Growth of calcium carbonate mediated by slowly released alginate†

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The presence of additives has demonstrated strong effects on the crystallization and morphology of calcium carbonate (CaCO₃). To understand the mediating function of alginate on the growth of CaCO₃, we design a novel method to add alginate molecules and inorganic ions mildly and continuously to the mineralization system: a Ca-alginate gel is used as a slow-releasing source of calcium ions and alginate molecules; the gel is gradually broken down by the diffusion of CO₂ to the solution, inducing the slow release of Ca²⁺ and alginate molecules. The slowly released alginate is involved in the nucleation and growth of CaCO₃, in the form of micro-sized lens-like particles with a vaterite polymorph and composed of fused nanoparticles. With the increasing reaction time, the lens-like CaCO₃ particles gradually develop into a hollow structure and finally turn into ring-shaped CaCO₃, in which the polymorph of CaCO₃ remains vaterite. The formation of the lens-like particles is the result of the partially-oriented aggregation of primary nanoparticles mediated by alginate. The further evolution of the morphology to ring-shaped particles is due to a dissolution–recrystallization process as well as Ostward ripening.

1. Introduction

Various biominerals are synthesized in living organisms through biomineralization process. These biominerals usually have an exquisite structure and possess outstanding mechanical and optical properties.¹⁻⁴ CaCO₃ is one of the most wide-spreading minerals in organisms, acting as a component of the skeleton or the protective shell. It exhibits a series of delicate control over the morphology, location of nucleation, polymorph and orientation mediated by biomolecules, especially proteins and polysaccharides.⁵⁻⁶ The precise control as well as wide applications of CaCO₃/organic compound(s) have inspired many researches focusing on the influences of additives to the crystallization/growth of CaCO₃.⁷⁻⁸ A number of additives, such as artificial polyelectrolytes⁹⁻¹¹ and biopolymers,¹²⁻¹⁵ have been proven to exert effects on the superstructures and polymorphs of CaCO₃. Recently, a new class of amphiphilic block copolymer, so-called double-hydrophilic block copolymers (DHBCs), has shown interesting effects in biomineralization.¹⁶,¹⁷ They are considered as a simplified model for some active proteins secreted by organisms.¹⁸ One of the blocks of DHBCs is designed for interacting with minerals and the other for solvating in water. Both the binding and solvating functions render DHBCs very influential on the morphology, polymorph and orientation of CaCO₃ and other minerals.¹⁹⁻²¹ However, it is still unclear how further non-natural additives can mimic the function of biomolecules in biomineralization.

Polysaccharides have been shown to play an important role in the growth of biominerals in organisms.¹⁻³,¹⁶,²² However, little is known of the precise mechanism on how polysaccharides affect CaCO₃ crystallization.²³⁻²⁵ From the viewpoint of chemical structure, many natural polysaccharides are block/random copolymers consisting of two or more glycosidic monomers.²¹ For example, alginate is composed of mannuronic (M) and guluronic (G) acid residues (Scheme 1). Basically, polysaccharides can be divided into two types: one type can form gel in the presence of calcium ions (Ca²⁺), such as alginate, and the other cannot, including cellulose. In the alginate molecule only GG-blocks and GM-blocks can bind Ca²⁺ in the form of “egg-box”, while MM-blocks act as the solvating moiety,²⁶⁻²⁸ similar to the structure of DHBCs. However, alginate was reported previously to exert little influence on the crystallization of CaCO₃ and did not alter the standard rhombohedral habit of calcite.²³ It has to be noticed that, in those studies, alginate was mixed with calcium chloride (CaCl₂) directly, and formed gel or aggregates with Ca²⁺, leaving only very few free alginate molecules in the solution that would only weakly influence the crystallization of CaCO₃. Very recently, alginate was used as oriented support for the growth of CaCO₃.²⁵ The oriented film could induce the formation of CaCO₃ particles with different shapes and polymorphs depending on initial concentration and reaction time.

