Biomimetic processes through the study of mineralized shells

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Abstract

Fabrication of mineralized structures is a widespread phenomenon among living organisms (e.g., shells, carapaces, spines, spicules, bones and teeth). These ceramic biocomposites consist of layered assemblies of minute amounts of macromolecules with well-ordered calcium-rich inorganic phases, resulting in the formation of products of unique morphologies and properties. The characterization of the mechanisms controlling the processes of biomineralization is crucial for the development of novel materials with desirable shape and texture properties. In previous reports on eggshells and mollusk and crustacean shells, we have studied the cell–shell interactions, the crystalline microstructure of the inorganic component, the localization of particular macromolecules and the capacity of various biomolecules to affect crystallization. Based on these comparative data, we propose that biomineralization can be described as a four-step process: (1) substrate fabrication, (2) crystal nucleation on the substrate or framework, (3) crystal growth in a gel and (4) mineralization arrest. These four steps open a new field for designing synthetic processes in order to fabricate new bioinspired composites with desirable properties.

Keywords: Biomimetics; Biomineralization; Fabrication

1. Introduction

Modern technologies require innovative approaches for controlled fabrication of crystalline materials with complex forms and novel properties [1–4]. Biomineralization is a widespread phenomenon among living systems (e.g., egg and mollusk shells, crustacean carapaces, echinoderm exoskeleton and spines, sponge spicules, pearls, corals, bones and teeth) [5–7]. This process leads to the formation of precisely controlled inorganic–organic composites, in which the minute organic component exerts substantial control on the mineralization process, which results in the formation of particles of uniform size, novel crystal morphology, specific crystallographic orientation and interesting properties [5–12]. For example, seashells exhibit mechanical properties that are 1000 times greater than those of the inorganic component alone [13,14]. Therefore, biomimetic design for the production of advanced composites with optimized novel properties has been explored and has led to recent advances in materials design inspired by biological processes [15–21]. A wide variety of strategies have been explored to control

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nucleation and growth of crystals based on molecular recognition at intrafaces or interfaces [4,22,23]. These methods include template-directed crystallization under compressed Langmuir monolayers, on self-assembled monolayers or nanocomposite films, on functionalized polymer surfaces, in surfactant aggregates and in cross-linked gels [22–31]. Understanding the mechanisms that regulate the fabrication of such highly ordered biocomposite ceramics may provide procedures for the synthesis of novel high-performance composite materials.

2. Eggshell formation and structure

Eggshells are natural composite bioceramics containing organic (5%) and inorganic (calcite) components (95%) and composed of a two-layered membrane and calcified extracellular matrix, which are sequentially assembled during the 22 h that the egg moves along the oviduct [1,32]. The structure and composition of the eggshell is shown in Fig. 1. The first layer to be formed in the eggshell comprises the shell membrane, a net of fibres composed by a core of type X collagen surrounded by a fuzzy material referred to as a mantle [33]. Although the shell membrane never mineralizes, due to an inhibitory effect of type X collagen, it acts as a substrate for the deposition of the mammillary knobs, which are the nucleation sites for calcite crystals [34]. These knobs are randomly deposited on the outer side of the shell membrane in the form of discrete organic aggregations (20–40 μm in diameter) containing mammillan, which is a proteoglycan containing oversulfated keratan sulfate [35–37]. Columns of calcite grow on the top of these mammillary knobs, and their crystal orientation and morphology are affected by ovoglycan, a unique dermatan sulfate proteoglycan [36,37]. The dermatan sulfate glycosaminoglycan chains of ovoglycan are polyanionic and acidic and have a high calcium affinity. When sulfation of these macromolecules is experimentally affected, the eggshell crystal-

