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Structure and Roles of the Various Layers in the Shells of Conch *Conus litteratus*

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Abstract

Mollusc shells are renowned for their mechanical strength and toughness. To better understand the mineralization process of the shell, structure of the body whorl and base of *Conus litteratus* (Conus shell) were in detail investigated by using scanning electron microscopy. Three-point bending tests were taken to demonstrate that each layer of crossed-lamellar structures is indispensable to enhance the whole strength of the shells. The results show that the conch shell is composed of hierarchical structure from nano scale to macro scale, and the basic constituent is long rod-shaped aragonite. Different positions of the shell have varied structures, and the base is more complicated than the body whorl. The mechanical properties of Conus are highly anisotropic and the arrangement of middle layer has a great influence on the bending strength. The outer and inner layers are very thin but play a protective role for the middle layer.

Keywords: crossed-lamellar, mechanical properties, aragonite, mollusc shell

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Nomenclature

TP	Transverse sample, loading on the P plane
LP	Longitudinal sample, loading on the P plane
TP-M	Middle layer of TP
LP-M	Middle layer of LP
TP-OM	Transverse sample that machined off the inner layer
TP-MI	Transverse sample that machined off the outer layer

1 Introduction

The shell material has attracted increasing attention of many scientists due to its high-toughness and intermediate-strength, which can surpass man-made ceramics[1–6]. Shells are ceramic/polymer laminated composites consisting of aligned, anisometric, calcium ($CaCO_3$) grains separated by extremely thin protein matrix[7,8]. Structure is essentially crucial to the mechanical properties when the components are consistent. The design principles found in these natural materials inspires scientists to synthesize composite materials owing optimized properties.

The novel structure and mechanical property of the nacre have become a new research trend during the past few decades[9–12]. Nacre is composed of a brick-and-mortar like structure and is considered to be stronger than the other structures in the shells[13]. However, another typical structure—crossed-lamellar is less strong but more widely exists in nature[14,15]. Crossed-lamellar has the property of fast growth and low content protein (less than 1%), and its repair processes are quicker than nacre when the shell is damaged[16]. Crossed-lamellar also has excellent mechanical properties that even surpass those of nacre in some respects. The researches of conch shell have provided clear evidence that the crossed-lamellar structure is the hardest[17] and its rupture work can reach 10 times as high as that of nacre[1,3]. Conus shells generally are made of three crossed layers on the macro[18]. Current researches of Conus mainly focus on the partial structure and mechanical properties, trying to explain why these mollusc shells have high fracture work. The main mechanisms of high toughness have been summarized: extensive crack deflection, fiber pull out and mineral bridges[15,19,20].

As is known to all, the great mass of the shell pro-

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vides stability against upheaval by tidal forces or predatory attackers, as well as protection from crab attacks that may involve forces as great as 800 N[7]. But scars are found in the body whorl in a number of conus shells (Fig. 1). To better understand the design of mollusc shells, the holistic structure of conus shells and mechanical properties that over different directions have been conducted in this paper. It proposes the possibility that the source of the scars in the shells.

2 Materials and methods

Conus litteratus was studied herein and the appearance is shown in Fig. 1. The sampling section is the body whorl of the conch shell, where symbols L, T and P represent longitudinal section, transverse section and stratification plane, respectively.

2.1 Scanning Electron Microscopy(SEM)

The intact longitudinal section of the body whorl and cross section of the base were prepared. The samples were polished by abrasive papers and etched by 1% HCl for 15 s. Subsequently, the structures of *Conus litteratus* on different positions were observed by SEM (S4800) after spray silver treatment. Fracture morphologies of some areas were also taken by SEM.

2.2 Three-point bending tests

These tests were desired to declare the role of each layer on promoting the whole strength of the shell. To minimize varying test results caused by the influence of age and environment, all longitudinal and transverse samples on each contrast experiments were cut out of one shell. Hacksaw with an abrasive blade were used to obtain rectangles with approximate sizes firstly. Successively, these precut specimens were divided into three groups and machined by raw abrasive papers to get the desired size for the following experiments. The length of 8 mm and different sizes of cross sectional areas were kept, considering the rectangular samples of transverse were too hard to take. The span was 6 mm for all the tests and the samples herein were tested by a head speed of 0.1 mm·min^{-1}. The results can only give comparison within group because of the samples are not standard.