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Scheme 1  The chemical structure of alginates.
However the mediating effect of alginate in the form of free molecules is still unclear.25

It is widely accepted that additives interact with a certain crystal face during CaCO3 growth, leading to different morphologies and polymorphs.17 To better understand the mediating mechanism of additives, various crystallization methods have been developed to slow down the growth of crystal, such as the vapor diffusion method29 and the use of dialkyl carbonate30 in which the carbonate ions (CO3^2-) are supplied slowly or in situ generated. It should be noted that biomolecules and inorganic ions are supplied continuously through certain pathways in organisms.4 On the other hand, biomolecules in organisms can selectively interact with the growing biominerals at different stages, which induced the so-called delayed addition method.31 Therefore, it is crucial to find a way to add inorganic compounds and organic additives slowly and continuously at the same time, for better mimicking the biomineralization in organisms.

Here we designed a novel strategy, with which Ca2+, CO3^2- and alginate were slowly and continuously supplied in the mineralization system and mineralization occurred at a relatively low rate. In brief, Ca-alginate gel was prepared first and was used as a source of alginate molecules and the Ca2+. CO2 vapor diffusion method was employed to supply CO3^2-. The experiment setup is shown in Scheme 2a, and possible processes are illustrated in Scheme 2b–e: the increasing concentration of CO3^2- captures Ca2+ from the Ca-alginate gel, leading to the formation of CaCO3 ion clusters or nuclei, as well as the gradual breakdown of the Ca-alginate gel and the release of alginate molecules to the aqueous solution; the free alginate molecules will in turn exert influence on the growing CaCO3.

2. Experimental

Chemicals

Sodium alginate (SA) with molecular weight of 120–190 kDa was purchased from Aldrich. CaCl2 anhydrous (AR) and ammonium carbonate (AR) were bought from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai). The ratio of mannuronic acid to guluronic acid (M/G ratio) in SA is 1.56. All chemicals were used as received.

Crystallization of CaCO3

Small pieces of glass substrates were cleaned and sonicated in ethanol, and further soaked with a H2O-HNO3 (65%)-H2O2 (1:1:1, v/v/v) solution, rinsed with deionized water and dried in N2 stream. Aqueous SA solution (2%) was centrifuged at 12000 rpm for 15 min to remove undissolved particles and 20 cm3 of the solution was cast to form a membrane at room temperature in a 55 × 55 mm dish. Ca-alginate membrane-like gel (Ivory-white in color) was prepared by immersing pieces of alginate membrane into 0.1 mol dm-3 CaCl2 solution for 6 h, and was then cut into 5 × 15 mm strips. One strip was suspended in 25 cm3 of deionized water in a small beaker (30 cm3) for mineralization with the CO2 diffusion method (Scheme 2a) modified from a classical procedure.28 One of the advantages of this procedure is that Ca2+ are gradually released from the Ca-alginate gel as the gel dissociates. One piece of glass slide was carefully put at the bottom of the beaker to collect particle deposits. Then the beaker was covered by Parafilm with pinholes and placed inside a large close desiccator (about 6.5 dm3) along with 3 g of crushed ammonium carbonate in a small glass bottle covered with punched Parafilm. The reaction was run at room temperature (around 20 °C). The glass slide with deposits was gently rinsed by deionized water and dried in N2 stream for further examinations.