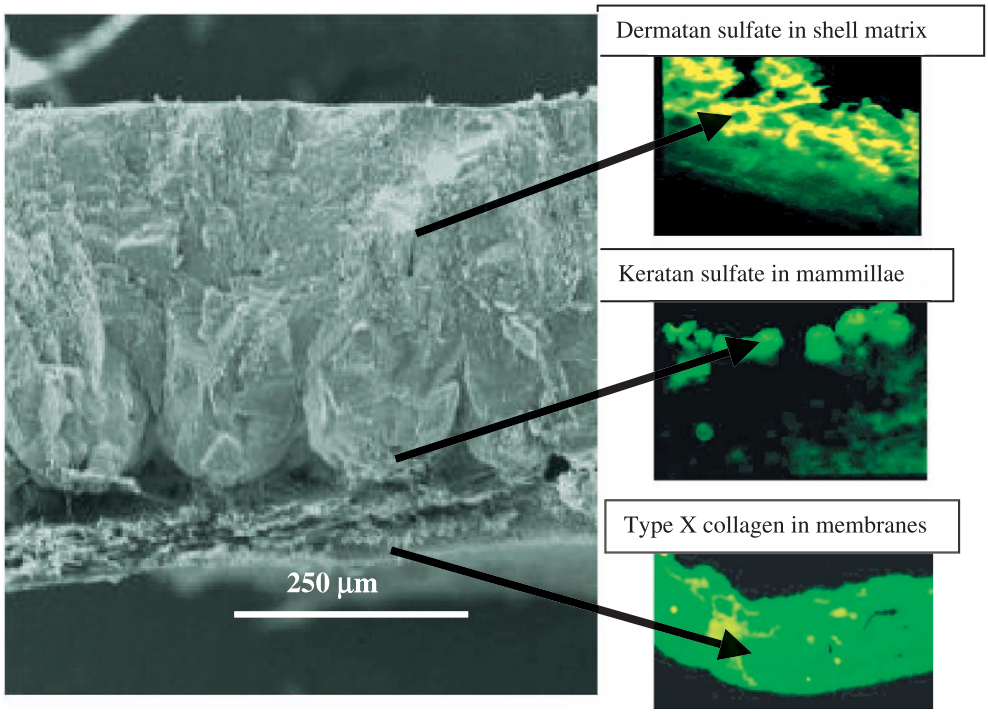

Fig. 1. Structural localization of macromolecules involved in eggshell formation. Left: Scanning electron micrograph of eggshell (170 ×). Right (top to bottom): Dermatan sulfate positive immunofluorescence in the shell matrix. Keratan sulfate positive immunofluorescence in the mammillae. Type X collagen positive immunofluorescence in the shell membranes (400 ×).

line calcite columns show severe structural alterations [37]. On the other hand, the sulfate content of these macromolecules affects the calcite crystal morphology [38]. These types of sulfated macromolecules have also been found in eggshells other than chicken [39]. Particular proteins secreted before oviposition are involved in the process of arresting eggshell formation [11,40]. Therefore, eggshell biomineralization is affected by particular macromolecules, which are produced by specialized cells in a spatiotemporally