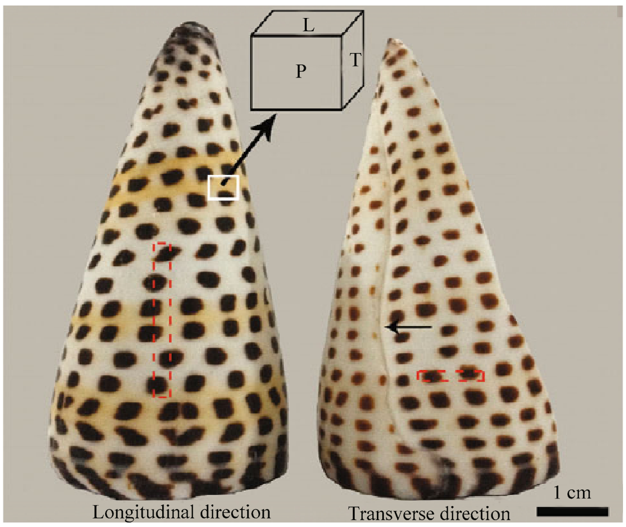

Fig. 1 The appearance of *Conus litteratus*, showing the cutting directions and positions of the test pieces; Scar is shown in the right one. L: longitudinal section, T: transverse section, P: stratification plane.

Integrated samples, which have the full thickness of the shell with the sectional areas about 2 mm × 2.5 mm, were tested on different planes in the first group. The loading was on P plane for the transverse and longitudinal samples (TP, LP). Fig. 5a gives an overview of the different test configurations. In the second group, all samples were machined off the inner layer and outer layer to remain the middle layer with cross sectional areas about 1 mm × 1 mm. The loading was applied on P plane for the transverse specimens (TP-M) and longitudinal specimens (LP-M). In the third group, the transverse samples were tested on P plane (TP). A part of them were removed the inner layer (TP-OM) or outer layer (TP-MI).

3 Results and discussion

3.1 Structure of the body whorl

3.1.1 Macrostructure

A mature conch *Conus litteratus* has a cone-like shell of proximate 60 mm – 70 mm high and of 30 mm – 40 mm diameter base, as shown in Fig. 1. The longitudinal section of the shell (Fig. 2a) indicates that the shell consists of several whorls growing from inside to outside. The most outside whorl is quite uniform in thickness and is also the thickest, and the middle position is about 2.0 mm – 3.0 mm. Due to this reason, we used the shell of this whorl as sample to measure the mechanical properties of the shell.

The uniform area of the shell is constituted of three layers along the thickness direction: outer layer (O), middle layer (M) and inner layer (I) (Fig. 2d). The middle layer is the thickest, about 1.5 mm, while the inner