Characterization

Scanning electron microscopy (SEM) was performed on a Philips XL-30 ESEM FEG instrument in high-vacuum mode operated at 5 kV acceleration voltage and the samples were sputtered with gold. Transmission electron microscopy (TEM)/scanning transmission electron microscopy (STEM) observation and acquisition of electron diffraction patterns were carried out on a FEI Technai G220 under 200 kV. Samples were deposited on carbon film-coated 300 mesh copper grids, and dried with N2 stream. The diameter of the electron beam was defined as 5 μm for electron diffraction (ED). Diffraction patterns were recorded at a distance of 320 mm from the sample. Thermogravimetric analysis (TGA) was performed with a TGA Q500 apparatus (TA Instruments) under air flow at a rate of 20 cm3 min⁻¹; a scanning rate of 20 °C min⁻¹ was employed with a temperature range from rt to 700 °C. Raman spectra were recorded at a Horiba Jobin Yvon LabRAM HR spectrometer equipped with CCD detector and an integrated Olympus BX40 optical microscope with 100×.
objective. The 632.8 nm Helium–Neon laser with the spot size of ca. 3 × 3 μm was used, while the scattered light was dispersed by a grating with 1800 grooves mm⁻¹.

3. Results and discussion

Slow release of alginate and Ca²⁺

The strategy we developed here targets to adding all the substances, namely Ca²⁺, CO₃²⁻ and organic additive (alginate molecules) to the mineralization system slowly and continuously. The mediating function of alginate is in synergy with the nucleation and growth of CaCO₃. This continuous addition method was designed on the basis of the competition between two equilibria, i.e., equilibrium of precipitation of CaCO₃ and equilibrium of gelation of Ca-alginate. In the precipitation equilibrium, the solubility-product constant (K_sp) of CaCO₃ is very low (3.4 × 10⁻⁹) at 25 °C. The CO₂ diffusion method used here contained a slow gas–liquid interfacial reaction step, which allowed the CO₃²⁻ concentration to increase gradually. It is known that Ca²⁺ interacts preferentially with the GG and GM segments in alginate and the structure of the gel is relatively loose, therefore Ca-alginate gel can be usually used to remove other heavy metal ions and even monovalent cations can destruct the gel. In this context, CO₃²⁻ is able to capture Ca²⁺ from the gel to induce the gel dissociation, enabling free alginate molecules to be continuously released from the gel till the gel is completely dissociated, and to be involved in the whole biomineralization process (including nucleation, aggregation and recrystallization) of CaCO₃. It should be noted that the process of releasing of alginate and Ca²⁺ is controlled by the rate of precipitation and solubility of CaCO₃ which is related to the alginate concentration and the pH value in the serum. The change of precipitation rate and solubility of CaCO₃ could impose effects on succedent of CaCO₃. This convenient method allowed alginate to influence the growth of CaCO₃ in the form of free molecules, rather than in the form of gel in the direct mixing method. These free alginate molecules could interact with a specific crystal face, as will be further discussed below.

Evolution of particle morphology

It was found that the morphology and polymorph of the CaCO₃ particles depended on the reaction time. After 24 h, only a few CaCO₃ particles (Fig. 1a) precipitated on the slide beneath the strip (the A zone in Scheme 2f). In the meantime, the ivory-white Ca-alginate gel strip became less turbid and the strip edges became fluffy, indicating the dissociation of the gel has occurred. These particles had a rhombohedral profile, and appeared to be mesocrystals as indicated by the truncation of the corners and the edges (right inset in Fig. 1a). Such rhombohedral particles were found in all the stages of experiment and were all located within zone A (Scheme 2f), which formed and remained constant from the early stage of CaCO₃ crystallization. As the powder XRD is not suitable for determining the polymorph of CaCO₃ here because of the small amount of the samples we could collect, micro-Raman was employed to characterize the particles. The Raman spectrum showed that these rhombohedral particles are typical calcite, e.g., 156 and 282 cm⁻¹ feature to lattice vibration of calcite, while the 712 and 1086 cm⁻¹ band to internal ν₄
vibration and $v_1$ vibration of calcite, respectively (curve a in Fig. 2). At this reaction stage, CO$_2$ captured a small amount of Ca$^{2+}$, which did not lead to an obvious damage to the Ca-alginate gel. The small amount of alginate molecules would exert little influence on the growth of CaCO$_3$ in the solution, leading to the thermodynamically stable polymorph calcite. In a recent investigation on additive-mediated mineralization, when the Ca$^{2+}$/additive ratio was high in the solution containing 10 mmol dm$^{-1}$ Ca$^{2+}$, namely the concentration of additive was low, calcite mesocrystals were obtained, similar to particles after 24-h reaction in this study (Fig. 1a).