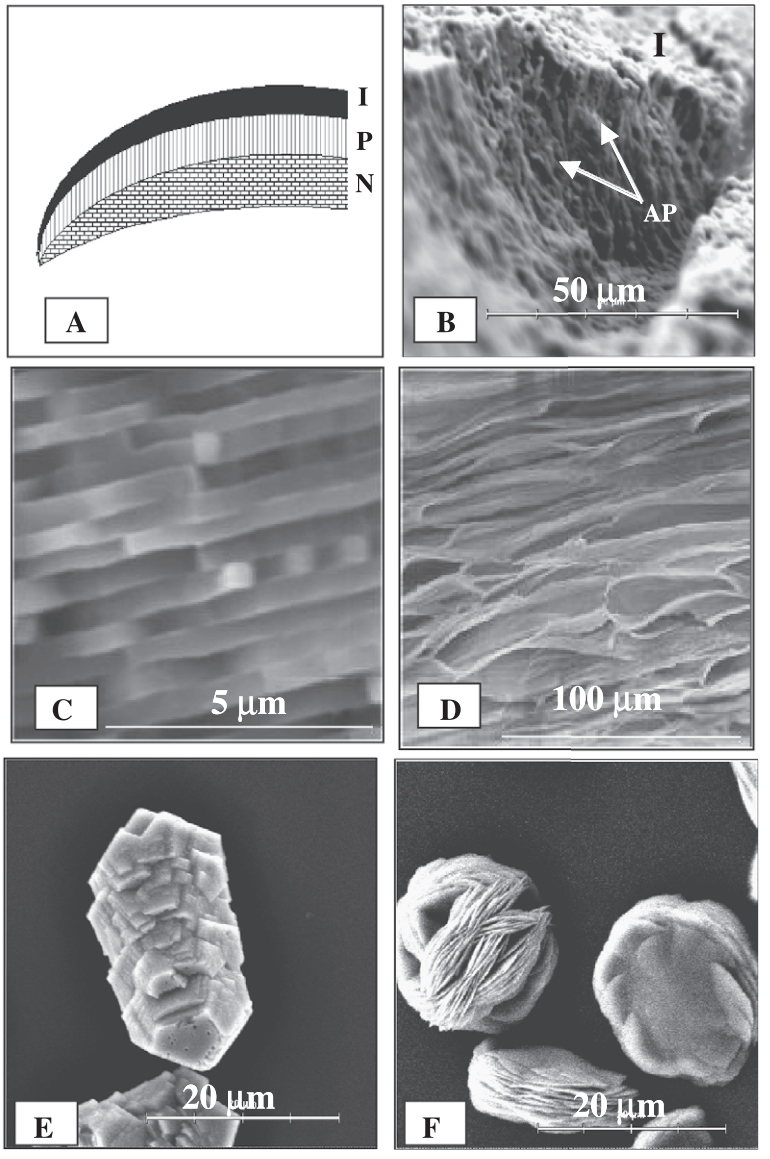

Fig. 2. (A) Schematic drawing of a bivalve mollusk shell. I: insoluble protein layer or periostracum, P: prismatic layer, N: nacre. (B) Prismatic layer. I: periostracum, AP: acidic proteins between prismatic calcite crystals. (C) Nacre layer showing brick wall of plate-like aragonite crystals. (D) Organic sheets and protein envelopes after nacre layer decalcification. (E) Calcite primatic crystal obtained in vitro influenced by soluble proteins extracted from the prismatic layer. (F) Aragonite crystal obtained in vitro influenced by soluble proteins extracted from the nacre layer.

dependent assembly line sequence as the egg passes along the oviduct [40,41].

3. Seashells formation and structure

Seashells are microlaminate composite bioceramics of mineral and biopolymers, which show exceptional regularity and a strength far exceeding that of the mineral itself. As in eggshells, the calcium carbonate phase of the seashell highly contributes to its mass (98%), while it is the integral organic matrix moiety (2% of the shell mass) that determines the precise structural formation, organization and properties of the mineralized composite [13,14,42].

Mollusk shells are mainly composed of layers of prismatic calcite crystals, brick-wall aragonite crystals or both types of construction (Fig. 2) [5–7]. The control of this polymorphism is exerted by a specific association of particular macromolecules [9,10,43]. Crystal nucleation occurs on an organic sheet (β-chitin or other organic matter of unknown composition) coated with hydrophilic, aspartate-rich macromolecules, while growth occurs within an organic

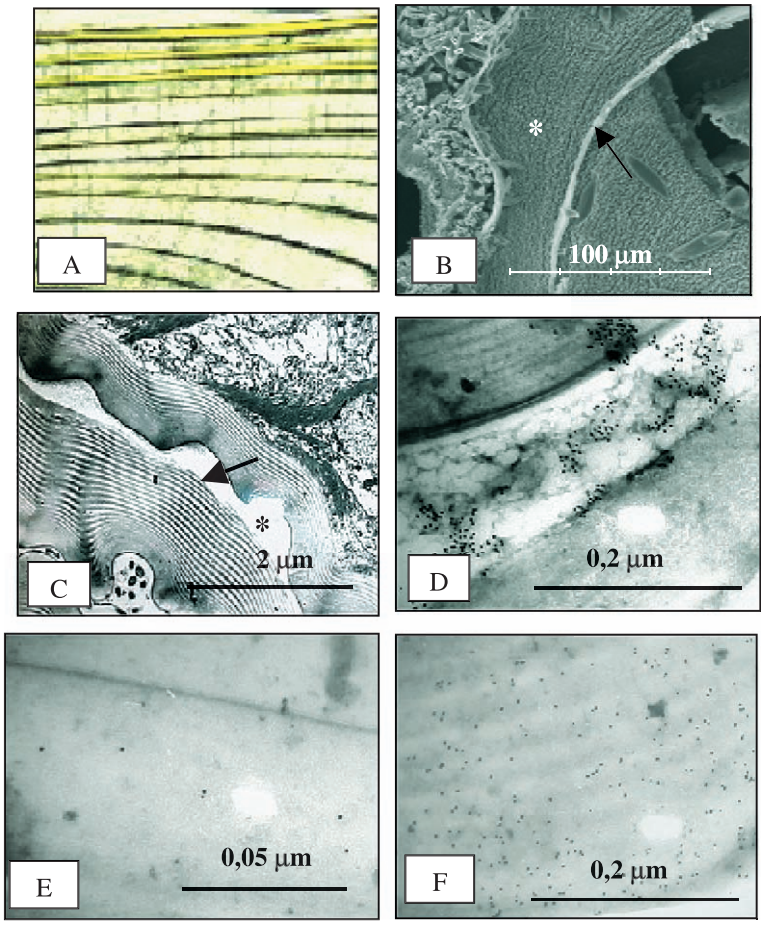

Fig. 3. (A) Polished transversal section of barnacle shell showing the layered structure, dark lamellae correspond to chitin (400 ×). (B) Scanning electron microscopy of partially decalcified shell showing calcite crystals between chitin sheets (arrow) and a granular organic material attached to them (*). (C) Transmission electron microscopy of a decalcified shell showing laminated sheets of chitin (arrow) and granular material between them (*). (D) Immunogold positive reaction with antichondroitin 4 sulfate antibody on the granular material. (E) Immunogold positive reaction with antidermatan sulfate antibody on the granular material. (F) Immunogold positive reaction with antikeratan sulfate antibody closely associated to chitin sheets.

envelope consisting of a silk fibroin-like protein gel containing acidic proteins [10,44,45]. Mineralization arrest is affected by the secretion of hydrophobic macromolecules such as those forming the periostracum or the scaffolding of the nacre layers. Therefore, crystal growth is modulated by specific proteins, while the final arrangement of crystals is determined by crystallographic constraints and space limitations [46].

