Alternate Deposition of Oriented Calcite and Amino Acid Layer On Calcite Substrates

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Alternate Deposition of Oriented Calcite and Amino Acid Layer On Calcite Substrates

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Material synthesis inspired by novel nacre architecture and mechanism is popular and has attracted more and more attention. In this paper, iso-oriented calcite tablets/layers and amino acid layers were formed alternately on calcite wafers. It is interesting that the neonatal calcite tablets/layers have the same crystal orientation with their inorganic substrates through amino acid layers. It is quite possible that the amino acid layers in this study could transfer crystal orientation from formed inorganic layers to neighboring neonatal layers due to their fixed and appropriate structures, which may imply the process of nacre formation, and the role of aligned organic matrix sheets in nacre. Moreover, it could provide a new way to produce oriented calcite tablets/layers.

1. Introduction

The study of biomineralized tissue offers valuable insights into the scope of material design. Of them, the detailed structure of nacre has attracted the interest of researchers for several decades. Nacre has a "brick and mortar" arrangement: the bricks are flat polygonal crystals of aragonite with preferred orientations, and the mortar is made out of polysaccharide and proteins.[1,2] Nacre is used as a model for studying "organic-matrix-mediated" biomineralization in which crystals are oriented, nucleated, and grown in a preformed structural substrates. Mann considered that there is a strong correlation between the spacing of arranged [Asp-X] (X means some organic groups) domains and the theoretical lattice arrangement of Ca^{2+} ions in the (001) surface of aragonite.[3] The structural match model has been used to account for the alignment of aragonite tablets with *c*-axes perpendicular to the matrix.[3] However, no result has presented sufficient evidence to describe how the interlammellar organic matrix acted to align the orientation of calcite tablets in successive laminae.[1,4−6] Previous investigations demonstrated that matrix sheets have pores that both allow the crystals to grow and keep them aligned crystallographically via mineral bridges.[7] However, Rousseau recently showed that the bridge from one tablet to the next was composed of crystallized organic.[8] The role of organic sheets between nacre laminae should be studied to explain this crystal orientation relationship.

On the basis of the organic-matrix-mediated phenomenon in biominerals, some research has focused on the promoting effect of organic templates on oriented crystal nucleation and growth. The main approach to investigate the interactions in vitro is to use molecular assemblages as organized organic surfaces for inorganic crystallization, for instance, Langmuir−Blodgett monolayer, self-assembled monolayer, and protein sheet extracted from organisms.[9−11] Fallini et al. showed that oriented crystallization of vaterite and aragonite occurs in uniaxially deformed gelatin films with the β-sheet structure assumed by poly L-aspartate.[12] Han and Aizenberg found the highly controlled synthesis of calcite with uniform nucleating plane, size, and morphology by combining the MUA-SAM-induced oriented nucleation with the addition of Mg^{2+} ions to the growth solution.[13]

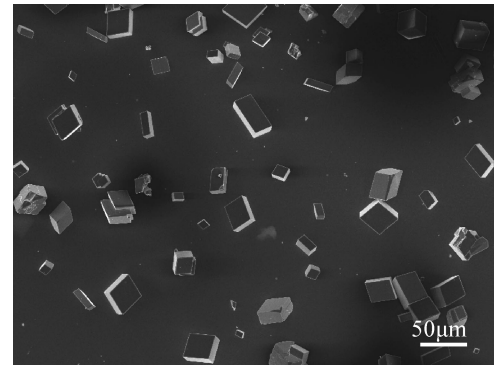

Figure 1. SEM micrograph of surface of pure calcite wafer soaked in $CaCl_2$ solution for 4 h, showing that some calcite crystals crystallized in solution fell on the wafer.

On the other hand, nacre fascinates many researchers because of its well-designed architecture.[14−16] Great efforts have therefore been taken to synthesize nacre-mimetic materials. These artificial nacre-mimetic materials usually have the complex structures and high mechanical performance, similar to nacre. $CaCO_3$ films have been prepared by many methods.[17−19] For instance, aragonite thin films have been deposited on chitosan matrices; vaterite thin film has been prepared by solvothermal treatment of calcium chloride and urea in the presence of ethylene glycol. Also, single-crystal calcite thin film was fabricated on modified polyethylene terephthalate (PET) templates.[20] The single crystals patterned at the micro- and nanoscale are important components in various electronic, sensory, and optical devices. Therefore, high-performance nacre-mimetic and thin films materials via a simple self-assembled process are attractive for researchers.

