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# Unique morphology and gradient arrangement of nacre's platelets in green mussel shells

Jun Xu, Gangsheng Zhang *

*College of Materials Science and Engineering, Guangxi University, Nanning, Guangxi 530004, China*

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ABSTRACT

Nacre has long served as a classic model in biomineralization and the synthesis of biomimetic materials. However, the morphology and arrangement of its basic building blocks, the aragonite platelets, are still under hot debate. In this study, using a field emission scanning electron microscope (SEM), a high-resolution transmission electron microscope (HRTEM), and an X-ray diffractometer (XRD), we investigate the platelets at the edges and centers of green mussel shells. We find that 1) flat and curved platelets coexist in green mussel shells; 2) the immature platelets at the shell edge are aggregates of aragonite nanoparticles, whereas the immature ones at the shell center are single crystals; and 3) the morphology and thickness of the platelets exhibit a gradient arrangement. Based on these findings, we hypothesize that the gradient in the thickness and curvature of the platelets may probably result from the difference in growth rate between the edge and the center of the shell and from the gradient in compressive stress imposed by the closing of the shells by the adductor muscles or the withdrawal of the periostracum by the mantle. We expect that the presented results will shed new light on the formation mechanisms of natural composite materials.



## 1. Introduction

Nacre, a natural composite consisting of approximately 95 wt.% aragonite and a small amount of biopolymer matrix [1,2], forms the inner shell layer of certain bivalves (e.g., pearl oysters), gastropods (e.g., abalones), and cephalopods (e.g., nautiluses), which can protect the soft bodies of these creatures from predators. By virtue of its well-designed structure, it exhibits outstanding mechanical properties of strength and toughness [3–5]. Therefore, this composite has attracted considerable interest in materials science as a potential source of guidance for the design of novel man-made materials.

A platelet, the basic unit of which nacre is composed, is a flat tabular crystal of aragonite, which is often hexagonal or polygonal with a crystallographic c axis vertical to its tabular (top or bottom) surface (Fig. 1a). These platelets, through close contact between their lateral surfaces, form a series of lamellae parallel to the inner shell surface, which are separated from each other by a thin organic substance. In adjacent lamellae, the platelets may stack in one of two patterns, sheet nacre or columnar nacre [3,6,7], in which the platelets stack in cross-sectional structures that either are similar to that of a brick wall or form vertical columns, respectively. The former exhibits a stair-like pattern on the top surface of the nacre, whereas the latter exhibits a stack-up pattern (Fig. 1b and c).

Many questions regarding nacre remain the subject of considerable debate. For example, it is unknown whether the platelets are flat or curved, and it is also unknown whether the thickness of the platelets is constant. With regard to the first question, it seems to be accepted as common knowledge that the platelets are flat, a statement that is either confirmed or supported by the large majority of publications [8–10]. Recently, however, Barthelat and Espinosa reported that the platelet is wavy at the microscale, with a dovetailed angle of $<5^0$ [11] (see the inset in Fig. 1a). In a cross-sectional view, such a dovetailed platelet looks like a double concave lens; however, its three-dimensional (3D) shape has not been reported. Thus, the manner in which such platelets can fill a 3D space without forming overlaps or voids remains a challenging issue to be addressed. Zhang and Li have found that in green mussel shells, the platelets are curved with a domed shape at the shell edge, but the detailed 3D platelet shape and the reason for its curvature have not been reported [12]. Zhang and Xu have also reported that the immature platelets at the shell edge are curved and have presented the corresponding nanostructure, but they did not report the 3D shape of mature platelets nor their formation mechanism [13]. Additionally, Pokroy et al. have reported that bow-shaped platelets form as a result of the strain gradients imposed by the gradual changes in platelet thickness that occur in *Perna canaliculus*, but the corresponding 3D platelet shape has not been reported [14]. With regard to the second question, to our knowledge, only one paper has reported a change in the platelet thickness from 0.5 μm to 1.1 μm, but the detailed gradient arrangement and formation mechanism have not been reported [14].

* Corresponding author at: College of Materials Science and Engineering, Guangxi University, 100 Daxue Road, Nanning, Guangxi 530004, China.
*E-mail address:* zhanggs@gxu.edu.cn (G. Zhang).

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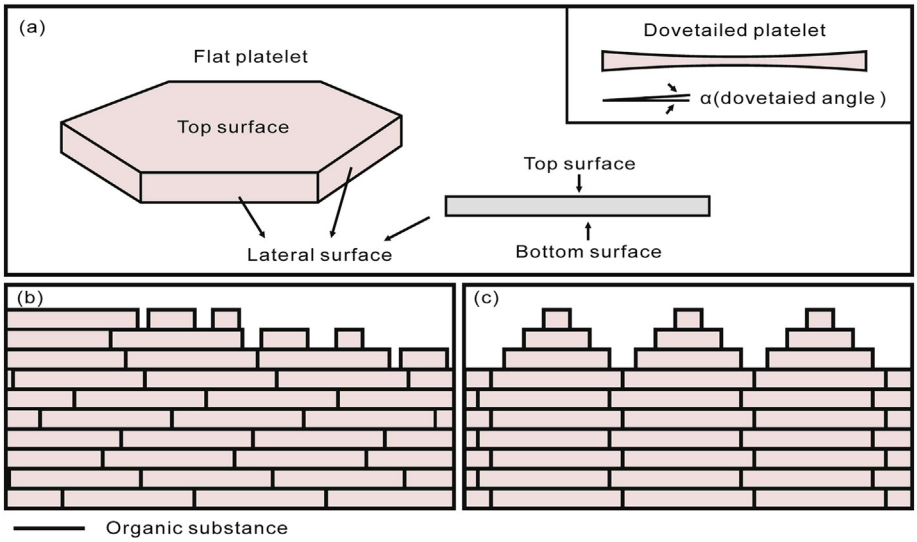


