Silk fibroin-mediated biomineralization of calcium carbonate at the air/water interface†

Wei Hao, a David Porter,‡b Xianting Wang a and Zhengzhong Shao*a

The synthesis of calcium carbonate (CaCO₃) with different morphologies and polymorphs at the air/water interface has been reported in previous studies while the influence of the structure of macromolecular additives on this type of mineralization is rarely investigated. Regenerated silk fibroin (RSF) from the Bombyx mori silkworm silk is analogous to the main protein contained in naturally formed nacre, which can form complex structures at the air/water interface due to the multi-block amphipathy. This study demonstrates how a range of CaCO₃ structures with different morphologies and polymorphs was obtained at the air/water interface mediated by RSF. The precursor-stabilizing ability of RSF allows the crystals to grow directly from amorphous calcium carbonate (ACC), which was observed by time-dependent experiments. The structures of RSF that exist at the interface determine the final morphologies and polymorphs of the crystals, which can be influenced by the concentrations of [RSF] and [Ca²⁺] and the molecular weight of RSF. A thermodynamically metastable aragonite phase may be mediated by the β-sheets of RSF formed at low concentrations, while stable calcite can be generated by the RSF amorphous structure at high concentrations. The synergy between RSF and CaCO₃ at the interface can provide a way to understand the function of organic materials involved in the biomineralization process and can be applied to manipulate the structures of synthetic hybrid materials.

Introduction

Calcium carbonate (CaCO₃) is one of the most abundant minerals in nature used as a structural or protective material. The preparation of highly regulated CaCO₃ with fine structures under ambient conditions has attracted much attention.1,2 During the biomineralization process, both the water-soluble fraction and the insoluble matrix of organic materials are considered to play essential roles.3 For example, the soluble acid glycoproteins combined with silk fibroin-like proteins in nacre, both in the amino acid sequence and secondary structure,4 contribute to the design and preparation of organic matrices (soluble and insoluble) are an active and fertile area of research. Insoluble templates such as rigid solid matrices (fibres, membranes, scaffolds),5 self-assembled monolayers,6 and Langmuir–Blodgett (LB) films at the air/water interface7 can all guide the inorganic crystal morphology to remarkable forms.

Amphiphilic molecules can self-assemble at the air/water interface and their conformation is different from that in the bulk solution. Thus they can be used to produce asymmetric inorganic particles or films7,8,10,11 and the chain length can affect crystallization.11 Particularly, various proteins can form more complex films at the air/water interface due to their high molecular weight and/or multi-block hydrophilic/hydrophobic segments. The structure and morphology of the protein self-assembled films depend on many factors, for example, concentration of the bulk solution, temperature and molecular weight.12,13 However, many previous studies have focused on solution-phase mineralization mediated by proteins while the influence of the self-assembled film structures on templated crystallization at the interface still needs to be studied.

Regenerated Bombyx mori silk fibroin (RSF) is similar to silk fibroin-like proteins in nacre, both in the amino acid composition and secondary structure.14 Work in our laboratory has shown that RSF dominated by β-sheet conformation in solution presents a strong preference for the formation of the β-sheet structures of RSF formed at the air/water interface and SEM images of calcium carbonate crystals, which can be influenced by the concentrations of [RSF] and [Ca²⁺] and the molecular weight of RSF. A thermodynamically metastable aragonite phase may be mediated by the β-sheets of RSF formed at low concentrations, while stable calcite can be generated by the RSF amorphous structure at high concentrations. The synergy between RSF and CaCO₃ at the interface can provide a way to understand the function of organic materials involved in the biomineralization process and can be applied to manipulate the structures of synthetic hybrid materials.
aragonite phase of CaCO₃. RSF is a hydrophilic/hydrophobic partitioning molecule which can separate its hydrophilic and hydrophobic residues on opposite sides of the interface when it is adsorbed at the air/water interface. Yang et al. have studied the behaviour of RSF at the air/water interface and found that the dynamic surface tension and structure of RSF are dependent on the solution concentration. In this paper, we focus on the effect of the concentrations of [RSF] and [Ca²⁺] and the RSF molecular weight on CaCO₃ templating in an ammonium carbonate diffusion system. A continuous change in the polymorphs and morphologies of the final CaCO₃ products could be linked to the structure of the RSF adsorbed layers at the air/water interface. Time-dependent observations were performed to reveal the crystallization process mediated by RSF. Based on these results, we suggest a possible mechanism for the mineralization at the air/water interface.

