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Control of calcium carbonate morphology by transformation of an amorphous precursor in a constrained volume

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Calcium carbonate crystals of defined morphology have been produced on transformation of an amorphous calcium carbonate precursor within a constrained volume.

The range of morphologies exhibited by inorganic crystals in biological systems is truly remarkable and provides a unique inspiration for synthetic crystal growth experiments.[1,2] Indeed, many biominerals display complex forms and curved surfaces that are as yet quite impossible to replicate in the laboratory. As an important step forward in understanding control of calcium carbonate growth *in vivo*, it has recently been demonstrated that certain biological calcite crystals actually comprise two mineral phases, calcite and amorphous calcium carbonate (ACC).[3–5] Examination of the mechanism of calcification in sea urchin embryos has further shown that in this organism, not only do ACC and calcite co-exist, but that ACC transforms into calcite over time.[3,4] The ACC therefore acts as a transient precursor to the more stable calcite phase,[3] and transformation of ACC to calcite within the constrained volume imposed by the spiculogenic cavity results in single crystals of calcite with a tri-radiate form and curved surfaces.

The role of ACC in the biological calcification process has not been entirely resolved, although it has been suggested that it may provide a temporary storage site in some organisms, or that in combination with a crystalline phase may provide a skeletal element with superior mechanical properties.[5,6] The experiments described here investigate whether ACC may also play an important role in morphological control. Indeed, it has been demonstrated that thin films of calcite can be formed in association with Langmuir monolayers *via* transformation of an ACC precursor film.[7] In common with the calcification mechanism active in sea urchins, in the current experiments ACC was contained within a restricted volume, and the morphological changes of the calcium carbonate particles on transformation to calcite were studied.

As a simple model system, 10 μm thick polycarbonate track-etch membranes were used as the crystallisation environment.[8] These membranes possess extremely regular cylindrical channels of diameter 3 μm, which are similar to the dimensions to sea urchin embryo spicules. ACC was precipitated in the membrane pores by placing a membrane between two half U-tube arms, and filling one tube with $CaCl_2$ solution and the other with Na_2CO_3 solution.† The experiments were carried out at 4–6 °C in order to stabilise ACC, by reducing the rate of transformation to calcite. Counter-diffusion of ions through the membrane pores resulted in precipitation of $CaCO_3$. The experimental conditions used generate ACC in bulk solution. Microscopic examination of the membrane after crystallisation revealed two populations of crystals. 15 μm crystals with intergrown, rhombohedral morphologies precipitated on the surface of the membrane. Particles were also observed within the membrane pores, and exhibited a circular cross section of the same dimensions as the membrane channel [Fig. 1(a)]. The morphologies of the intra-membrane crystals were further examined subsequent to dissolution of the membrane. Again, the two populations of crystals were observed but were readily distinguished on the basis of size and morphology. The intra-membrane crystals were of dimensions 3 × 10 μm and displayed cylindrical morphologies and curved surfaces, as shown in Fig. 1(b). Thus, both the size and shape of these crystals had been clearly dictated by the geometry of the membrane channels in which they had formed.

The crystals deposited on the membrane surface were adhered strongly to it, which compromised opportunities for *in situ* analysis of the intra-membrane crystals. To confirm that the intra-membrane crystals formed from an initial ACC phase, they were isolated by dissolution of the membrane at early stages of the experiment. Microscopic examination of the separated crystals clearly showed that the cylinders formed *via* an ACC precursor [Fig. 2(a)]. Spherical particles of diameter 0.5 μm, which are characteristic of ACC,[9,10] were observed to initially coat the walls of the pores, before filling in the entire volume and generating the final cylindrical form. Identical particles were produced in a control experiment by mixing the $CaCl_2$ and Na_2CO_3 solutions at low temperatures in the absence

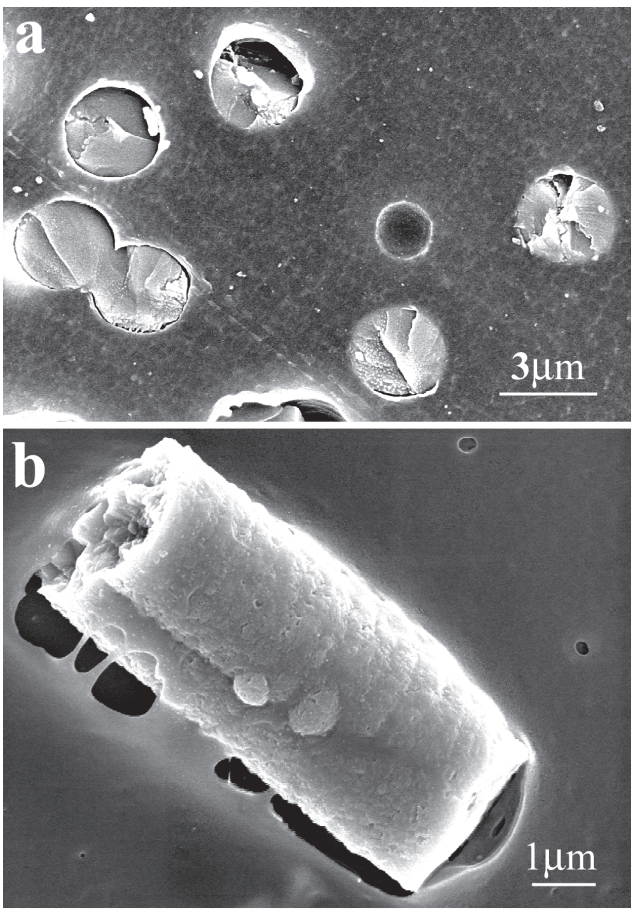

Fig. 1 (a) Calcium carbonate crystals grown within membrane pores. (b) Intra-membrane crystal isolated from the membrane, showing cylindrical morphology and dimensions identical to the membrane pores. Both images are of samples after 24 h.