After a 48-h reaction, the Ca-alginate gel completely dissociated and the lens-like particles in the range of 4 to 10 μm (mostly around 5 μm) were found sporadically in the whole range (within and outside zone A) of the glass slide (Fig. 1b). The surface of the particles appeared to be rugged and granular, comprising loosely-packed, discernible 30-nm nanoparticles (Fig. 1b). After 72 and 100 h, more lens-like particles could be found all over the glass slide (Fig. 1c and d). The particles after a 72-h reaction still had a granular surface and individual nanoparticles were bigger (about 50 nm) and more densely packed (Fig. 1c). The surface of the particles obtained after 100-h reaction, however, was covered by a scale-like structure (Fig. 1d), which appeared to be composed of numerous fused primary nanoparticles. After 144-h reaction, some of the lens-like particles appeared to have crevasse in the middle (Fig. 1e) and a ribbon-like structure developed at the particle surface, due to the fusion of nanoparticles. At 192 h, most of the lens-like particles turned into ring-shaped particles (Fig. 1f), and larger holes as well as a filament-like structure were observed clearly (inset of Fig. 1f).

Micro-Raman spectra (Fig. 2) were investigated for representative lens-like particles collected at different reaction times. Clearly, all the lens-like particles are mainly composed of vaterite, with characteristic bands, e.g., 1074 and 1090 cm$^{-1}$ feature to internal $v_1$ vibration, while the 267, 303 cm$^{-1}$ band to lattice vibration, and the 754 cm$^{-1}$ corresponds to internal $v_4$ vibration (weak here) (Fig. 2, curve b–f). During the morphology development of the lens-like particles, the polymorph remained as vaterite, while in some other cases there was a transformation from vaterite to calcite following a solution-mechanism after a period of time in solution. The alginate here exerted a strong stabilizing effect on the vaterite polymorph, which may originate from the special interaction between alginate and Ca$^{2+}$.

These lens-like particles obtained from different reaction times were examined by TEM as well. The structure of the particle obtained after 48 h was relatively loose (Fig. 3a) and the primary nanoparticles, partially fused together, could be clearly seen. After a 100-h reaction, the lens-like particle became more compact and had a higher electron density in the center (Fig. 3b). At the edge of the particle, the scale-like profile can be observed, similar to the SEM image in Fig. 1d. The particle appeared to be hollow when the reaction time was 144 h (Fig. 3c), as expected from the crevasse observed by SEM (Fig. 1e). Finally, the lens-like particle changed into ring-shaped structure after a 192-h reaction (Fig. 3d), with many filaments around the “hole”.

In Fig. 3, corresponding electron diffractions (ED) of the single lens-like particle are shown in the insets. The diameter of the electron beam was set to 5 μm, which can cover the whole particle. For the ring-shaped particle obtained at 192-h reaction, a 1-μm beam was used. These ED patterns can be indexed as a hexagonal vaterite crystal viewed from the [001] zone axis. All diffraction patterns of these lens-like particles showed periodic isolated spots, diffracting as a single crystal, which indicates that these seemingly randomly-arranged lens-like aggregates (rough surface, made up of nanoparticles or nano-scales, Fig. 1) were highly oriented aggregates; in other words, the crystallographic axes of the nanoparticles in each lens-like particle were parallel. All the ED patterns of single lens-like particles demonstrated identical diffraction patterns along the [001] direction of vaterite. This was likely due to the anisotropic nature of the lens shape, which tended to lie flat on the TEM grid. In crystallography, the vaterite which belongs to the hexagonal crystal system usually does not expose its (001) faces, because these faces are only composed of Ca$^{2+}$ or CO$_3^{2-}$ (Fig. S1 in the ESI†) and have high energy. Nonetheless, vaterite can be stabilized via the adsorption of additives at (001) faces (the surface energy is thus lowered). Although the pH value in our system increased from 6.5 to 9.3 as a result of dissolution of ammonia, the negative charges in alginate molecules were maintained because the $pK_a$ of mannuronic (M) acid and guluronic (G) acid is 3.38 and 3.65, respectively. Therefore, alginate can interact with the Ca$^{2+}$ layer at (001) faces through electrostatic attraction; in addition, chelating interaction (egg-box model) has been postulated, which was suggested to induce the vaterite polymorph exclusively (Fig. 3).
Mechanism of particle formation