Experimental section

Materials and preparation of regenerated silk fibroin solution

RSF aqueous solutions were prepared from natural Bombyx mori silkworm cocoons. To obtain RSF with various molecular weights, we used different degumming methods following established procedures. The silk cocoons were first treated with 0.5 wt% Na₂CO₃ or NaHCO₃ boiling solution to remove sericin and the degummed silk was then dissolved in 9.5 mol L⁻¹ LiBr aqueous solution. After filtration, the RSF/inorganic solvents were dialyzed against deionized water for 72 h to remove inorganic ions. The dialyzed solutions were then clarified by spinning in a centrifuge at 6000 rpm for about 8 min. The supernatant with about 4 wt% (w/w) RSF was collected and stored at 4 °C. Different concentrations of solutions were prepared at room temperature. The molecular weights were determined by rheological measurements, described in detail elsewhere. The labels and preparation processes of different RSF solutions are summarized in Table 1.

Crystallization of CaCO₃

For the preparation of CaCO₃, the glass substrates and beakers were subjected to ultrasound in ethanol and deionized water (DIW) for 30 min, further soaked in H₂O–HNO₃ (65 wt%–H₂O₂ (1:1:1, v/v/v) solution, rinsed with DIW and finally dried in an oven at 80 °C. Mineralization was carried out in a 10 mL beaker containing a mixture solution of an appropriate amount of RSF (RSF-83 was employed if not further noted) solution in the range of 0.0001 wt% to 2 wt% and CaCl₂ solution in the range of 1 mM to 10 mM. The beaker was covered with parafilm with six pinholes and then transferred to a large closed desiccator (about 6.5 L). 3 g of freshly crushed ammonium carbonate was placed in another 10 mL beaker covered with parafilm with 6 pinholes and placed into the desiccator for CO₂ release. The mineralization temperature was around 25 °C. After a designated time, a glass substrate or a copper grid was used to carefully collect the CaCO₃ particles produced at the air/water interface. The obtained CaCO₃ particles were dried in air for further characterization. For laser confocal fluorescence microscopy characterization, the particles were washed with DIW and obtained using centrifugation. This process was repeated 3 times and the sediments were dried in air before observation.

Characterization

The morphologies of the CaCO₃ particles were observed with a TS 5136MM scanning electron microscope (SEM) at 20 kV after sputtering with gold (about 5 nm to 20 nm in thickness). High-magnification images were obtained by using an S-4800 FE-SEM at 1 kV. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) images, selected area electron diffraction (SAED) patterns and electron diffraction (EDX) spectra were obtained by using a JEM-2100F transmission electron microscope operated at 200 kV. The polymorphs of CaCO₃ were detected by using a Renishaw inVia Reflex Raman spectrometer equipped with a Leica 2500 optical microscope, a 633 nm helium/neon laser, and a CCD detector. Visual observations were performed by polarized optical microscopy (Olympus BX61). Fluorescence observations were performed by laser confocal fluorescence microscopy (Olympus FV 1000), and the excitation wavelength was 405 nm.

Results and discussion

RSF is recognized as a multi-block amphiphilic macromolecule, thus it can adsorb on the surface and change the surface tension. Yang et al. used a video-enhanced drop shape tensiometry technique to characterize the surface activity; they found that the equilibrium surface tension of RSF solutions was constant above a bulk concentration of 2 wt% and ascribed this to the formation of aggregates in the bulk solution. In the diluted RSF solution (≤2 wt%), the adsorption mechanisms and structure of RSF depend on the bulk concentration. In our system, we measured the RSF solution surface tension at different concentrations of CaCl₂ (1 mM, 5 mM and 10 mM) using the Wilhelmy plate method (ESI, Fig. S1†). It was found that increasing the concentration of RSF from 0.0002 wt% to 2 wt% in the bulk solution can decrease the surface tension at all concentrations of CaCl₂, and the decrease in surface tension slows down at higher concentrations of RSF which may be ascribed to the formation of aggregates in the bulk solution. This trend is in accordance with Yang’s research and may affect the mineralization process. Therefore, RSF solutions with concentrations less than 2% were selected as additives for the mineralization of CaCO₃.