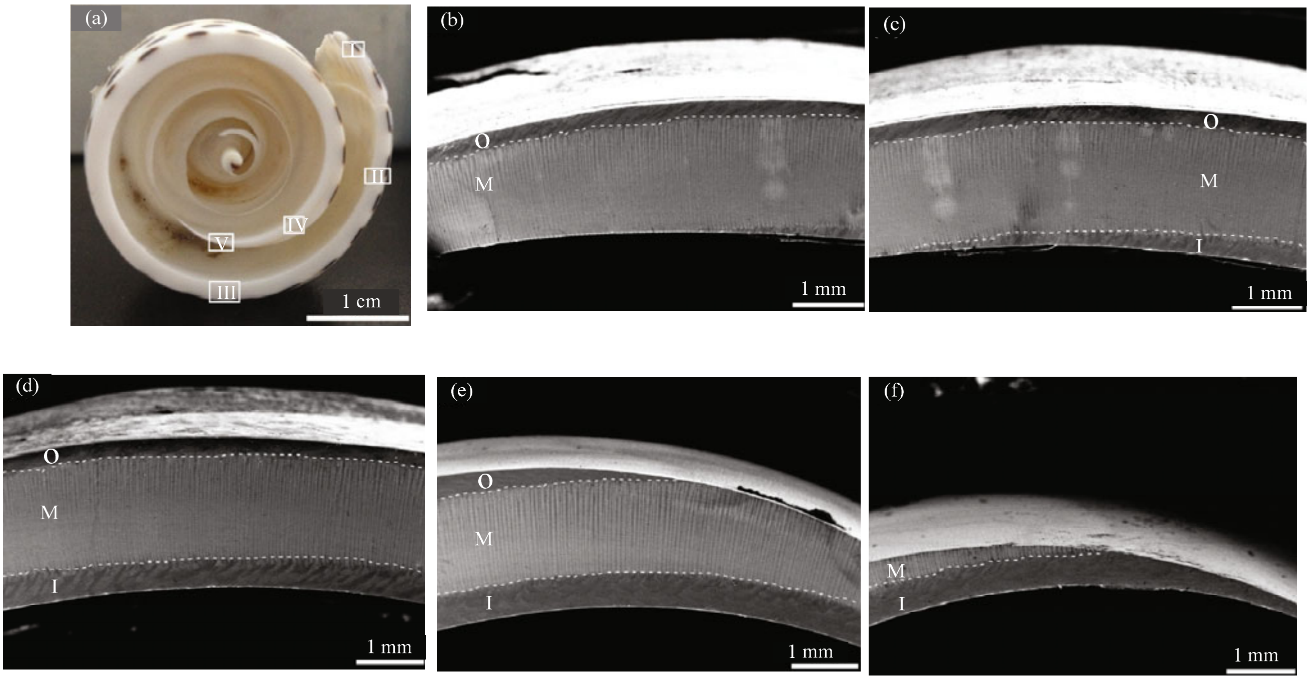

Fig. 2 (a) Longitudinal section of *Conus litteratus*; (b) area I, shows the lip consists of two layers; (c) area II, inner layer starts to appear; (d) area III, shows the uniform area consists of three layers; (e) area IV, the thickness of outer layer starts to decrease and disappear; (f) area V, middle layer vanishes, leaving the inner layer with a few dozen micros thick only. The sample was etched by 1% HCl for 15 s.

layer and the outer layer are about 0.5 mm and 0.3 mm, respectively. These results are consistent with the observations of Rodriguez-Navarro[2].

From the whole longitudinal section of the shell, it is interesting to notice that the lip of the conus shell is not identical in thickness and constituted layers to the rest of the last whorl of the shell. At the lip part (Fig. 2c), the inner layer grows thinner and finally disappears. In the final part of the lip where the inner layer is missing, the middle layer grows thinner as it is approaching to the tip of the lip (Fig. 2b). As we know, the lip is the growth front of the cone shell. The characteristics of the lip structure may reflect the growth pattern of the shell, which will be shown in the next section. This structure makes the lip to be the most vulnerable part of the shell. Meanwhile, the thickness of the outer layer decreases from outside to the inside in the second whorl (Fig. 2e). The outer layer and inner layer vanish gradually, until leaving the inner layer only (Figs. 2e and 2f). The most inside two whorls are only consisted of inner layer, which are a few dozen microns thick.

3.1.2 Microstructure

The fracture morphologies of area III in Fig. 2a were further studied and the results are shown in Fig. 3. Obviously, these three macro layers are structurally identical except the adjacent layer containing the first order lamellae is rotated by 90˚ (Fig. 3a). The micro layer consists of parallel lamellae (first order lamellae, Fig. 3b), which are in turn packed into second order lamellae (Figs. 3b and 3c). The long rod-shaped aragonite (third order lamellae, Fig. 3d) is the original constitution of the shell, and it in turn packs into second order lamellae. These lath-like aragonite crystals are parallel to each other within the first order lamellae form a high angle (about 105˚) with those in adjacent first order lamellae. The long rod-shaped aragonite with a rectangular cross-section, are about 80 nm thick and 150 nm – 250 nm wide. These microstructure characteristics are almost identical to those observed in Conch shells (*Busycon carica*) reported by Li *et al.*[21]. They pointed out that this long aragonite consists of nanoparticles, which offers a great convenience for the third order lamellar to grow along different directions[21]. The first order lamellae have large range thickness of 10 μm – 25 μm, some of them even thin and disappear (Fig. 3b, the right dashed box). The first order lamellae of the outer layer and inner layer are longitudinally arranged, while those of the middle layer are arranged parallel to horizontal direction.