Besides the classical laws of crystallization via a layer-by-layer adsorption of solute atoms or molecules, some non-classical pathways of crystallization have been proposed through colloidal intermediates. Ostwald ripening and aggregation of primary particles are widely accepted. The Ostwald ripening mechanism describes the growth of larger crystals at the expense of smaller ones because smaller ones have a higher chemical potential. The aggregation mechanism describes the assembly of nanoparticles into higher-level particles (secondary particles) with different shapes, which is usually followed by fusion of nanoparticles and reorientation/recrystallization to form large single crystals. At the surface of these lens-like particles obtained with shorter reaction times in our experiment (48 and 72 h), nanoparticles were clearly observed. As reaction time increased, the appearance of the lens-like particle surface changed from nanoparticles to a scale- or ribbon-like structure. These phenomena indicate that the formation of lens-like particles involves aggregation of nanoparticles and subsequent recrystallization and fusion.

To better understand the formation of the lens-like particles, the serum phases in the reaction system (excluding the deposits on the slide) at different growth stages were collected for additional investigation. The serum of a 24-h reaction was dried for STEM and TEM observation. Particles of two different sizes (around 150 and 30 nm) were clearly seen (Fig. 4a), and both were CaCO\textsubscript{3} particles from EDX analysis (inset in Fig. 4a). Furthermore, the electron diffraction pattern (inset in Fig. 4b) revealed that these particles were mainly in amorphous state (ACC), suggesting emphatically that the lens-like CaCO\textsubscript{3} particles grow from particle-by-particle aggregation. Besides the particles, a lot of fiber-like structures which may correspond to alginate molecules were also distinguished and linked the particles together (indicated by long white arrows in Fig. 4a and white arrows in Fig. 4b). The coverage by alginate molecules may help the ACC particles to produce the special short-range order structure or even be away from crystallizing within a certain period of time. Generally, ACC precursor particles are favored at high supersaturation and formed as the first species. Nevertheless, the ACC particles observed could be an artefact during the sample preparation, because the degree of supersaturation might be relatively low in the system we carried out. However, there are some factors that may contribute to the growth of ACC in our system. First, besides the increasing reactant (ions), the increasing additive concentration also leads to the formation of ACC. The increasing alginate content in the serum in the first stage could be one kind of promotion for the ACC. Second, in our experimental design, there is likely a gradient of Ca\textsuperscript{2+} concentration away from the gel in the serum during the dissolution of gel; in other words, in the solution around the gel the Ca\textsuperscript{2+} concentration may be relatively high. Third, the pH value of the serum increased to 9 within 24 h. The driving force for precipitation is higher at higher pH, which would induce the formation of ACC. Besides the extensive element analysis and electron diffraction confirming the existence of ACC, the granular surface of lens-like particles indicated the formation of ACC.

