate-determining step is the surface diffusion of the growth units on the supercritical nuclei that are formed at the active growth sites provided by the polymeric substrates.

Calvert and co-workers reported the crystallization of CaCO_3 using agarose gel.^{28,72} The gel was doped with soluble calcium salts. The gel was then mineralized by diffusion from a solution of carbonate or phosphate and dried to make a stiff material. Wada et al. studied the effect of divalent cations upon nucleation of aragonite crystal in an agar gel using a double-diffusion method.⁷³ Recently, we developed a similar method for preparation of CaCO_3 /poly(vinyl alcohol) (PVA) hydrogel composite materials.⁷⁴ The crystallization of CaCO_3 in the PVA gels was performed by a permeation supply method which is illustrated in Figure 11. Calcium ions permeated into the hydrogel from one side of the hydrogel disk, and carbonate ions permeated from the opposite side of the hydrogel disk. Permeated Ca^{2+} ions meet with carbonate ions at the inside of the hydrogel disk and induced crystallization of CaCO_3 in the gel. The SEM observation of the obtained CaCO_3 /hydrogel composite shows the formation of spherical vaterite particles in the gel (Figure 12). It can be speculated that hydroxyl groups of the PVA hydrogel and microenvironment of the hydrogel structure induce the vaterite crystals.

Synthetic polymers can be imprinted with motifs of crystal surfaces so as to template the growth of specific crystal phases.⁷⁵ The synthesis of the polymers involves the adsorption of functional monomers to a calcite surface, followed by copolymerization with a cross-linker to create an imprint of the crystal surface. Subsequent removal of the calcite template yields a polymer matrix

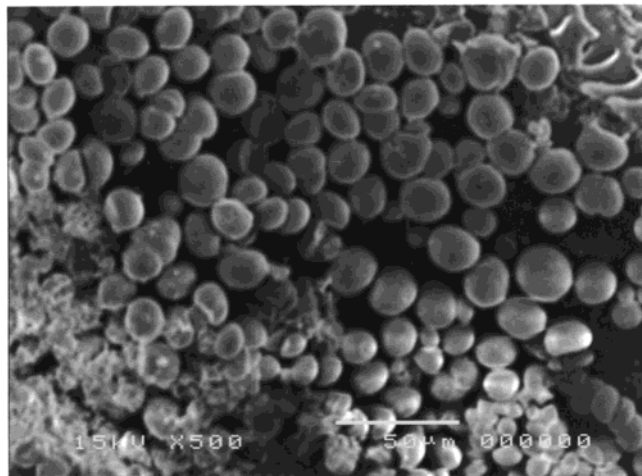


Figure 12. Scanning electron micrograph of a vertical sectional view of the PVA hydrogel after calcium ions and carbonate ions were permeated into the gel for 6 h.

with a surface functionality mirroring the crystal face and able to promote the nucleation of calcite. A schematic representation of the imprinting and directed nucleation processes reported here is shown in Figure 13. They used 6-methacrylamidohexanoic acid as a functional monomer and divinylbenzene as a cross-linker to convert calcite-templated monomer assemblies into a polymer matrix.

Self-organized Media

Microscale phase separation of reaction media has importance in the synthesis of inorganic materials with unusual form and hierarchical structure, analogous to the exquisite biomineralized architectures fashioned by many single-celled organisms such as coccoliths and radiolarians. Thin cellular frameworks of either meso-

porous or macroporous aragonite can be formed from an oil–water–surfactant microemulsion saturated with calcium bicarbonate, with the pore size determined by the relative concentrations of water and oil.⁷⁶ These structures originated from rapid mineralization of aragonite with a self-organized foam of oil droplets acting as a structural template. When micrometer-sized polystyrene beads are used as the substrate for the microemulsion, hollow spherical shells of the honeycomb architecture can be produced. Hirai et al. used a double water-in-oil-in-water emulsion system as a biomimetic environment for the preparation of calcium carbonate particles.⁷⁷ Calcium ions were extracted from an external aqueous phase containing calcium nitrate into the organic membrane phase and stripped into the internal aqueous phase containing sodium carbonate, where the precipitation of calcium carbonate occurred. The polymorphic form of the particles was changed from aggregated vaterite to micron-sized single-crystalline rhombs of calcite by raising the pH of the internal aqueous phase.

Another example for templated crystallization of calcium carbonate was carried out in foam lamellae.⁷⁸ A foam provides a high surface area of gas bubbles dispersed in a liquid, which provide increased interfacial area/unit volume of solution. The stabilizing surfactant or other amphiphile adsorbed at the gas/liquid interface and these interfaces acts as a template for crystallization.