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<th>Table 1</th>
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In the controlled mineralization without any additive, the CaCO$_3$ particles produced at the air/water interface were conventional rhombohedral calcite with little vaterite (Fig. S2†). The addition of RSF to the mineralization solution greatly changed the polymorphs and morphology of the CaCO$_3$ particles. Fig. 1 shows how the morphologies of CaCO$_3$ particles varied with the concentration of RSF and Ca$^{2+}$. The polymorphs of the particles were detected by Raman spectroscopy (Fig. S3†) and summarized in Table 2.

The two main crystal polymorphs obtained at the air/water interface are calcite and aragonite. In general, large calcite crystals can be obtained at relatively high bulk concentrations of RSF and Ca$^{2+}$ (shown in the darker grey areas in Table 2), while aragonite particles become the dominant polymorph under conditions of low bulk component concentrations (displayed in a lighter grey color in Table 2). The dominant crystal polymorphs showed a gradual transition from calcite to aragonite with decreased [RSF] at a fixed Ca$^{2+}$ concentration. The coexisting point of the two polymorphs shifted to lower RSF concentration with increasing Ca$^{2+}$ concentration.

With decreasing RSF concentration, the morphology of the calcite particles changed from flower-like structures (Fig. 2a and b) to rhombohedral structures with a stepped indentation in the upper surface and rounded corners (Fig. 2c and d). When the RSF concentration is extremely low, Ca$^{2+}$ cannot reach the critical nucleation point on the RSF chains, resulting in conventional rhombohedral calcite similar to that obtained in the control experiment, due to thermodynamic stability (Fig. 2e, f). Classical crystallization might be dominant in control experiments and at low concentrations of RSF.

The continuous morphology and polymorph variation can be observed in the aragonite crystals obtained with decreasing RSF concentration when [Ca$^{2+}$] = 1 mM. The two ends of bundle-like aragonite, namely the fan-shaped sectors, Fig. 2c and d). When the RSF concentration is extremely low, Ca$^{2+}$ cannot reach the critical nucleation point on the RSF chains, resulting in conventional rhombohedral calcite similar to that obtained in the control experiment, due to thermodynamic stability (Fig. 2e, f). Classical crystallization might be dominant in control experiments and at low concentrations of RSF.

The continuous morphology and polymorph variation can be observed in the aragonite crystals obtained with decreasing RSF concentration when [Ca$^{2+}$] = 1 mM. The two ends of bundle-like aragonite, namely the fan-shaped sectors,
gradually became wider and the final size became larger. When the RSF concentration was further decreased, the low content of RSF at the air/water interface caused the formation of irregular bundle-like aragonite. When the Ca$^{2+}$ concentration increased to 5 mM and 10 mM, both the size and the quantity of these calcite particles increased at high RSF concentration, and the fan-shaped sectors of the aragonite became wider. Besides the bundle-like aragonite morphology, large hemisphere-like aragonite particles were produced at high component concentration, which are printed in bold and italic type “A” in Table 2. The size of hemisphere-like aragonite obtained under calcite-dominant conditions is much smaller than under other conditions.

To gain insight into the formation process of the aragonite products, we looked in detail at the development of the crystal morphology under two selected sets of conditions. First, under the conditions of $[\text{RSF}] = 0.1$ wt% and $[\text{Ca}^{2+}] = 5$ mM, the growth process of hemisphere-like aragonite is shown in Fig. 3. After mineralization for 2 h, nothing can be observed by the unaided eye; however, some relatively dark particles can be seen in the TEM image (Fig. 3a). The selected area electron diffraction (SAED) pattern of the corresponding area indicates that the particles are aragonite (inset in Fig. 3a). These particles are also present in the SEM image (Fig. 3b) but their structure can be destroyed by low-energy (1 kV) electron irradiation (inset in Fig. 3b), which suggests the partially amorphous nature of these particles. When the reaction time increased to 4 h, some hemi-gourd-like aragonite particles with an indentation in the middle of the upper surface are detected (Fig. 3c). When the mineralization was extended, the aragonite particles became larger. We can see from the back view after 6 h (Fig. 3d) and side view after 8 h (Fig. 3e) of the hemisphere-like aragonite that the crystals are composed of nanoneedles, which are similar to the units of aragonite obtained in the bulk solution at high component concentration. The size of the aragonite further increased after crystallization for 10 h (Fig. 3f). Some nanowires connecting two particles (Fig. 3g) and a few irregular calcite particles (Fig. 3h) can be observed in the last stage of mineralization. We can see the nanowires located between two hemisphere crystals (Fig. 3i and j); however, they are not on the same height level as the crystal's upper surface (Fig. 3g) and some of them are broken (Fig. 3g). These nanowires are speculated to be the dry state of the mother liquor between two particles after they are collected from the air/water interface. Besides the nanowires, irregular material can also be found adhered to the nanoneedles at the edge of the hemisphere when mineralization was extended to 72 h (Fig. 3k).