The serum phases were dried at 100 °C and the solid contents (non-volatile matter content) for the 24, 48, 72, 100, 144 and 192-h serums were 0.13, 0.25, 0.28, 0.32, 0.41, and 0.45 mg cm\textsuperscript{-3}, respectively. The TGA curves of these solids show several weight loss stages (Fig. S2 in the ESI†). The weight loss between 150 and 500 °C was contributed to the decomposition of alginate\textsuperscript{8} and above 600 °C was due to the decomposition of CaCO\textsubscript{3} to CaO and CO\textsubscript{2}. Combining the solid content and the corresponding TGA data, the alginate concentrations in the serum phases after 24, 48, 72, 100, 144, 192-h reaction were determined to be 0.06, 0.20, 0.23, 0.27, 0.35, and 0.39 mg cm\textsuperscript{-3}, respectively. The theoretical amount of all alginate was 0.40 mg cm\textsuperscript{-3}. After the complete dissolution of the Ca-alginate gel (48 h), the increase of the alginate content from 72 h to 192 h was attributed to its release from the lens-like particles, as a result of recrystallization and fusion. This also indicates that the alginate was involved in the lens-like particles and the lens-like particles were CaCO\textsubscript{3}/alginate hybrids. It is interesting to point out that decomposition of CaCO\textsubscript{3} was only shown for the solids from the serum phases of the 24- and 48-h samples, containing 0.07 and 0.03 mg cm\textsuperscript{-3} of ACC particles, respectively; the absence of CaCO\textsubscript{3} in the serum phases for other samples at longer reaction times clearly indicates that the ACC nanoparticles have assembled into much larger lens-like particles.

It has been widely accepted that surface-stabilized nanoparticles (acting as a building block) can assemble into various ordered micro-scale structures. A few examples of such aggregation-based growth have been reported for CaCO\textsubscript{3} particles. For the formation of lens-like particles, Gehrke and Xu suggested the stacking of the preformed hexagonal plates and aggregation of nanoparticles. In our experiment, we did not observe the stacking of plates, as indicated by the finding of the interesting “embryo” of lens-like particle (Fig. S3a in the ESI†). The strands of nanoparticles which were loosely linked were identified clearly and the whole particle already had a lens shape. In our opinion, such embryo would induce the subsequent gathering of nanoparticles on it, which further developed into lens-like particles with morphologies shown in Fig. 1. Now the question is why the nanoparticles aggregate into lens-shaped secondary particles.

Usually, aggregation of nanoparticles is energetically favored because of the reduction of the interfacial energy. In the meantime, the organic molecules on the nanoparticle surface may

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**Fig. 4** (a) STEM image of the serum at a 24-h reaction; inset shows the element analysis of these particles. (b) TEM image of big and small particles at higher magnification; inset shows the electron diffraction pattern of these particles.
facilitate the formation of aggregates with an ordered architecture.\textsuperscript{44} The alginate molecules in our system may be one of the driving forces in the aggregation of the lens-like particles (Fig. 4). On the other hand, ED patterns showed that the [001] direction is perfectly parallel to the principal axis of the lens-like particle, which suggest that the nanoparticles themselves take effect for the aggregation as well. It has been reported that the nearly spherical nanoparticles can give rise to vectorial aggregation due to the perfectly ordered structure (such as crystalline structure) in the nanoparticles before aggregation,\textsuperscript{37,45} e.g., the vaterite nanoparticles formed hexagonally shaped plates via oriented attachment because of the symmetry and retaining their mutual orientation. In another case, the homogeneous ACC nanoparticles (without any ordered structure) would assemble into spherical aggregates via non-oriented aggregation.\textsuperscript{46} The lens-like shape, which has the oblate character of a plate and the round profile of a sphere, was considered as the compromise between a hexagonal plate and a sphere. This indicated that the aggregation phenomenon in our case could take place through the middle path, namely through "partially oriented aggregation". ACC nanoparticles may form some weakly ordered structure before aggregation,\textsuperscript{40,47} which would lead to aggregation in a partially oriented character, forming convex lens shaped particles. These nanoparticles roughly aligned with each other based on the weakly ordered structure. In the aggregation-based system, the formation of ordered structure in primary nanoparticles and aggregation will both take place. It seems that the shape of the final aggregates relies on the sequence with which formation of the ordered structure in nanoparticles and aggregation happen. If aggregation happens first, the aggregates are probably spherical-shaped;\textsuperscript{48} contrariwise, a plate-shape aggregate would form;\textsuperscript{45} if they happen at the same time, the compromise shape such as lens-shaped aggregate could be generated.

