Fabrication and Growth Mechanism of Pumpkin-Shaped Vaterite Hierarchical Structures

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Supporting Information

ABSTRACT: CaCO₃-based biominerals usually possess sophisticated hierarchical structures and promising mechanical properties. Recent researches imply that vaterite may play an important role in the formation of CaCO₃-based biominerals. However, as a less common polymorph of CaCO₃, the growth mechanism of vaterite remains unclear. Here we report the growth of a pumpkin-shaped vaterite hierarchical structure with a sixfold symmetrical axis and lamellar microstructure. We demonstrate that the growth is controlled by supersaturation and the intrinsic crystallographic anisotropy of vaterite. For the scenario of high supersaturation, the nucleation rate is higher than the lateral extension rate, favoring the “double-leaf” spherulitic growth. Meanwhile, nucleation occurs preferentially in (1120) as determined by the crystalline structure of vaterite, modulating the grown products with a hexagonal symmetry. The results are beneficial for an in-depth understanding of the biomineralization of CaCO₃. The growth mechanism may also be applicable to interpreting the formation of similar hierarchical structures of other materials.

Crystals with complicated hierarchical structures typically exhibit unique physical or chemical properties compared to their single crystalline counterparts.¹⁻³ In nature, CaCO₃-based biominerals such as nacre, sea urchin spine, and egg shell are excellent examples showing that ordered structures extensively enhance the mechanical performance of crystals.¹⁻³ Understanding the formation mechanisms of self-organized CaCO₃ structures has attracted much attention, in an attempt to realize controlled growth of advanced functional materials as well as to clarify biomineralization process.¹⁻¹²

The main inorganic component of CaCO₃-based hard tissues such as shells and nacres is typically aragonite or calcite polymorph.¹⁻³ Vaterite, the third polymorph of CaCO₃, is less common in biological tissues because it usually spontaneously converts to calcite or aragonite in water.¹ Therefore, it has attracted less attention for a long time and its crystallographic structure and growth habits are not well understood.¹⁻⁶ Recently, in situ study shows that CaCO₃ crystallization starts with the formation of amorphous calcium carbonate (ACC), which then directly transforms into vaterite.¹⁻⁶ The results suggest that vaterite could be a precursor in the formation of different CaCO₃-based biominerals, and play a key role in establishing the final hierarchical structure. Various hierarchical structures of vaterite have been fabricated in vitro.⁹⁻¹¹ In spite of quite different morphologies, these products reveal similar microstructures. They are composed of lamellar crystalline plates highly resembling the nacre structure and exhibit a sixfold symmetric feature.⁹⁻¹¹ Since vaterite might act as a precursor of nacre (and other CaCO₃-based biominerals),⁹⁻¹² this lamellar growth could be deterministic of the unique nacre structure. The structures were also found to depend primarily on supersaturation,⁹,¹⁰ which shows a promise of tunable growth of products with different microstructures. Thus, for a better understanding of the formation mechanism, tunable growth, of CaCO₃-based hierarchical structures, in-depth study on the growth mechanism of the hierarchical structures of vaterite is highly expected.

Here we report the growth of a pumpkin-shaped vaterite hierarchical structure with a sixfold symmetrical axis and lamellar microstructures. Based on structural characterization and in situ observations of the growth process, we demonstrate that this unique morphology is generated via a “double-leaf” spherulitic growth with the branching regulated by the competition of the lateral extension rate and the nucleation rate of lamellar plates. The intrinsic crystallographic anisotropy of vaterite determines preferential branching sites and is responsible for the symmetric properties of the final products. We also propose that the spherulitic growth might be closely correlated with the intrinsic dipole field.

The samples are prepared via a (NH₄)₂CO₃ diffusion growth method.¹³ Analytical grade CaCl₂ (NH₄)₂CO₃, and Na₂CO₃ purchased from Sigma-Aldrich were used without further purification. For typical experiments, 10 mL CaCl₂ aqueous solution (5 mM in concentration) was filled in a growth cell. The cell was covered by a glass disk and placed at the center of a Petri dish. A container with 1 g (NH₄)₂CO₃ was placed in the
Petri dish next to the growth cell as a source of CO₂. The growth time was 5 min to 24 h for different batches. Products were grown on the cover glass as well as in the solution. The growth process was real-time monitored by an optical microscope (Nikon Eclipse 71i) with a 50X long-focus objective lens and a CCD camera. All the products were thoroughly washed with deionized water and dried at room temperature before characterization. A field-emission scanning electron microscope (FESEM, Zeiss Ultra 55) and a high resolution transmission electron microscope (HRTEM, FEI Tecnai F20) were used for morphological and structural observations. Fourier transform infrared (FTIR) spectra and powder X-ray diffraction (XRD) spectra were measured by a Bruker Vertex 70 V FTIR analyzer and a Bruker AXS D8 Advance powder XRD analyzer with monochromatic Cu Kα radiation, respectively.