For the second set of conditions, when $[\text{RSF}] = 0.02$ wt% and $[\text{Ca}^{2+}] = 1$ mM, bundle-like aragonite particles can be produced at the air/water interface (Fig. 4). The SEM image of the sample obtained at 2 h is shown in Fig. 4a, from which some unstable nanoparticles are observed. After mineralization for 4 h, some “shuttle-like” structures were formed, on which new spikes evolved at one end and an indentation in the upper surface can be seen. In a subsequent step, the structures started to branch with an expansion in size over the following hours, finally forming well-defined bundle-like aragonite (Fig. 4c–f). We can see from the front view (Fig. 4g) and back view (Fig. 4h) of the bundle-like aragonite collected at 72 h that the crystals consisted of nanoneedles, which are bigger in size than the units in hemisphere particles (Fig. 3d and e). These units are similar to those of aragonite collected in the bulk solution at low component concentration. An obvious boundary can be seen on the upper surface of bundle-like aragonite (lower left corner inset of Fig. 4g), which is highlighted by the dotted white lines (top right corner inset of Fig. 4g). The structure of the depressed section in the middle is looser than that of the remaining parts. Moreover, the nanoneedles are smoother on the back side than those on the upper surface (Fig. 4h).

The growth process of the aragonite particles is very similar to that of polymer spherulites, which consist of radial growth fibers, and they have strong birefringence. Clear Maltese crosses can be observed in the hemisphere-like aragonite particles obtained in our experiments, which can further verify the similarity between the aragonite and polymer spherulites (Fig. 5).
TEM was performed to further investigate the structure of the hemisphere-like aragonite particles. The nanoneedles at the edge of the crystals mineralized for 24 h show a well-defined characteristic of the crystals viewed along the [010] direction, and the long axis of the needles is [001] (Fig. 6a, b). This crystal lattice structure is in accord with the aragonite produced in the bulk solution.\textsuperscript{15} RSF has proven to be extremely versatile in its control over the nucleation and growth of aragonite because of the excellent match in the ionic spacing in the \(\beta\)-strand conformers and the aragonite (010) plane.\textsuperscript{15}\textsuperscript{\textdagger} TEM shows that the upper surface of the particle parallel to the air/water interface is the aragonite (010) plane. We suppose here that the ionic spacing of the \(\beta\)-structure (\(\beta\)-strand or \(\beta\)-sheet) is the same as in the RSF chains existing in both the bulk solution and the air/water surface layer (or subsurface layer). Then, it could be speculated that \(\beta\)-sheets (or \(\beta\)-strands) in RSF chains at the interface could be adsorbed onto the aragonite crystal (010) face by electrostatic interactions between the negatively charged oxygen atoms of the C\(\equiv\)O groups and positively charged Ca\(^{2+}\), resulting in the aragonite crystal growing along the [001] long axis.

In the TEM image of the crystal mineralized for 72 h, we can see some irregular material near the nanoneedles that shows relatively low contrast (Fig. 6c). No crystal lattice can be observed in the HRTEM image of the irregular material (Fig. 6d) as well as in the nanowires connecting two particles, which was already found by SEM (Fig. 6e and f). SAED of the nanowires shows a dispersion ring. Elemental analysis indicates that they are mainly composed of Ca, O and C (Fig. 6f). Therefore, the material near the nanoneedles and nanowires connecting two particles is speculated to be amorphous calcium carbonate (ACC).