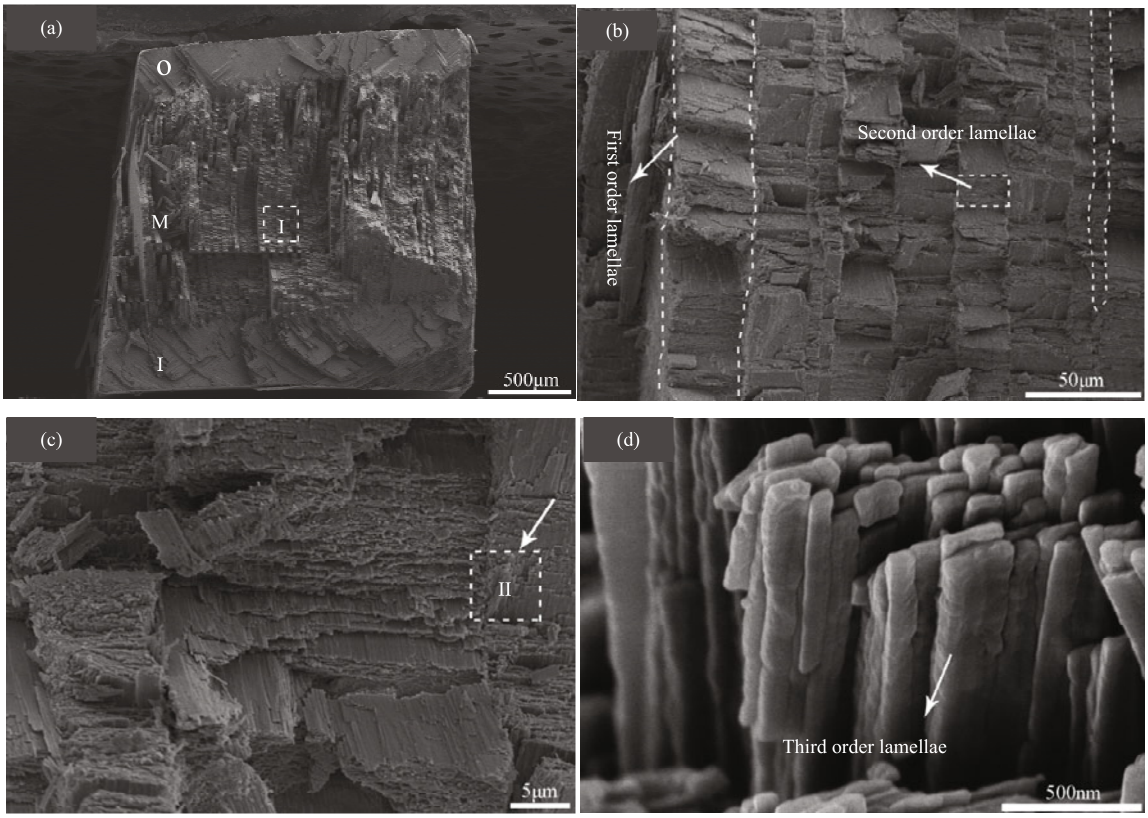

Fig. 3 Fracture morphologies of area III on the last whorl. (a) Longitudinal section of the shell; (b) details of the middle layer, magnification of the selected area I, showing the first order lamellae and second order lamellae; (c) detail of the second order lamellae in alternating first order lamellae; (d) detail of selected area II, showing the third order lamellae.

3.2 Structure of the base

The hardest and thickest part of the shell is the base. To reveal the intact structure of the shell, the structure of whole base section were observed. The component of the base is consistent with the body whorl, but its structure is more complex. The base consists of repetitive unit that can be divided into six parts from the right to the left (Figs. 4b and 4c). The area I in Fig. 4b is a zone that fills the gap between two adjacent whorls and the area VI is a transition area at the top of the base, where contacts the soft tissue directly. The area V is a transition zone for I, II and VI. The II–IV areas are the main part of the base, and they have different orientations. The II–IV areas have different growth patterns at the vertical direction: the area III and IV are restrained when they stretch upward, and area II only reserved, which is the inner layer that we observed at the longitudinal section (Fig. 4c).

3.3 Three-point bending strength of conch *Conus litteratus*

Fig. 5b shows the bending strength of integrated rectangular samples. The transverse samples (TP) behaved very bad, whose values are about 70 MPa. The bending strength of longitudinal test pieces performance is far better than the transverse ones.

The middle layer is the thickest part of the samples, and its arrangement has a significant impact on the bending strength. In order to better understand the bending results that loading on different direction, the middle layer was taken out to have individual tests and the results are displayed in Fig. 5c. The test pieces of TP-M have the lowest strength of about 10 MPa, some samples even fractured directly before tests without values, leaving smooth fracture plane. When the loading was applied on TP-M, the pieces were broke off along the bonding surface of the two adjacent first order lamellae (Fig. 6a). LP-M test pieces have better performance that can exceed 200 MPa. The first order lamellae of LP-M were fractured at the same time (Fig. 6b), with the phenomenon of fiber pull out.