In the present work, we propose the biomimetic synthesis of iso-oriented calcite tablets and single-crystal-like layers via the alternate epitaxial growth of oriented calcite layers and amino acid layers. It is interesting that the neonatal calcite tablets/layers grown on amino acid layers have the same crystal

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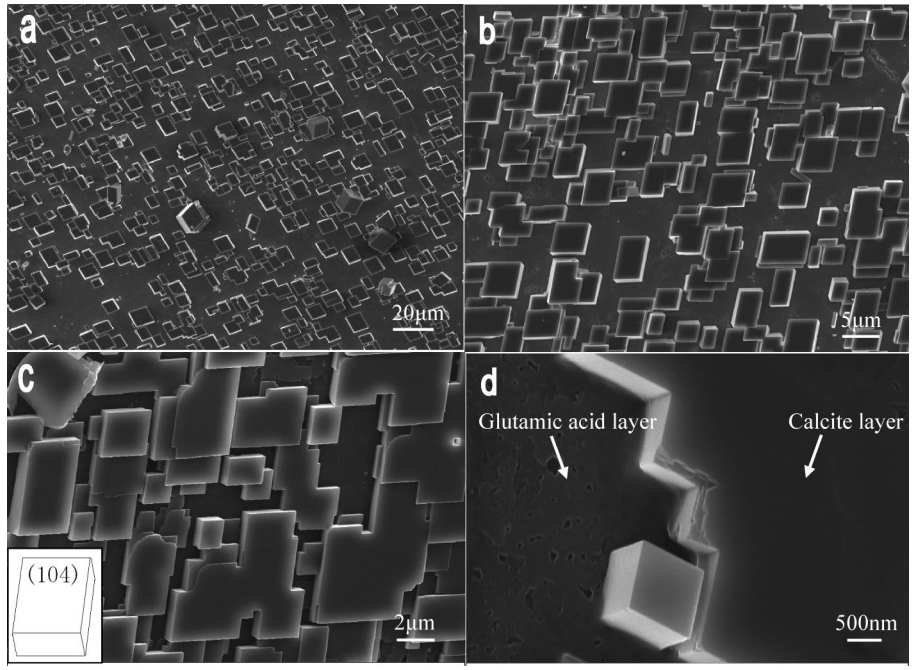

Figure 2. SEM micrographs of calcite tablets and calcite layers deposited on amino acid layers. (a) Calcite tablets grown on glutamic acid layer for 1 h; (b) calcite tablets grown on arginine layer for 1 h; (c) the bigger and conjunctive calcite tablets grown on glutamic acid layer for 1.5 h; (d) the calcite layer grown on glutamic acid layer for 2 h.

orientation as their inorganic substrate, which may imply the process of nacre formation. This work also offers a simple method for synthesizing layer-by-layer compositions, which are composed of organic layers and inorganic single-crystal-like layers.

2. Experimental Section

Surface modification. Four kinds of amino acids (glycine, glutamic acid, lysine, and arginine) were dissolved respectively in distilled water with the concentration of 1 mM. Calcite wafers with (104) exposed surface (Geologic minerals, provided by museum of China University of Geoscience) were overlaid with amino acid solution for 10 h at 4 °C. The amino acid solutions should be fresh to prevent amino acid molecules from synthesizing polypeptides. The modified wafers were dipped in water to wash for several seconds and dried in air for use. The silicon wafers were modified using the same method.

Crystallization. In our experiment, the method of synthesizing crystals was according to the report of Aizenberg.[21] The crystals were grown by slow diffusion of NH_4HCO_3 vapor into cell-culture dishes containing 5 mL of 8 mM $CaCl_2$ solution in a closed desiccator. The calcite wafers with and without amino acid coatings were put on the bottoms of the culture dishes as crystal growth templates at room temperature. The deposition time was alterable in this experiment. This crystallization process for silicon wafers with amino acid modification is the same as for calcite wafers. The samples were dipped in water to wash for several seconds, and dried in air for use.

Double layer preparation. Just repeat surface modification and crystallization processes alternately.

Analysis methods. The morphologies and structures of the depositions were observed using scanning electron microscope (SEM, JEOL-1530) and X-ray diffraction (XRD, R-AXIS SPIDER). The glutamic acid layer was analyzed by X-ray photoeletron spectroscopy (XPS, PHI-5300), Auger Electron Spectrometer (AES, PHI-700), and Surface Tensiometer (Krüss-K12).