Figure 1A shows a typical scanning electron microscope (SEM) image of the products grown on the cover glass of the growth cell for 5 h with the concentration of Ca²⁺ = 5 mM. (A) Low magnification image showing a mixture of spherical and rhombohedral particles, which are vaterite and calcite, respectively. (B) Top-view image of one vaterite particle showing it has pumpkin-like morphology and six segments. (C) Zoom-in image of one segment showing it is composed of convex-lens-like plates. Each “lens” is composed of tightly packed nanoparticles. (D) Image of a 24 h product showing the “lenses” have turned into plates with well-shaped edges. (E and F) SEM images of the 24 h products after etching in deionized water for 5 min. (E) Image of the equatorial cross section of a pumpkin, indicating the six segments and radially aligned “necktie” units. The inset is a zoom-in image showing the well-shaped tips and the boundaries between the “neckties”. (F) Image of the longitudinal cross section indicating symmetrical branching features. The details are shown in the inset.

Figure 1. (A–C) SEM images of a typical product grown on the glass substrate for 5 h with C(Ca²⁺) = 5 mM. (A) Low magnification image showing a mixture of spherical and rhombohedral particles, which are vaterite and calcite, respectively. (B) Top-view image of one vaterite particle showing it has pumpkin-like morphology and six segments. (C) Zoom-in image of one segment showing it is composed of convex-lens-like plates. Each “lens” is composed of tightly packed nanoparticles. (D) Image of a 24 h product showing the “lenses” have turned into plates with well-shaped edges. (E and F) SEM images of the 24 h products after etching in deionized water for 5 min. (E) Image of the equatorial cross section of a pumpkin, indicating the six segments and radially aligned “necktie” units. The inset is a zoom-in image showing the well-shaped tips and the boundaries between the “neckties”. (F) Image of the longitudinal cross section indicating symmetrical branching features. The details are shown in the inset.

Figure 2. (A) Powder XRD pattern of the product grown for 5 h. The peaks of vaterite and calcite are annotated with “V” and “C”, respectively. (B) FTIR pattern of the product. While the peaks in 1440, 1087, and 870 cm⁻¹ are characteristic of all the polymorphs of CaCO₃, the peak at 745 cm⁻¹ clearly indicates the presence of vaterite. The weak peak at 850 cm⁻¹ should be caused by a small amount of ACC.¹⁴

are a mixture of the vaterite and calcite polymorphs. Selected area electron diffraction (SAED) results presented below further confirm that the spherical particles are vaterite and the rhombohedra crystallites are calcite. The enlarged micrograph of the spherical particle shows that it resembles a pumpkin, with six fluffy segments arranging in a sixfold symmetric style (Figure 1B). A hexagonal pit is visible at the junction of the segments. Further investigation of these pumpkin-shaped products reveals that each segment is formed by micrometer-sized convex-lens-like plates (Figure 1C), which are composed of tightly packed nanoparticles of ~40 nm. When the pumpkins grow for 24 h, the “lenses” will transform into plates with well-shaped edges (Figure 1D). One can see that unlike the smooth side surfaces, the top surfaces remain rough and manifest nanocrystals. The morphology is quite similar to the aragonite plates in nacre.²

Unlike full pumpkins formed in solution, those grown on the cover glass are typically hemispheric. The growth habit allows us to visualize their “cross sections” easily. The samples were peeled off the substrate with carbon conductive tapes, and etched for 5 min in deionized water to reveal the details. Figure 1E is a SEM image of the equatorial cross section of a pumpkin grown for 24 h, clearly showing the six-segment structure. One can see that the cross section is composed of multiple radial “growth lines”. The growth lines are a result of the stacking of the building blocks that gradually broaden along the growth directions (see inset of Figure 1E). The longitudinal cross section shows similar growth features (Figure 1F and the inset). The continuous branching occurs and a symmetric pattern is developed across the longitudinal cross section, with two pits formed at the poles. This pattern is characteristic of the “double-leaf” type spherulites.¹⁵