The effect of each layer on the bending strength was further studied, and the results are displayed in Fig. 5d. The experiment results indicate that the bending

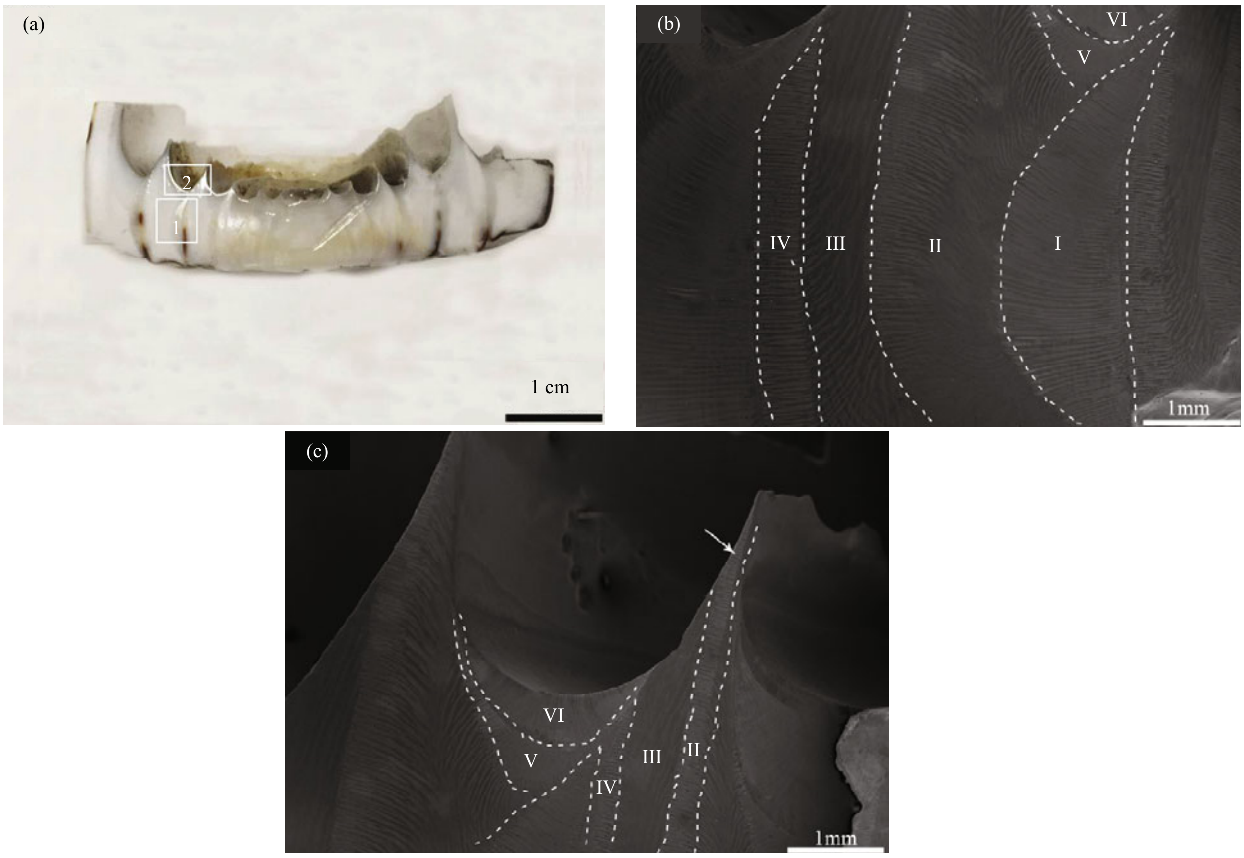

Fig. 4 (a) The cross section appearance of the base; (b) the morphology of area 1, showing the repetitive unit of the base consists of six parts; (c) the morphology of area 2. The sample was etched by 1% HCl for 15 s.