3. Results and Discussion

Figure 1 shows an SEM micrograph of a bare calcite wafer soaked in supersaturated calcium carbonate aqueous solution for 4 h where several calcite particles that formed in solution fell on the calcite wafer. Other similar crystals were observed on the rest part of cell (beyond substrate), so the result is that lots of calcite tablets on substrate did not depend on the substrate. The crystals crystallized in solutions, and calcite wafer did not play any role. In this condition, large numbers of Ca^{2+} and CO_3^{2-} were absorbed directly to calcite surface steps,[22] which can be considered as the growing-up process of calcite substrate.

We observed different phenomenon for the $CaCO_3$ deposition under similar conditions when the calcite surfaces were modified by glutamic acid molecules before deposition. From SEM images, it can be seen that $CaCO_3$ crystals grown on calcite substrates soaked for 1 h in 1 mM glutamic acid solution appear as rhombohedral tablets. These similarly sized rhombohedral islands were nucleated at random positions but with the same orientation as the underlying substrate, as shown in Figure 2a. The crystalline tablets were identified to be calcite oriented with (104) surface. It is notable that the calcite tablets have the same crystallographic orientation as the underlying calcite substrate. Calcite tablets were arranged orientedly on the modified surface, which indicated that calcite tablets were not formed in solutions evidently. As expected, the calcite tablets grew with increasing deposition time, as shown in Figure 2c. When the calcite tablets grew and contacted each other, a calcite layer was formed, creating a (104) surface. Figure 2d shows the calcite layer grown on glutamic acid layer. Figure 3 shows a two-layered film of calcite and glutamic acid. The upper calcite layer has successfully grown on the underlying layer, again with the same crystal orientation.

Similar results were obtained on other calcite wafers whose (104) surfaces were coated by other three amino acid layers (glycine, lysine, and arginine). The differences among these

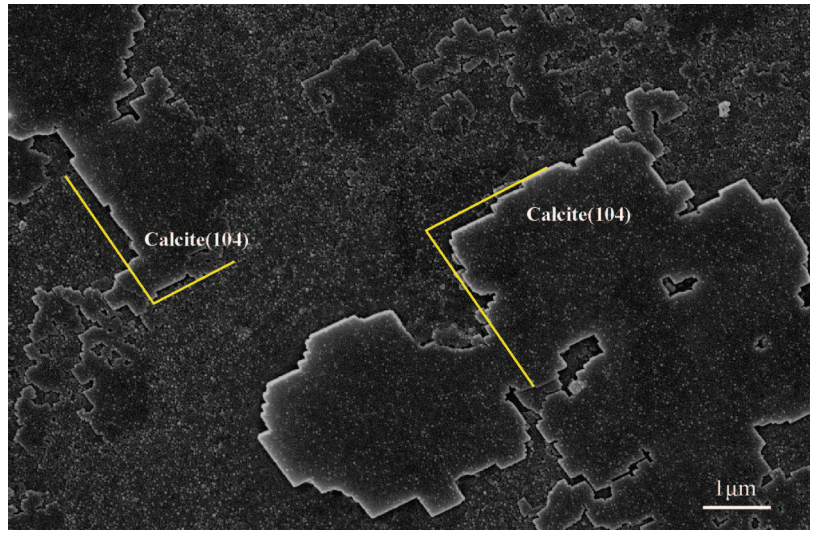

Figure 3. SEM micrograph of double layered structure of calcite and glutamic acid. The first calcite layer was deposited for 4 h, and the second calcite layer was deposited for another 3 h. (Nanogold particles observed were caused by double-spraying.)