To investigate further how the pumpkins are formed, the growth was monitored in situ by an optical microscope, and the structures grown at different stages were examined by SEM. Figure 3A shows the snapshots taken during pumpkin formation (see also Supporting Information Movie S1). It is clear that the pumpkin evolves from a spherical core. The sixfold symmetric morphology becomes obvious as the small core grows larger, and the contrast suggests that the thickness of the corners gradually surpasses the central region. The peripheral parts continuously thicken with a faster speed and curl toward the center, and finally a “pumpkin” with six segments is formed. Parallel fringes on the segments of the pumpkin represent the ordered piles of the plates shown in Figure 1. Figure 3B–I shows top- and side-view SEM images of
the structures at different growth stages. At the very early stage of growth, the typical morphology of the products is an ellipsoid (Figure 3B and F). Gradually, six edges are formed, indicating a hexagonal symmetric feature (Figure 3C and G). Comparing Figure 3F and G, we can see that the lateral growth rate is higher than that of the vertical growth. Besides, the lateral growth is also direction-dependent, leading to formation of six edges. At the intermediate stage, layered growth at the circumference of the lens surpasses the central part, and six segments start to emerge from the corners (Figure 3D and H). At the late stage of growth, the segments are well developed and the overall morphology resembles a pumpkin (Figure 3E and I).

To figure out the origin of the hexagonal symmetry of the pumpkins, products grown for 24 h were further studied. SEM micrograph of a representative pumpkin is shown in Figure 4A. The rectangle marked the contact region of the core part and two segments, which is magnified in Figure 4B. It reveals that the area is covered by hexagonal plates with well-shaped edges. The plates are larger at the outer part than at the central part. One noteworthy phenomenon is that all the plates are highly ordered, with their sides parallel to the core-segment contact lines. This result implies that the plates resemble single crystals and the symmetric features of both the “pumpkins” and the plates should be closely related to the hexagonal symmetry of the $P_{6}_5/mmc$ vaterite structure.16

The microstructures of the products at different growth stages are examined. Transmission electron microscope (TEM) image shows that the products grown for 5 min are mainly spherical particles of $\sim$200 nm (Figure 4C). Selected area electron diffraction (SAED) shows smeared rings characteristic of an amorphous phase (inset of Figure 4C), indicating the formation of ACC at the initial growth stage.3 SAED pattern for a 10 min sample shows diffraction arcs (inset of Figure 4D), demonstrating the occurrence of crystallization. This sample is an ellipsoid composed of nanoparticles similar to that shown in Figure 3B and F. Based on the vaterite structure data reported by Kamhi (JCPDF #72–0506),16 the pattern is indexed by (0111) and (1010) diffractions, respectively. The SAED pattern for a 10 min sample shows diffraction arcs (inset of Figure 4D), demonstrating the occurrence of crystallization. This sample is an ellipsoid composed of nanoparticles similar to that shown in Figure 3B and F. Based on the vaterite structure data reported by Kamhi (JCPDF #72–0506),16 the pattern is indexed by (0111) and (1010) diffractions, respectively. The diffraction arcs demonstrate that nanoparticles in the ellipsoid are crystallized and stacked with similar orientations. Figure 4E shows a TEM image of a slab taken from a 24 h spherulite via sonication treatment. The SAED patterns of the plates in the slab show sharp diffraction spots (e.g., Figure 4F), indicating that each plate is a single crystal. The SAED pattern is also indexed with the vaterite structure proposed by Kamhi.16 Yet, there are some weak diffraction spots that cannot be indexed by this vaterite structure (denoted with $d$-values in the figure). The phenomenon has been observed previously by several groups, and Kabalah-Amitai et al. suggested recently that it corresponds to a superstructure of the classic structure.5 Based on the
SAED result, the side surfaces of the plates are assigned to \{{\overline{1}120}\}, and the top surface is \{0001\}.

Control experiments were also performed to study the influence of supersaturation on the product morphology. As shown in Figure 5A and E, when the Ca\(^{2+}\) concentration is reduced to 0.1 mM, no branching occurs even for 24 h growth. Instead, lens-like plates with a hexagonal symmetry are formed. With \(C(Ca^{2+})\) of 0.2 mM and 1 mM, different levels of branches are observed, and the products resemble pancakes (Figure 5B and F) or flowers (Figure 5C and G), respectively. When \(C(Ca^{2+})\) is increased to a very high concentration (e.g., 100 mM), the product turns into a more complicated hierarchical morphology. Figure 5D reveals that the products are spherical particles with pits similar to the “pumpkins” harvested with \(C(Ca^{2+})\) of 5 mM (see, e.g., Figure 1B). However, they are composed of multiple \(~10\mu m\)-sized “mini-pumpkins”. As shown in the inset of Figure 5D, each “mini-pumpkin” is composed of micrometer-sized plates. The longitudinal cross section of the product shown in Figure 5H confirms the complex structural features. This observation clearly shows that the branching rate, and therefore the overall hierarchical morphology, of the product strongly depends on the supersaturation.