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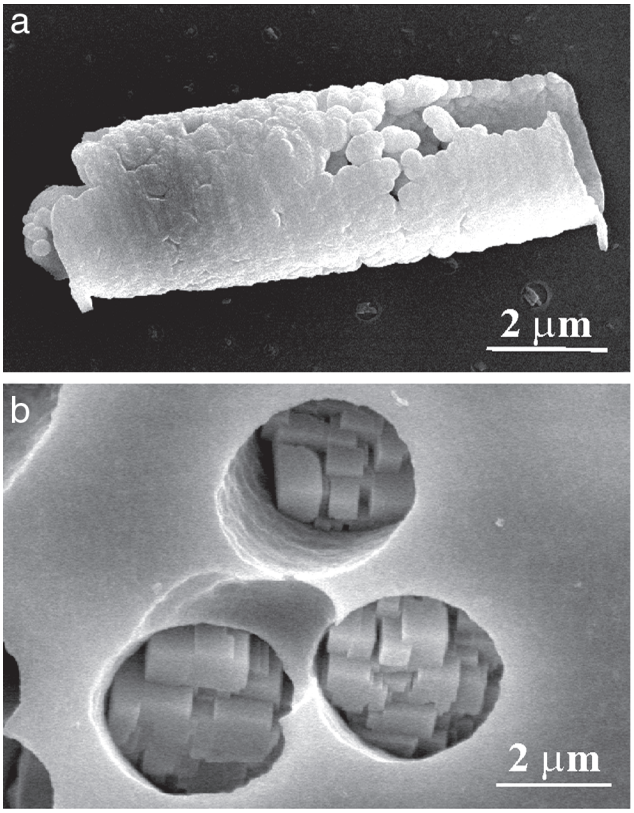

Fig. 2 (a) Intra-membrane particle, isolated after 15 min, showing its formation from spherical ACC particles. (b) Crystals grown within membrane pores showing crystalline faces characteristic of calcite (sampled after 24 h).

of the membrane, and were confirmed to be ACC using FTIR and XRD.

Comparison of the images of the intra-membrane particles at early stages [Fig. 2(a)], and after longer times [Fig. 1(b)] shows that the structure of the cylindrical particles alters with time. While the distinct spherical morphologies of the amorphous precursor particles are initially clearly observed within the cylindrical particle, continued incubation in solution results in transformation to a cylindrical particle with indentical overall morphology but with uniform structure. Examination of the ends of the mature cylindrical crystals frequently shows blocky crystal faces typical of rhombohedral calcite [Fig. 2(b)]. It has not as yet been impossible to conclusively identify the crystals as calcite, or to probe whether they are single crystal or polycrystalline as they are too small to investigate using standard laboratory XRD. However, selected area electron diffraction, carried out in a transmission electron microscope (TEM) was performed on the mature crystals in an attempt to obtain a diffraction pattern from a thinner edge area. Some diffraction patterns were obtained, all of which could be fitted to calcite. In addition, examination of the cylindrical particles between crossed polars in an optical microscope demonstrated that they were anisotropic, which again suggests crystallinity.

In order to investigate whether the precursor ACC phase was necessary for the precipitation environment to impose its structure on the final morphology of the crystal, the experiments were repeated at room, rather than low temperature. In the absence of the membrane, calcite precipitated immediately from solution. Precipitation within the membrane pores resulted in crystals of irregular, as opposed to cylindrical morphologies.

These experiments demonstrate that transformation of ACC within a constrained volume can produce a crystalline particle of morphology imposed by the environment. Both the ACC precursor phase and the constrained volume appear to be essential to this mechanism of morphology control. Biology obviously does not apply low temperature as a route to producing ACC. However, there is increasing evidence that organisms synthesise organic macromolecules specifically for stabilising ACC with respect to the thermodynamically stable phase calcite.[5,7,11] We therefore suggest that deposition of ACC within a vesicle may provide organisms with a route to producing crystals with elaborate forms and curved surfaces, such as the remarkable fenestrated calcitic skeletal plates of sea urchins.

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Notes and references

† A polycarbonate track-etch membrane (pore diameter 3 μm) was placed between two, identical, half-U tube arms, which were clamped at the membrane position to form a U-tube. A small volume of cold water was introduced into the U-tube to wet the membrane. Equal volumes of 0.1 M solutions of Na_2CO_3 and $CaCl_2$ in Millipore water, which had been previously filtered and cooled to 4–8 °C were then simultaneously poured into each of the U-tube side-arms. The apparatus was placed in a refigerator at 4–6 °C for a period ranging from 15 min to 24 h. On completion of the experiment, the membrane was washed with water and was dried using compressed air. The $CaCO_3$ crystals produced in association with the membrane were examined using scanning electron microscopy (SEM) either *in situ* in the membrane, or after dissolution of the membrane in chloroform. All samples were Au-coated prior to examination in a JEOL 6300F scanning electron microscope (SEM), fitted with a field emission source and operating at 10 kV. Selected-area electron diffraction was also carried out on the $CaCO_3$ crystals after isolation from the membrane. A drop of an ethanolic solution of the isolated crystals were placed on a C-coated, Formvar-covered Cu TEM grid, and was examined in a JEOL 2010 transmission electron microscope (TEM) operating at 200 kV.

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