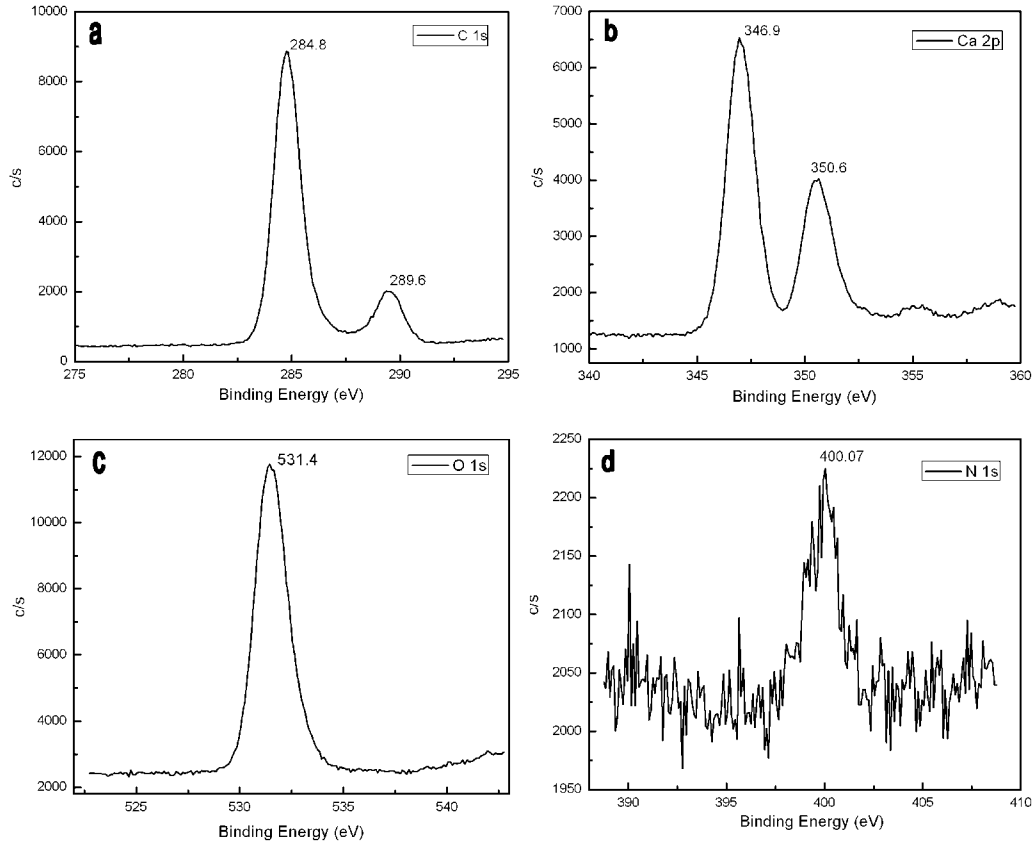

Figure 4. XPS spectra of typical elements of glutamic acid layer deposited on calcite wafer. (a) C 1s; (b) Ca 2p; (c) O 1s; (d) N 1s.

calcite tablets grown on different amino acid layers are the density and dimensions, as shown in Figure 2a,b.

Calcite surfaces coated with glutamic acid were analyzed by XPS, as shown in Figure 4. Carbon, oxygen, calcium, and nitrogen were the main surface components. The atomic concentrations of these elements were 61.49 (C), 30.38 (O), 7.27 (Ca), and 0.85 (N). Compared with pure calcite surface, the surface carbon and oxygen content of calcite wafer coated with glutamic acid increased and the calcium content decreased, which indicats that glutamic acid molecules form an organic layer on the calcite surface. The existence of nitrogen is further evidence of glutamic acid on calcite wafer. The C (1s) area is made up of two peaks at 284.8 and 289.6 eV. The value of 284.8 eV was adopted as standard C (1s) binding energy, and the peak at 289.6 eV is assigned to carbon in C−O and C═O in carbonate groups.[23] The 289.6 eV peak of modified calcite surface was stronger than that of pure calcite surface, which indicates that the amount of C−O and C═O increases due to the existence of glutamic acid. The O (1s) at 531.4 eV is associated with to C−O and C═O in carbonate groups.[24] The N (1s) signal of the amine groups at 400.07 eV can be assigned to nitrogen in R-NH_2.[25] The XPS of Ca bonded to COO− is consistent with that of Ca in $CaCO_3$ at 346.9 and 350.6 eV.[26] The strong bonding

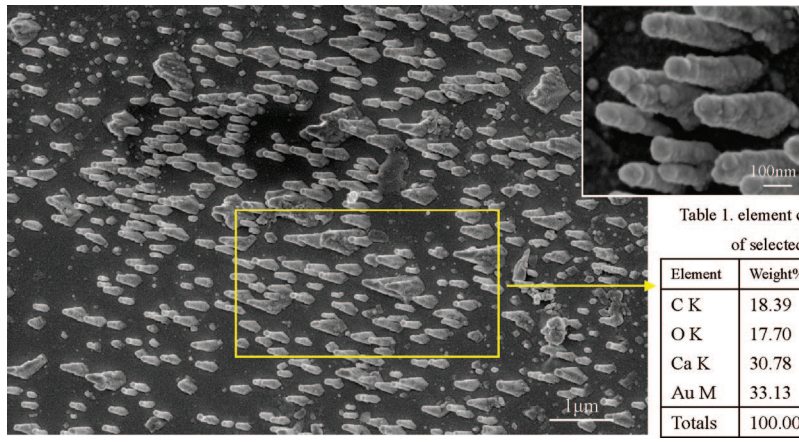

Table 1. element concentration of selected area

Element	Weight%	Atomic%
C K	18.39	42.85
O K	17.70	30.96
Ca K	30.78	21.49
Au M	33.13	4.71
Totals	100.00	

Figure 5. SEM micrograph of glutamic acid layer deposited on calcite substrate in 1 mM glutamic acid solution for 24 h.