Based on the experimental results shown above, self-assembly of the pumpkin-shaped vaterite hierarchical structures can be understood in the framework of a spherulitic growth controlled by supersaturation and the intrinsic crystallographic anisotropy of vaterite. As schematically presented in Figure 6A, when CO\(_2\) diffused into the growth cell reaches the critical concentration, the growth is ignited with the formation of ACC particles. The particles transform to the vaterite phase within a very short time: vaterite nanocrystals nucleate within the ACC particles, which are prone to oriented contact in order to lower the total free energy.\(^6\) When the crystallized region expands enough, the highly oriented nanocrystals start to show an anisotropic influence on newborn nuclei due to the crystalline structural anisotropy. Vaterite possesses a hexagonal symmetric crystal structure with polarized \(\{0001\}\) faces.\(^7\) As a result, oriented nucleation takes place more in the six equivalent \(\langle1120\rangle\) directions than in the \(\langle0001\rangle\), so as to gradually shape the growing products from initial spheres to ellipsoids, convex lenses, and then convex lenses with hexagonal edges.

Depending on supersaturation, crystallographic misorientations may appear between daughter nuclei and mother cores, and they may gradually accumulate and lead to different degrees of branching to tune the morphology of the final product.
products. To be more specific, the branching rate is determined by the competition between the lateral extension rate (ER) and the nucleation rate (NR) of the lamellar plates. It has been well established that the nucleation rate of the crystallite depends nonlinearly on supersaturation; for the scenario of diffusion-limited growth and rough interfacial growth, the extension rate of a growing interface is linearly related to the supersaturation.\textsuperscript{17} It follows that the nucleation rate increases much more rapidly than the lateral extension rate for the scenario that the supersaturation is higher than a certain value. At lower supersaturations, NR is smaller than ER, and the newly formed crystallites tend to epitaxially nucleate on the crystalline facets in order to minimize the interface energy.\textsuperscript{17}

This condition favors formation of lenses as shown in Figure 5A and E. When the supersaturation becomes sufficiently high, NR surpasses ER, and multiple nuclei will be formed simultaneously at the growth front, leading to the branched growth. Since nucleation and growth are faster in the ⟨1120⟩ directions, branching occurs preferentially in these directions to form six segments, and finally leads to formation of the pumpkins with a hexagonal symmetry (Figure 1B). Simultaneously, two hexagonal pits form at the junctions of the segments.

In addition, the vaterite pumpkins are nearly identical to the “double-leaf” type spherulites common in various minerals, alloys, and polymers.\textsuperscript{15,18–23} The “double-leaf” growth is recognized to depend on varies factors such as the number of nucleation sites, structure of the material, and supersaturation.\textsuperscript{15} Yet, its microscopic mechanism is still an open question. In experimental work on fluorapatite “double-leaf” spherulites grown in gelatin, electron holography revealed that branching was highly influenced by the distribution of the intrinsic dipole field.\textsuperscript{25} Note that vaterite has the space group of P6\textsubscript{5}/mmc and its ⟨0001⟩ are highly polarized crystallographic faces, we suggest that the intrinsic dipole field originated from the polarized ⟨0001⟩ faces strongly affect the misorientations of branches. That is, a dipole field is generated from the convex lens-shaped cores that compose of the oriented vaterite nanocrystals. The dipole field increases with the lens size, and gradually becomes intense enough to direct orientations of newborn nanocrystals. Then, at the growth front of convex lenses, the ⟨0001⟩ of nuclei tend to tilt according to local dipole field, whereas the lateral orientations remain essentially the same as the substrates (Figure 6B). Eventually new branched lenses with their ⟨0001⟩ aligned by the dipole field and lateral orientations similar to the cores will be formed. The continuous branching shapes the products from lenses into pancakes, and finally forms the typical pumpkins (Figure 6A).

In conclusion, the growth of pumpkin-shaped vaterite hierarchical structures with hexagonal symmetry is investigated. The formation mechanism of the structure could be summarized as follows. Controlled by the hexagonal crystal structure, nucleation of vaterite particles occurs preferentially in ⟨1120⟩, modulating the grown products with hexagonal symmetry. For the scenario of high supersaturation, the nucleation rate is higher than the lateral extension rate. Therefore, spherulite growth becomes dominant and produces pumpkin-shaped hierarchical structures with sixfold symmetry. The results are beneficial for an in-depth understanding of the biomineralization of CaCO\textsubscript{3}. The growth mechanism may also be applicable to interpret the formation of similar hierarchical structures of other materials.\textsuperscript{18–22}