**Fig. 1.** Schematic illustrations of the morphology and arrangement of aragonite platelets. (a) Morphology of a flat platelet in a perspective view and a cross-sectional view. The inset shows the cross section of a dovetailed platelet, as proposed by Barthelat and Espinosa ($\alpha < 5^0$) [11]. (b) Sheet nacre. (c) Columnar nacre.

Most studies focus on mature (well-developed) platelets and neglect the immature ones. This study bias has contributed to the lack of clarity regarding platelet morphology and arrangement. In this study, we focus on the platelet morphology and arrangement in bivalve green mussel shells, particularly the 3D morphology and arrangement. We will demonstrate that flat and curved platelets coexist in green mussel shells. Interestingly, their morphology and thickness are highly dynamic, exhibiting a gradient arrangement.

## 2. Materials and methods

### 2.1. Materials

Fresh green mussel shells were collected on the coast of Beibu Gulf in Guangxi, southern China. The shells were classified into three groups based on their size: small (approximately 3.1 × 1.6 cm in size), moderate (Fig. 2, approximately 7.3 × 3.3 cm in size), and large (approximately 10.4 × 4.7 cm in size). We observed at least three shells in each category and found the results to be the same, which is reasonable given that the growth of the shells is genetically controlled.

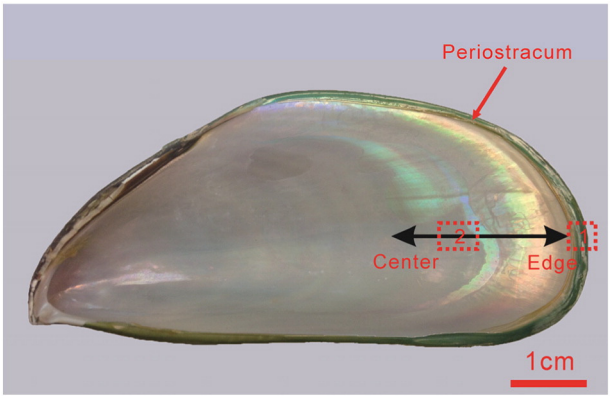


**Fig. 2.** Inner view of one shell valve of a green mussel shell. Region 1 corresponds to the shell edge, which is covered with a green and transparent periostracum, whereas region 2 corresponds to the shell center, which is not covered with periostracum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 2.2. Sample preparation

The shells were cleaned with distilled water after the soft bodies of the organisms were removed, and they were then air dried at room temperature for 2 h. Shell fragments were mechanically broken away from the edge and center of the shell (regions marked in Fig. 2) and were then trimmed into small blocks with dimensions of approximately 4 mm × 3 mm for the subsequent experiments.

Some small blocks were subjected to an NaClO solution treatment, in which they were soaked in 6 vol.% NaClO solution for 10 min and then washed with distilled water.

Some small blocks were subjected to ultrasonic treatment, in which they were first placed in a 50 ml beaker filled with distilled water, which was then transferred into an ultrasonic cleaning device (KUDOS sk2200). The blocks were ultrasonically treated for 5 min at 100 W of ultrasonic power. Finally, the blocks were washed with distilled water.

### 2.3. Analysis and testing

#### 2.3.1. Scanning electron microscope (SEM)

For SEM analysis, the as-prepared blocks were coated with a 10 nm gold film and observed using a Zeiss Ultra FESEM and a Hitachi-SU8020 FESEM at an acceleration voltage of 8–10 kV and a working distance of 11.5 mm.

#### 2.3.2. Transmission electron microscope (TEM)

For TEM analysis, thin slices of nacre (approximately 70 nm thick) were cut in a direction parallel to the inner shell surface using a diamond knife on a microtome (Reichert-Jung Ultracut E) and were then placed onto a carbon-coated Cu grid. TEM images and selected area electron diffraction (SAED) patterns were then obtained using an electron microscope (JEOL JEM-2010) at an acceleration voltage of 200 kV.

#### 2.3.3. X-ray diffraction (XRD)

For XRD analysis, powder samples were scraped from the inner surfaces of the shells. Their diffraction patterns were recorded using a diffractometer (Rigaku D/Max-2500) in a θ-2θ configuration with Cu Kα radiation (λ = 0.15406 nm). The operating voltage and current were 40 kV and 200 mA, respectively, and the scan speed was 2°/min. At least three specimens from each region indicated in Fig. 2 were prepared for analysis, and the results were found to be identical.