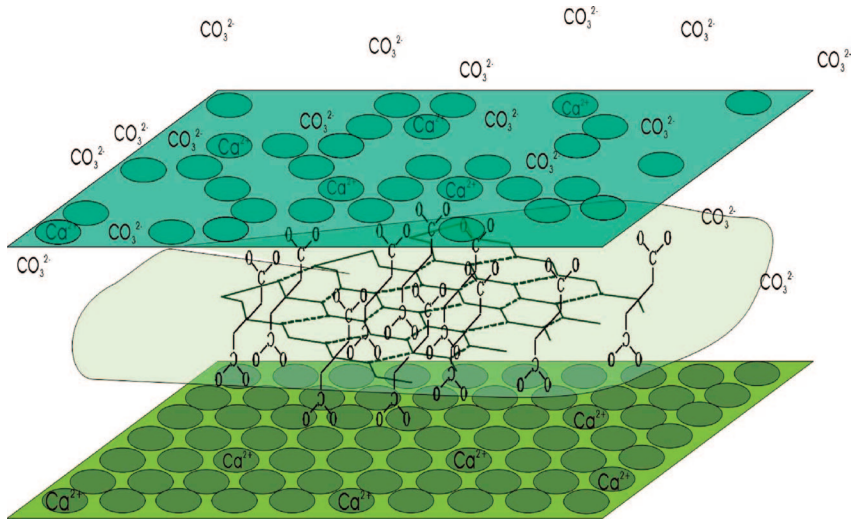

Figure 6. A schematic representation of the formation of oriented calcite layer on an amino acid layer or hillocks. See detailed description in the text.

of Ca^{2+} and COO^- is universally proved in various inorganic−organic interfaces.[27] This suggests that glutamic acid molecules are mostly chemisorbed on the calcite (104) surface. The atomic concentration of N in five independent points of this surface was tested using Auger electron spectrometer (AES) (see Supporting Information), which indicates that glutamic acid molecules were evenly deposited on calcite wafer. The average concentration of N is 2.95 atom %, higher than that in the XPS result, mainly since the scan depth of AES is shallower. The contact angle of water on modified calcite wafer is 44.5°, while that on pure calcite wafer is 67°, which indicates that the surface of calcite wafer was modified.

Figure 5 shows the glutamic acid layer deposited on a calcite substrate in 1 mM glutamic acid solution after 24 h (a comparative long time). We did not observe the similar phenomenon at the initial 10 h deposition. If there is no layer formed first, we could observe the hillocks at the initial time, so we think that the layer formed first, and then the hillocks grow on this layer, which seems more reasonable. About the stability of this amino acid layer, we think that the bonds of Ca^{2+} and COO^- are strong and common; it is not easy to remove amino acid from calcite surface just by clearing in water. After cleaning and drying the modified calcite substrate, we tested the existence of amino acids in all points we chose, which indicates the stability of amino acid layer indirectly. Besides, if amino acid dissolved in solutions, the organic molecules would induce crystals with different polymorphs and morphologies, which indicate that the amino acid did not dissolve in solution.

For comparison, the same synthesis process by which calcite tablets were formed on calcite/amino acid substrates was conducted on silicon (001) wafers. No calcite tablets were observed on the silicon (001) wafers. Lattice geometry and electrostatic potential at inorganic−organic interfaces are the key concept in oriented crystal nucleation.[28,29] The silicon atoms arrangement on (100) surface is far different from that of calcite (104). Therefore, silicon (100) is nearly impossible to play the similar action of structure matching as calcite (104) in the same system. On the other hand, Ca^{2+} and CO_3^{2-} in calcite surface have reasonably strong electrostatic bonds with amino acid groups, as the similar case of calcium-containing biomaterials. But silicon atoms of single crystal silicon are uncharged even in solution, which implies that silicon atoms are hard to be combined with carboxyl COO^- or amidogen NH_3^+ in amino acid solutions.