After the aggregation, the orientation of the nanoparticle was further adjusted by recrystallization, which may result in the change of the ED patterns of lens-like particles (Fig. 3). At the early stage (48 h), the diffraction spots were slightly elongated (inset in Fig. 3a), suggesting the presence of some misorientation between the nanoparticles. After further fusion which is indicated by the change of the surface morphology of the lens-like particles, from granular to scale-like structures (Fig. 1), the alignment of nanoparticles tended to be perfect, indicated by the sharp diffraction spots in Fig. 3b–d.

As of the formation of hollow CaCO\textsubscript{3} particles, several mechanistic models have been proposed. Yu and Loges suggested the recrystallization and so-called "3D coffee-stain effect" led to the hollow structure, respectively,\textsuperscript{48,49} where one less dense polymorph transforms to a denser material. In our lens-like particles, the ACC nanoparticles were consumed from the inside and more dense vateritic shell developed. At the same time, few cracked lens-like particles were observed occasionally (Fig. S3b–d).\textsuperscript{†} They showed that the fusion happened from outside the lens-like particles. The inner nanoparticles had a much smaller size, just similar to the primary nanoparticles observed in Fig. 4. The size difference between the nanoparticles outside and inside made us believe that the Ostwald ripening process also contributed to the formation of the hollow and ring-shaped structure.\textsuperscript{50}

In combination with the discussion about the aggregation of lens-like particle and formation of hollow structure, we hypothesize a possible growth mechanism for the CaCO\textsubscript{3}/alginate hybrids (Fig. 5). Generally, alginate molecules were released in the solution while CO\textsubscript{3}\textsuperscript{2−} bound Ca\textsuperscript{2+} and formed ion clusters\textsuperscript{47,50} or ACC nanoparticles. The special interaction between alginate and Ca\textsuperscript{2+} could induce weakly ordered structures (e.g., vaterite short range order structure) in ACC nanoparticles, which would lead to the "partially oriented aggregation" of ACC nanoparticles and result in a lens-shaped aggregate. Afterwards, recrystallization and fusion initiated from the outside of the lens-like particles with consumption of the relatively amorphous inside part gave rise to a hollow and, finally, to a ring-like structure. Ostwald ripening was also attributed to the morphology development.

The continuous releasing method employed here can be a study model. It will be useful for the polymers which easily form a gel with a calcium ion in mineralization experiments. It is worth pointing out that no extra Ca\textsuperscript{2+} and alginate molecules are introduced to the reaction system during the ripening process (after the complete disassociation of Ca-alginate gel). If another fresh strip of Ca-alginate gel is introduced to the system once the former gel has disassociated completely, the growth of CaCO\textsubscript{3} could be different and also interesting, which is under investigation in our lab.

4. Conclusions

Here we designed a novel strategy for investigating the influence of alginate, a kind of gelation polysaccharide, on the crystallization of CaCO\textsubscript{3}. The results showed that the mixture method of ions and additives is important and should be highly considered
in the mineralization experiments. With our strategy, CO$_3$$^{2-}$, Ca$^{2+}$ and alginate are released into the reaction system slowly and continuously. Alginate molecules influenced the growth of CaCO$_3$ at a very early stage and can stabilize vaterite CaCO$_3$ for a long period. The slow rate of mixing Ca$^{2+}$ and alginate was better mimicking the biomineralization in organisms. The growth of lens-like particle in our experiment adopted an aggregation-based pathway. We suggested that the “partially oriented aggregation” led to the lens shape, and the fusion and ripening was the main reason for the formation of hollow and ring-shaped structure.

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**Notes and references**