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Enhanced toughening of the crossed lamellar structure revealed by nanoindentation

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ABSTRACT

Gastropods shells have evolved to resist the threat of increasingly stronger predators that smash, peal, and crush their shells. Their shells are most commonly constructed from a crossed lamellar microstructure, which consists of an exquisitely architected arrangement of aragonitic mineral and organic encompassing at least four orders of hierarchy. It is this careful control of mineral and organic placement within the entire crossed lamellar structure that yields a four-order of magnitude increase in fracture toughness versus abiotic aragonite. We investigated the effect of differing microstructural orientations on their influence of inter-3rd order lamellar fracture behavior using nanoindentation from the inner layer of the *Strombus gigas* shell. We observed a significant influence of lamella (plank) orientation and nanoindenter probe on the mechanical properties. The ± 45° arrangement of mineral planks found within biological crossed lamellar composites provides a significant enhancement of isotropic resistance to penetration by sharp objects such as jaws and claws. In addition, the ± 45° arrangement is able to resist higher loads before failure. This combination of features from the crossed lamellar architecture helped enable species with this shell structure to survive predation for hundreds of millions of years and will also help provide insights into designs of future generations of composites.

1. Introduction

Over the course of 400 million years of evolution, gastropods have evolved tough and hard shells in order to provide protection from durophagous (shell breaking) predators (Carter, 1989). From this evolution, two common architected microstructures have arisen: the evolutionary older nacreous structure (Signor III and Brett, 1984) and the crossed lamellar design, the latter of which emerged as the dominant gastropod shell microstructure after the Mesozoic marine revolution (Salinas and Kisailus, 2013). An extant example of the crossed lamellar structure is found in the shell of the *Strombus gigas*, a large (> 30 cm) Atlantic gastropod, which possesses a thick and heavily mineralized shell to defend against pealing (Giant Hermit Crab, *Petrochirus diogenes*) and rolling/flipping predators (Horse Conch, *Triplofusus giganteus*) (Fig. 1A) (Linnaeus, 1758). Both nacre and crossed lamellar based shells primarily consist of aragonitic calcium carbonate and a small fraction of organic (polysaccharide and proteinaceous) material (~1–5% (Addadi et al., 2006)). These organics afforded a pathway to control the growth and arrangement of the $CaCO_3$ mineral components (Levi-Kalisman et al., 2001; Belcher et al., 1996; Falini et al., 1996; Weiner and Traub, 1984) and thus enabled gastropods to evolve numerous shell microstructures with increasing toughness versus its geologic counterpart (Currey, 1976). Aragonitic gastropod shells containing the crossed lamellar microstructure are 3–4 orders of magnitude tougher than abiotic aragonite (Currey, 1976; Currey and Taylor, 1974).

Previous investigations of nacre have revealed numerous microstructural toughening mechanism (Espinosa et al., 2011) (e.g., nano-asperities (Song et al., 2003), interlocking tablets (Barthelat, 2007)). Clearly, the interfaces between stiff mineral and compliant organic matrix in lamellar systems affords additional toughening over the bulk mineral. In addition, it has been found that the hierarchical nature of the crossed lamellar structure at the nanoscale provides significantly more toughening over the nacre structure (Li and Ortiz, 2014). This was ascribed to crack bridging, and crack deflection (Kamat et al., 2004; Kamat et al., 2000; KuhnSpearing et al., 1996). Additional work has investigated the quasi-static and dynamic response of crossed-lamellar shells (Menig et al., 2001) and comparisons made between crossed lamellar and nacre structures (Min Lin et al., 2006). Previous

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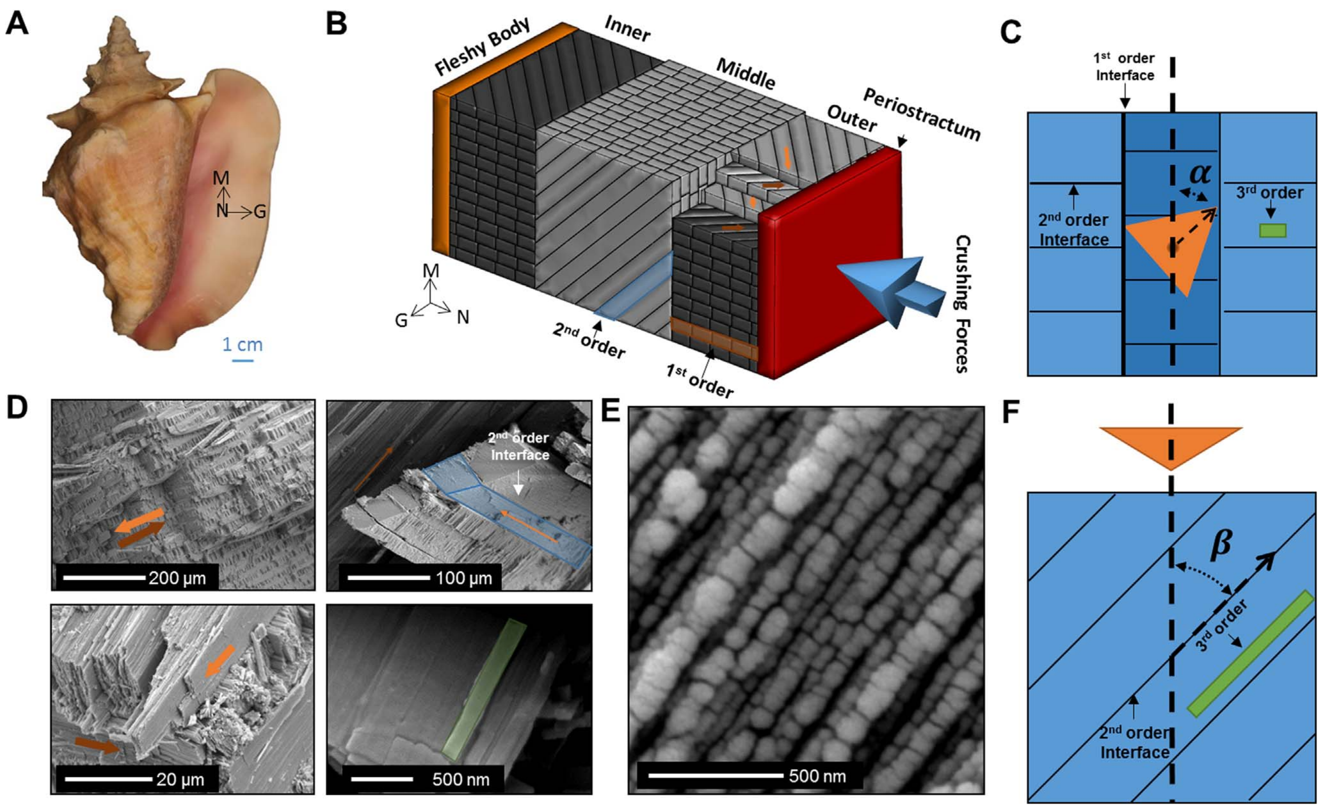