In this model, we observed the alternate deposition of amino acid layers and calcite layers on calcite substrates; also, new calcite crystals have the same orientation with their inorganic substrates through amino acid layers. Ca^{2+} of calcite surface has a strong binding with COO^- of amino acid. In our opinion, assembled way of this organic layer is not exact, it is not a monolayer, and four kinds of amino acid have their own assembled ways, which are perhaps similar but not the same. Every assembled way has its

intrinsic structure, it is reasonable that all these four amino acid could induce oriented crystals with different densities. From theory explanation, bonds between Ca^{2+} and amino acid is strong, and amino acid can form peptides spontaneously, so amino acid and $CaCO_3$ must combine each other in certain form, which is also basic theory for biomineralization. About the self-ordering glutamic acid film, the way that we used to form amino acid layer is a common way to prepare the self-assembling layer. Recently, most SAMs are prepared through depositing in low concentration solutions. Generally, SAMs can be observed using atomic force microscopy (AFM), but this amino acid layer is hard to be proved due to the small molecular weight of amino acids. Here, we do not think the layer is completely self-ordering, but there must be ordering domains to induce the oriented growth. These amino acid molecule layers or initial hillocks provide nucleation sites as well as crystallographic direction for new nucleus. The surface of amino acid layers orderly absorbed Ca^{2+} in solution to form oriented calcite tablets due to the structure match and electrostatic potential between them, see the schematic representation in Figure 6. This model was established based on some basic theories and experimental methods including (1) the stable bonding of Ca^{2+} and COO−,[27,30−32] (2) the phenomenon of ordering growth, and (3) the common self-ordering layer forming method. The crystal orientations of the neonatal tablets depend on the structure and electrostatic potential of the medium existing between neighboring layers. Simple amino acid molecules exert an important action on transferring the crystal orientation from one calcite layer to the next. Besides, the surface tension has decreased since the amino acid is hydrophilic, a decreased contact angle implies a higher surface energy, and thus a lower supersaturation is needed to nucleate crystals on the surface. Therefore, modified wafer surface provide high surface energy, low binding energy, COO−Ca bonds, and nucleation sites (layer or initial hillocks). For the similar condition in nacre, the neighboring aragonite tablets of several successive layers generally have the similar crystal orientations.[4−6]

Actually, interlamellar organic matrix of nacre has the complicated components. If the organic matrix is organized to form a fixed structure under the biological control, it could successfully transfer the crystal orientation of successive nacre layers, as the case of amino acid molecules in this model. Up to now, we are still far from fully understanding the organic matrix structure and the mechanisms by which it controls crystallization. Levi's study showed that the interlamellar sheets are composed mainly of highly ordered and aligned β-chitin fibrils.[33] Rousseau also observed the crystallized organic matrix using HRTEM.[11] Therefore, it is highly possible that both this aligned organic matrix sheets in nacre and the amino acid layers with fixed-structure in the present model could transfer crystal orientation from formed inorganic layers to neighboring neonatal layers due to their fixed and appropriate structures.

Through this novel method, it is possible to design two kinds of layered compositions by altering the deposition time of Ca^{2+} and CO_3^{2-}: nacre-biomimetic and layer-by-layer calcite/amino acid compositions. The layer-by-layer organic/inorganic composition with single-crystal-like inorganic layers is strikingly novel. Apparently, the dimension of calcite tablets and the thickness of calcite films can be easily controlled by altering the deposition time. Compared with some nacre-biomimetic methods, the present method does not put forward a faster synthesis way, but this process has a better performance on crystal orientation regulation of nacre using calcium carbonate system. In addition, this method is not limited to a specific material but is also applicable to other kinds of appropriate inorganic and organic materials with precisely controlled process. The key is interface recognition and the structural match between organic and inorganic medium.

4. Conclusion

In summary, we have succeeded in the formation of oriented calcite tablets/layers and amino acid layers via the alternate heteroepitaxy growth. These iso-oriented calcite tablets and single-crystal-like layers have the same orientation with their inorganic substrates through the amino acid layers. The amino acid layers are contributed to work as medium conveying crystal orientation information from one layer to the next due to their fixed and appropriate structures. This is a possible explanation for crystal orientation relationships between the neighboring aragonite tablets in successive nacre layers. This method of synthesizing oriented tablets and layer-by-layer compositions has potential application for the fabrications of high-performance organic/inorganic composite materials.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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