Barnacle shell is also composed by a layered structure of calcite crystals (Fig. 3) [47]. The crystalline layer is sandwiched between two sheets of chitin, which are coated with a polyanionic sulfated proteoglycan (keratan sulfate), probably acting as the nucleation site, while crystal growth occurs in a dermatan and chondroitin 4 sulfate polyanionic gel [48]. Mineralization arrest is affected by the deposition of a new chitin sheet.

4. A model of shell mineralization

A commonly used strategy in shell biomineralization is the elaboration of a well-organized extracellular organic matrix, which regulates where, when and in what form mineralization will occur [1]. Three general biological processing principles have been identified, which govern the composition, architecture and methods of assembly of bioceramics and which have implications for material scientists and engineers [1]: (1) Biomineralization occurs within specific subunit compartments or microenvironments, which implies stimulation of crystal production at certain functional sites and inhibition or prevention of the process at all other sites. (2) A specific mineral is produced with a defined crystal size, shape and orientation. (3) Formation of macroscopic shape is accomplished by packing many incremental units together, which results in unique composites with layered microarchitectures that impart exceptional material properties. In some natural systems, remodeling of the original mineral structure occurs.

The geometric shape (habit) of a crystal is determined by the external expression of a selected set of symmetry-related faces [4]. Although the unit cell symmetry governs the spatial relation between the faces, the final form of a crystal is determined by the relative rates of growth along different crystallographic directions. Faces perpendicular to the fast directions of growth have smaller surface areas, and slow-growing faces therefore dominate the morphology. Thus, the preferential adsorption of organic molecules to specific faces can specify a face-selective nucleation, change the crystal surface energies and the process of growth and finally modify the crystal habit [4,15,25].

From the comparative studies of the structure and formation of shells, it is possible to propose a four-step mechanism of biomineralization consisting of a precise spatiotemporal arrangement of sequentially deposited macromolecules (Fig. 4). The first step is the fabrication of an inert laminar substrate or framework, which compartmentalizes a microenvironment where mineralization will take place. This scaffolding consists of a nonmineralized, well-ordered hydrophobic organic material and usually is composed of β-chitin, type X collagen or other not well-characterized biopolymers. The second step is the fabrication of particular polyanionic macromolecules, which are deposited on the previously formed inert scaffolding and where nucleation of the calcium crystals takes place. These macromolecules are aspartate- or glutamate-rich proteins or keratan sulfate-rich proteoglycans. The third step is the fabrication of a gel structure consisting of silk fibroin-like proteins or proteoglycans and containing acidic proteins or dermatan sulfate. This gel not only controls polymorphism but also the diffusion-controlled growth, face-growing rates and habit of the crystal formed. The fourth step is the arrest of crystal formation and is related to the

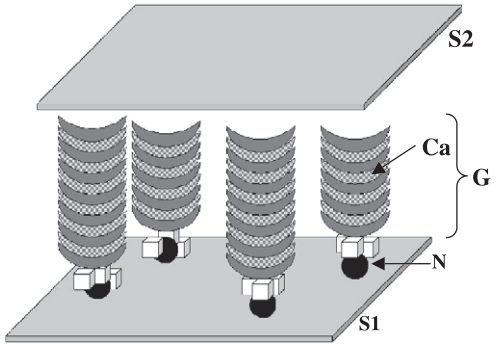

Fig. 4. A four-step model of shell mineralization. Crystal nucleation occurs on an organic sheet (S1) coated with polyanionic nucleation sites (N). Crystal growth (Ca) occurs within a polyanionic gel (G). Mineralization arrest is associated with the deposition of another organic sheet or specific macromolecules (S2).

fabrication of a new inert scaffolding or the deposition of particular hydrophobic inhibitory proteins (e.g., the eggshell cuticle).

5. Conclusions

Substantial progress has been made in using basic principles of biomineralization to accomplish controlled processing of engineering materials. Inorganic and organic substrates have been chemically modified to have charged surface groups, which successfully induce growth of specific ceramic films. Despite the successes, no processing system has yet been devised that approaches the exquisite molecular control evident in nature. Mimicking biological processes is not only a matter of fabricating films or soluble macromolecules with specific affinities or molecular recognition in the form of charge, stereochemical and structural matching but equally important is the spatiotemporal sequence, concentration and ionic strength, in which such molecules must be present during the assembly line process of crystal formation. The process of biological remodeling is still a principle that remains to be developed into a practical engineering process. Contrary to materials science, biomineralization has evolved over eons. As such, there is still much to learn from the assembly of biocomposite ceramics.

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