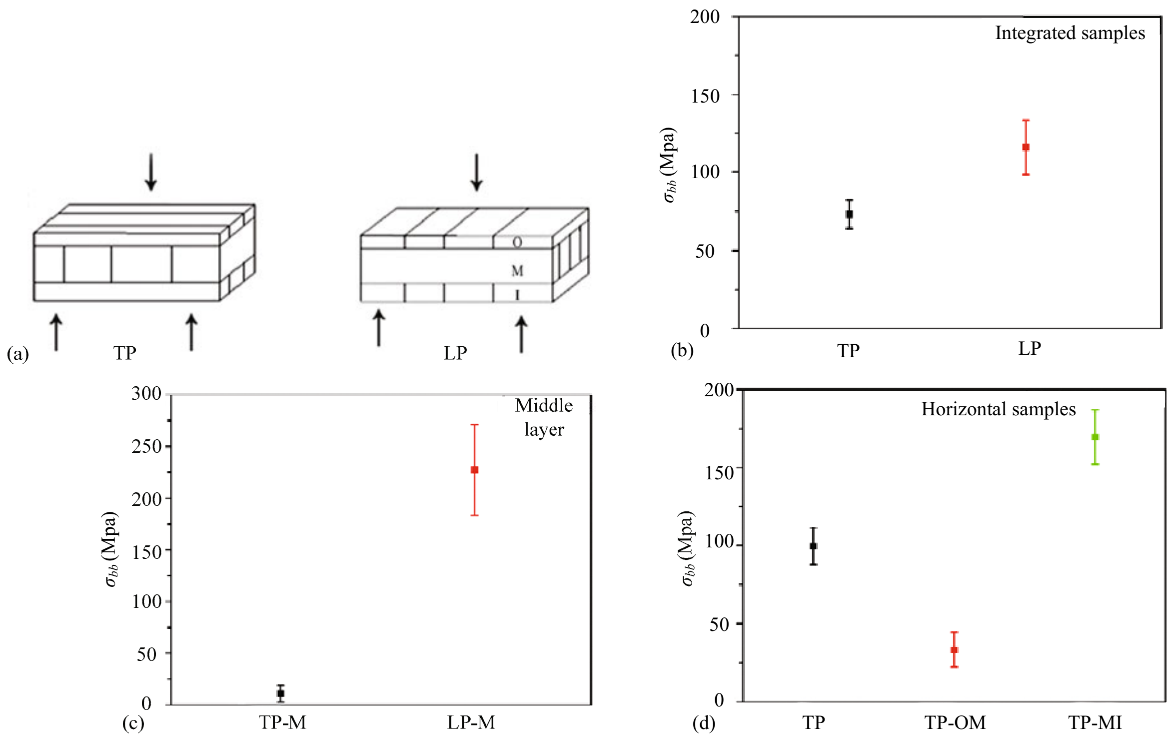

Fig. 5 (a) The schematic diagrams of three-point bending tests. The results: (b) integrated test pieces; (c) middle layer; (d) transverse samples that some of them were peeled off inner layer or inner layer.

strength of the samples without inner layer (TP-OM) is close to that of TP-M. The strength of integrated samples (TP) is approximate two times as high as TP-OM. Without inner layer, the cracks extend along the bonding surface of first order lamellae directly when the loading was applied on P plane. But without outer layer, the test pieces show greater bending strength that even exceeds the integrated samples. The effect of outer layer is not so obvious, but the inner layer plays an extremely important role in the horizontal direction.

Fig. 7a gives a partial stereogram of *Conus litteratus*, which shows a very simple situation that shells may encounter. The out surface of red area I, bearing the maximum tensile strength, is the most dangerous. With the protection of outer layer, whose conditions are comparative to LP-M, the shells can endure bigger external force before failure, or the cracks can easily form and expand through the weak bonding surface under the action of tensile strength.

Previous studies have shown that the biopolymer render Conch shells joint increase in strength, ductility and fracture energy, on the contrary, without organic matrix, the strength of shell will decrease sharply[21,22].