To further unravel the interaction between RSF and the crystals, we observed the particles obtained at the air/water interface under fluorescence microscopy. Our lab has found that RSF that has undergone the treatment of degumming and LiBr dissolution exhibits blue fluorescence under 405 nm excitation which may be caused by some specific amino acid residues or some metal elements combined with RSF.
In the control experiment, we collected the CaCO₃ particles produced without RSF and then soaked them in 1 wt% RSF solution for 12 h. The particles obtained in this way were washed with DIW three times and collected by centrifugation. After drying in air, no fluorescence was observed in the dark field images (Fig. 7a and b). This suggests that conventional calcite is not fluorescent and RSF which adhered to calcite can be removed by washing with DIW. Calcite produced under high component concentration shows blue fluorescence after washing with DIW (Fig. 7c, d). This fluorescence is not the photonic crystal coloration due to the heterogeneous structure of CaCO₃ but results from the synergistic reaction of RSF and CaCO₃. Thus it is reasonable to conclude that these calcite particles are mediated by RSF. Similarly, hemisphere-like aragonite also exhibits fluorescence after washing, and it seems that the fluorescent groups in the upper surface are greater than in the back side (Fig. 7e, f). This suggests that CaCO₃ particles just form beneath the air/water interface, where the interfacially self-assembled RSF film can adsorb on the crystal upper surface and result in more fluorescence.

Proteins may have two effects on biomineralization, one is nucleation of the desired mineral phase and the other one is control over the growth of the mineral. Unlike in the control experiment and at extremely low concentrations of RSF, the mineralization under other conditions may experience a “non-classical crystallization” process. The initial stage of this process involves liquid/liquid phase separation which leads to an emulsified state of a highly hydrated liquid amorphous calcium carbonate (LACC). In our system, a continuous “white liquid film” can be observed at 5 mM and 10 mM CaCl₂ solution under relatively high RSF concentration at 72 h mineralization (Fig. S4a and b†) but was not found in the control experiment without RSF (Fig. S4c and d†). This emulsified-like film might be ascribed to LACC. Wolf et al. pointed out that acid proteins can stabilize LACC through “depletion stabilization” and “de-emulsification” processes. In the presence of RSF, significant LACC may be stabilized for a month (or even longer) at the air/water interface in this experiment. When removed from solution, LACC shrinks to form solid amorphous calcium carbonate (SACC) in the form of the irregular material adhering to the crystals (Fig. 6c) or nanowires connecting two particles (Fig. 3i and 6e) due to the rapid dehydration. SACC nanowires exhibit blue fluorescence in confocal fluorescence microscopy images (Fig. S5†), which might indicate that RSF co-exists with ACC. Moreover, we can observe the hemisphere-like aragonite and flower-like calcite at high RSF and Ca²⁺ concentrations. This radial growth process is commonly seen in polymer-induced ACC films, especially under high supersaturation.

Crystals collected at the air/water interface were produced directly from ACC, with no vaterite phase observed in the mineralization process, which is different from those obtained from the bulk solution. This suggests that the polymorph could be determined at the very beginning of the mineralization process. The structure of RSF at the air/water interface may play important roles in the selection between calcite and aragonite.

Here we propose a formation procedure for the CaCO₃ particles at the air/water interface assisted by RSF (Scheme 1). RSF can be adsorbed at the air/water interface and exhibits specific structures depending on the bulk concentration. Ellipsometry data indicated that a Langmuir RSF film is 11.6 to 11.9 Å thick. Thus we speculated that the RSF chains templating CaCO₃ are on the layer and sub-layer of the air/water interface. For diluted RSF solution (<0.02 wt%), the RSF molecules tend to pack laterally on the adsorbed layer and seek more energetically favorable conformations, e.g., helical silk III or laminated silk II. These RSF molecules may act as anchors to form compact multi-layers. A more stretched conformation at the interface results in more interaction zones between the RSF chains on the surface layer and sub-surface layer. The laterally packed RSF chains on the sub-surface can more easily form β-sheet (or β-strand) structures through hydrogen bonding. β-sheets possess an intrinsic preferential ability to induce the crystallization of the aragonite phase, which may be adsorbed on the aragonite crystal (010) face, and promote crystal growth along the [001] long axis, resulting in bundle-like aragonite.

For the concentrated RSF solution (≥1 wt%), RSF chains may adopt a hairpin-like conformation due to the crowded surface, resulting in the reduced interaction zones between the surface and the sub-surface layers. The more disordered
and large compact aggregates at the air/water interface are loosely interconnected,13 which may inhibit the formation of compact β-sheet structures. Thus, calcite crystals are preferred at high concentrations of RSF.

When the RSF concentration was between 0.02 wt% and 1 wt%, RSF chains were crowded at the air/water interface and it was more difficult to form β-sheet structures than in the diluted RSF solution. Under these conditions, Ca2⁺ concentration can affect the conformation of RSF, and thus decide the morphology and polymorphs of CaCO₃. Higher Ca²⁺ concentration may partially inhibit the formation of β-sheet structures, probably by introducing strong electrostatic interaction between RSF chains.27 Thus, more calcite can be obtained at high Ca²⁺ concentration. Coexisting aragonite crystals produced under high Ca²⁺ concentration have a hemispherical shape, which can indicate the compact and isotropic structure of RSF at the crowded air/water interface.