Conclusions

Over the past decade, considerable work has been made on mineralization of calcium carbonate in the presence of various organic templates and additives. These interesting efforts have led to fundamental developments in areas relating to the biomineralization

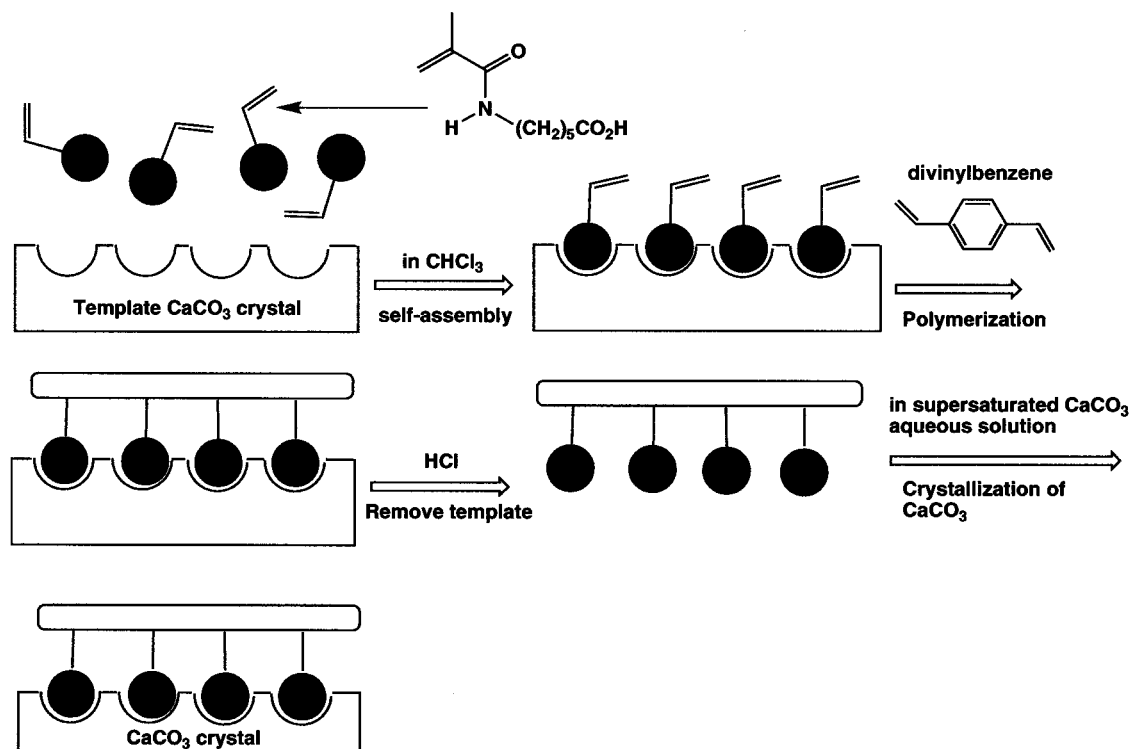


Figure 13. Schematic representation of nucleation of calcite at a crystal-imprinted polymer surface. Adapted from ref 75.

process. However, there still remain many unknowns as to how the natural organisms produce inorganic–organic hybrid materials. Different crystal polymorphs, calcite, aragonite, vaterite, and amorphous calcium carbonate, are formed and stabilized by organisms. Thermodynamically stable forms, calcite and aragonite, are mainly produced by organisms.¹³ Some organisms stabilized vaterite and amorphous calcium carbonate in their tissues. While there can be no doubt that these crystal structures are determined by local conditions in natural systems, specific biological requirement of the organisms to manipulate both the polymorph and the orientation of the mineral is open to question. The core of these questions is derived from a genetic basis for the diversity and evolution of biominerals. In our opinion, this point might be clarified during continuous efforts of fundamental biomineralization studies.

Natural inorganic–organic hybrid materials are manufactured through multistep processes. First, the extracellular macromolecular substrates are pre-organized for regiospecific nucleation and the subsequent development of biominerals with controlled microarchitecture. Second, inorganic ions are selectively transported to produce localized high concentration within discrete organized compartments. Third, crystallization of inorganic materials is selectively induced at the site of biomineralization. Since the final concentration of the ions is still below supersaturation, induction of crystallization occurs by a specific matrix. Crystal morphology, size, polymorph, and orientation are controlled by local conditions and, in particular, the presence of “matrix” proteins or other macromolecules. Finally, the crystal growth is determined by the pre-organized cellular compartments. Although active research has resulted in the development of new methodologies for controlled crystallization of minerals in aqueous solution, most biomineralization studies mainly focused on the third stage. The first and the second stages are closely related to the research field of so-called “supramolecular chemistry”. The main question is how to construct organized well-defined structures by self-assembling processes.

The abalone shell is a composite of calcium carbonate plates sandwiched between an organic layer as described in the beginning of this review (see Figure 1). The organic component, comprising just a few percent of the composite by weight, is thought to hold the key to nacre’s fracture toughness. Although recent efforts showed that ceramics laminated with an organic material are more fracture-resistant than nonlaminated ceramics, the synthetic materials made of interlocking ceramic tablets bound by a few weight percent of ordinary adhesives do not have a toughness comparable to nacre.⁷⁹ Recent atomic force microscopic analysis suggested that a modular elongation mechanism might prove to convey toughness to natural fibers and adhesives.⁸⁰ Such a kind of synthetic adhesive has not been prepared so far. A modular mechanism might work for synthetic polymers, as long as it is possible to compact the length of the polymers using intermediate-strength bonds that break sacrificially with external force. Polymer scientists should make such polymeric materials, which would lead to industrial applications.

From these opinions, the continuous cooperation of organic and polymer chemists with inorganic and

biochemists is desirable for discovering new concepts and methods for constructing composite materials and crystalline forms analogous to those produced by nature. We expect that these continuing and fundamental efforts to clarify the biomineralization process by nature would lead to the next industrial revolution.

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