# A Crucial Process: Organic Matrix and Magnesium Ion Control Amorphous Calcium Carbonate Crystallization on ß-Chitin Film

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**Statement of Purpose:** Nacre, as a natural inorganic-organic composite, has excellent mechanical properties due to its complicated hierarchical structure (Jackson AP. Proc R Soc Lond B. 1988; 234: 415-440.). Therefore, the study on the formation mechanism of nacre tablets can provide theoretical basis for designing new organic-inorganic composite with high performance. On the formation mechanism of individual nacre tablets, there are three important hypotheses, including (i) single crystal growth, (ii) a coherent aggregation of nanograins, and (iii) phase transformation from amorphous calcium carbonate (ACC) to stable aragonite. Recently, most evidences show that nacre is formed via a precursor phase of amorphous calcium carbonate (Nassif N. Proc Natl Acad Sci USA. 2005; 102: 12653-12655; Weiner S. Front Mater Sci China. 2009; 3: 104-108.). This strategy thus appears to be widespread. The challenge now is to understand how ACC is temporarily stabilized and then destabilized to transform into a crystalline product in nature.

**Methods:** ACC particles were synthesized first according to the previous study. Aragonite pearl of the freshwater mollusc Hyriopsis cumingii (Zhejiang province, China) was chosen as raw materials to extract water soluble matrix (WSM). WSM and magnesium ion were used as stabilizer and chitin film was acted as a substrate. Then we performed a biomimetic study to investigate the crucial process from ACC to crystalline calcium carbonate. Morphology and elemental composition of calcium carbonate crystals were observed on a SEM with EDX. Raman spectrometer, FTIR and TEM were used to determine the polymorph.

**Results:** The synthesized calcium carbonate was characterized by SEM, XRD, FTIR and TGA-MS. SEM image reveals that calcium carbonate is completely consisted of spherical particles with diameter of about 300 nm and the surface of particles looks smooth. XRD, FTIR and TGA-MS results show that the calcium carbonate particles are amorphous.

To investigate the effect of WSM from freshwater pearl on ACC crystallization on chitin film, ACC powders were soaked into the solution with or without WSM on silicon slice and chitin film, respectively. In the absence of WSM, only calcite was formed either on silicon slice or on chitin film despite the difference in the crystal morphology. Introducing WSM into the mineralization solution resulted in the formation of aragonite accompanied by calcite on silicon slice. In contrast, the rod-like aragonite crystals predominantly grew when the template is chitin film.

Similarly, ACC powder was immersed in low Mg solution (10 mM) and high Mg solution (40 mM) respectively to study the effect of magnesium ion on ACC crystallization on chitin film. A trend was found that aragonite morphology changed from rod-like aggregate to quasi-spherical aggregate with an increase in $Mg^{2+}$ ion concentration, which indicated that magnesium ions acted as a shape modifier. In this case, the deposition of Mg-calcite was determined by the substrate on which ACC crystallization happened. On chitin film, no Mg-calcite crystals grew in low Mg and high Mg solutions either.

Time-dependent morphology evolution process was carefully examined to understand the process of ACC crystallization under the control of WSM or magnesium ions on chitin film. Then the possible mechanism of ACC phase transformation was proposed and simply illuminated (Fig. 1). Despite all this, it must be pointed out that the exact mechanism for ACC crystallization with different morphologies is extremely complicated and needed to be further studied.

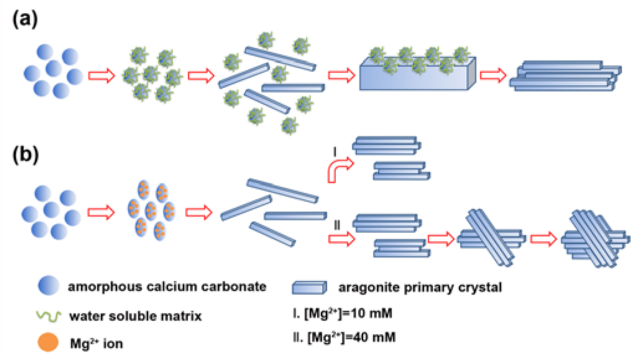


Figure 1. Schematic illustration of ACC crystallization process occurring on chitin film under the control of (a) water soluble matrix or (b) magnesium ions.

**Conclusions:** In summary, ACC particles with diameter of about 300 nm are synthesized first. Then we have investigated the phase transition process from ACC to crystalline phase on chitin film by adding WSM or magnesium ion in aqueous solution. WSM may stabilize ACC, and the combination of WSM with chitin film offers the ability to enhance aragonite nucleation and control the pure aragonite phase, leading to form the rod-like aragonite crystal aggregates. Comparatively, the collaborative effect of magnesium ion and chitin film not only induces to form the aragonite crystal aggregates, but also inhibits the transformation from ACC to Mg-calcite. A possible growth mechanism of the aragonite crystal aggregates with different morphologies is thereby proposed. This study may give some useful clues for understanding the biomineralization mechanism of calcium carbonate in nature. Furthermore, the present work provides some new insights into preparation of $CaCO_3$ aggregates with complex shape and fine structure assembled from amorphous precursor.