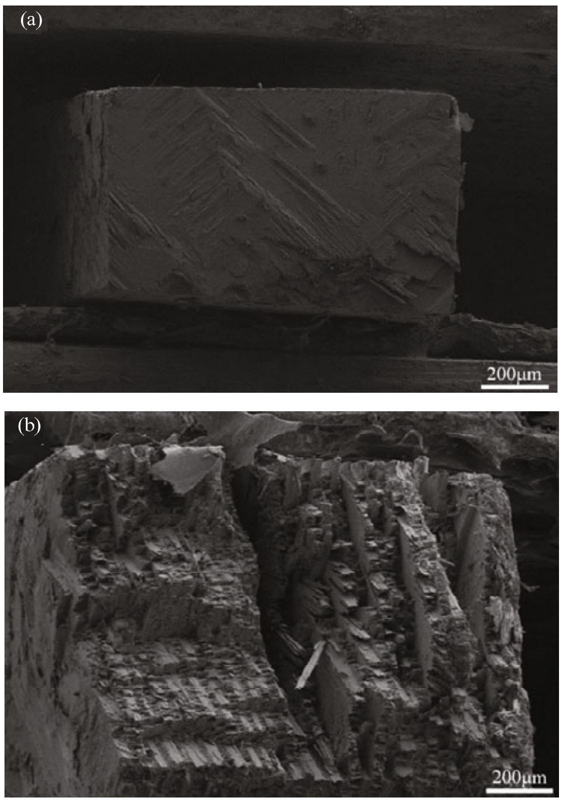

Fig. 6 Fracture morphologies of the middle layer. (a) TP-M; (b) LP-M.

This conclusion is also appropriate for nacre. Recently, large amount of researches have proved that the third-order lamellar, which consists of aragonite nanoparticles, exhibits plastic deformation and its maximum strain can arrive to 0.7% upon mechanical loading[22,23]. Actually, there are various kinds of shapes for mollusk, and we found that shells of cone-shape generally are made up of crossed-lamellar, while those of bivalve usually are composed of nacre or hybrid structure. These structure designs have stood the test of the nature and seashells possess enough ability to resist external force, such as tidal forces and predatory attackers, and this ability should due to the ordered arrangement of different scales[24].

Under normal circumstances, the ability of withstanding external force is directly proportional to the thickness of shells. For Conus shell, they are better at withstanding the loading in the transverse direction than in the longitudinal direction. The type of loading exerted by crabs is equivalent to bending in the transverse direction and the inner layer is adaptive[18]. Most species of Conch shells are very thick, and the thickness is generally uniform throughout the last whole. However, the phenomena of scars that exist in the shells are very common, and these may have a close relationship with the overall structure of Conch shells. By the experiments of three-point bending tests, it can be proposed that the relatively thin outer lip can be broken off when the shells get in trouble of crabs, especially where missing inner layer. The Conus later repairs its shell, but a record of the attack is left as a scar (Fig. 1). According to these experiments, we can easily arrive at the verdict: the outer

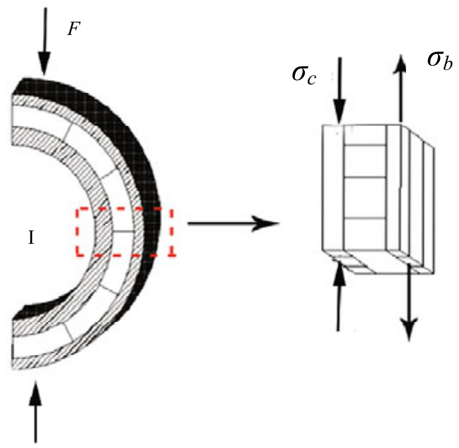

Fig. 7 The stereogram of *Conus litteratus* that squeezed by extrusion force and stress analysis of the selected area I.

layer and inner layer play a protective role for the middle layer and enhance the overall strength of the shell. Since we already know the structure of lip is different from the last whorl, we can confirm the three crossed layers of *Conus litteratus* on the macro do not grow at the same time. We may research the sedimentary sequence of each layer in the future work.

4 Conclusion

Seashells are highly organized nanocomposites designed to be extraordinarily tough while remaining hard and strong. The structure of *Conus litteratus* differs in different positions. The Conus shells are composed of many layers of aragonite with the crossed-lamellar structure. The body part consists of several whorls. The lip part has two macro layers and the last whorl with full thickness has three macro layers, while the most inner part has only one macro layer with about a few dozen microns thick. The structure of the base consists of repetitive unit that can be divided into six parts. But the basic component of the shell is long rod-shaped aragonite with about 80 nm thick and 150 nm – 250 nm wide. The mechanical properties of the shells are highly anisotropic, and the strength of the shell is relative to the arrangement of each layer. Conch shells are better at withstanding the loading in the transverse direction than in the longitudinal direction. Without inner layer, the strength of the transverse samples declined sharply. The lip part, where the inner layer is missing, is the most vulnerable part of the shell. The scar on the shells mostly is a record of attack. The out layer and inner layer are relative thin but their roles can't be ignored.

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