Fig. 1. (A) Image of *Strombus gigas* (with shells ranging up to 36 cm in length). (B) Schematic of cross-section of shell consisting of three different crossed lamellar layers (Inner, Middle, Outer) with the dip directions (2nd order lamellae) aligned ± 45° from **N** (the radial direction). In the Inner and Outer layers, the 1st order lamellae are aligned normal to **M** (the marginal direction), in the Middle layers they are aligned normal to **G** (the shell growth direction). (C) α, s the angle between the 1st order interface and a vertex of the cube corner indenter probe, ranging from 0°or 120° in our experiments. (D) SEM micrographs of the different hierarchical features of the crossed lamellar system: 0th (Top left), 1st (bottom left), 2nd (Top Right), 3rd (Bottom Right). (E) SEM micrograph from polished and lightly etched (NaOCl) 3rd order lamellae, highlighting 4th order features (F) β, the angle of inclination of the 2nd order lamellae with respect to the indentation axis.

nanoindentation studies (Romana et al., 2013; Yang et al., 2011) have shown that the hardness variations found in the different layers of the crossed lamellar structure are related to the crystallographic orientation of the aragonite mineral within each layer.

However, very little is known about the effects of this structure at the mesoscale. Here, we probe the complex microstructure of the crossed lamellar design found in the gastropod *Strombus gigas* using nanoindentation in order to understand how this dominant architecture enabled survival against these jaw and claw-bearing predators. The results from these studies will also help provide insights into designs of next generation composites.

2. Ultrastructure of *Strombus gigas* shell

The shell of *Strombus gigas*, a large gastropod native to the tropical western Atlantic Ocean, is composed of three distinct crossed lamellar macro-layers: inner, middle, and outer, each of which are composed of four different hierarchal orders of features spanning from nanometers to millimeters in scale (Fig. 1B).

Each of the three shell macro-layers is composed of numerous parallel 1st order lamellae. The 1st order lamellae are sheets composed of unidirectional layers of 2nd order lamellae, alternating in direction between two perpendicular orientations, typically ± 45°. The 2nd order lamellae are rectangular beams that are 10–20 µm thick (t_{2nd}), 20–50 µm wide (w_{2nd}), and up to several hundred micrometers in length (l_{2nd}). Each 2nd order lamella is composed of tens of thousands of 3rd order lamellae. The 3rd order lamellae have a rectangular plank-like structure that are approximately 150 nm wide (w_{3rd}) by 100 nm thick (t_{3rd}) and several micrometers in length and are surrounded by a thin organic sheath (Fig. 1D). The 3rd order planks are formed from numerous assembled 4th order particles. (Fig. 1E).

The remarkable toughness is the result of a variety of structural features, which work to deflect and arrest fractures. During loading by 3-pt bending of the shell, multiple cracks are initiated within the inner layer due to a difference in toughness between the "weak" inner and "tough" middle layers (Kamat et al., 2004). Each macro-layer has a "weak" direction, which when loaded in tension, results in 1st order lamellae delamination. The direction of 1st order lamellae is nearly perpendicular between adjacent macro-layers, causing a fracture that propagates along the weak direction and thus deflected at the interfaces of adjacent 1st order lamellae. Within a 1st order lamella, there are two principle directions along which cracks can propagate: along the weak interfaces between 2nd order lamellae or through 2nd lamellae. Fractures along the weak direction of 1st order lamellae are bridged by the perpendicular 2nd order lamellae in the adjacent 1st order lamellae. The interfaces between 1st order lamellae are rough and interdigitated, which increases their adhesion strength and dissipates forces between neighboring 1st order lamellae (Kamat et al., 2004, 2000; KuhnSpearing et al., 1996).

As previously mentioned, a hardness variation was observed in the different layers of the crossed lamellar structure. In addition to the effects of crystallographic orientation, the hierarchical nature of the crossed lamellar structure significantly influences its mechanical properties. There is an increase in the rate of crack nucleation within the 3rd order lamellae compared to abiotic aragonite, due to the hierarchical internal 3rd order nanostructure (Romana et al., 2013). In this study, we use a combination of nanoindentation and finite element analysis to examine the fracture mechanisms and resistance to penetration of the inner crossed lamellar layers of *Strombus gigas* for four different alignments of 3rd order lamellae (β) (Fig. 1D) along with 17