The aragonite particles produced here have two morphologies (bundle-like and hemisphere-like), which both grow directly from ACC. Generally, lower concentrations of RSF and Ca²⁺ result in bundle-like aragonite (Morph I). The main evolution process from ACC is schematically illustrated in Scheme 1. The aragonite was assembled by much smaller nanoplates at the initial stage. With longer reaction times, the nanoplates preferred to tilt at both ends and further aggregate to form a bundle-like structure. The size of the fan-shaped sectors depends on the component concentration, where lower ACC or RSF concentration may result in the smaller edges of structures.

Hemisphere-like aragonite (morph II) can be produced at high component concentration. Larger amounts of ACC and RSF chains can be inserted into the branches of the crystals at the very initial stage, resulting in a hemisphere or hemi-gourd-like aragonite.28 With longer reaction times, the crystal size becomes larger, and the radial growth leads to the formation of well-defined hemisphere-like aragonite. Moreover, SACC can be observed after the product has been collected in the form of an irregular material or nanowires.

Besides the calcite produced at extremely low RSF concentration, all of the particles obtained have a concave upper surface. This might be caused by the less ordered arrangement of the aggregated macromolecules,9 due to the high molecular weight and multi-block structure of RSF, or by the balance of gravity and pulling force coming from the surface tension of the solution.29 Thus the indentation in the center of the upper surface might be the nucleation point of the crystals at the core of the fans, and the crystals obtained at high RSF concentration may have a deeper indentation due to the aggregation of RSF and the low surface tension of the solution.

Our lab has found that molecular weight (MW) can influence the behavior of silk proteins at the air/water interface, which is reflected in the surface tension results. High MW results in higher surface activity but lower adsorption efficiency. After mineralization under the same procedure, the morphology and polymorphs of CaCO₃ particles vary with the MW of RSF. The morphology of CaCO₃ particles mediated by higher MW RSF (RSF-185) is shown in Fig. S6,† and the crystal polymorphs are summarized in Table 3. The mineralization results are similar to those mediated by RSF-83 at higher concentrations of RSF, while at lower concentrations, the typical rhombohedral calcite is the dominant crystal instead of the aragonite mediated by RSF-83. This phenomenon can be ascribed to lower adsorption of high MW RSF. Moreover, aragonite particles mediated by RSF-185 are “plumper” and bigger than those mediated by RSF-83 due to the entanglement of high MW RSF. In contrast, aragonite mediated by low MW RSF (RSF-36) is smaller and thinner in morphology, with no hemisphere-like aragonite, and is found in greater quantities under higher RSF concentration (Fig. S7†). This

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Note: the same meaning for the capital letters A and C as that in Table 2.
Table 4 CaCO₃ polymorphs obtained at the air/water interface with different concentrations of RSF-36 and Ca²⁺

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*Note: the same meaning for capital letters A and C as that in Table 2.

could be due to the lower adsorption and lower degree of entanglement of RSF-36. The polymorphs of the particles are similar to those mediated by RSF-83 (Table 4).

Conclusion

A series of CaCO₃ structures with continuously varying morphology and polymorphs were obtained at the air/water interface through the mineralization mediated by regenerated silk fibroins. The crystals can be formed from ACC directly with the face through the mineralization mediated by regenerated silk fibroins. The crystals can be formed from ACC directly with the aid of protein stabilization. Higher concentrations of RSF-36 and Ca²⁺ resulted in calcite while low concentrations induced the aid of protein stabilization. Higher concentrations of RSF fibroins. The crystals can be formed from ACC directly with the mineralization mediated by regenerated silk fibroins. The crystals can be formed from ACC directly with the mineralization mediated by amphiphilic proteins at the interface, providing a new way to understand the structure of the mineralization results. This work demonstrated the conformation of the protein at the air/water interface is critical for the mineralization results. This work demonstrated the control of RSF over the mineralization of CaCO₃ at the air/water interface, providing a new way to understand the structure of RSF adsorbed at the interface. Moreover, the mineralization mediated by amphiphilic proteins at the interface may become a new approach for producing materials with metastable polymorphs and complex morphologies.

Acknowledgements

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Notes and references