STRUCTURE-MECHANICAL PROPERTY RELATIONSHIPS IN A BIOLOGICAL CERAMIC-POLYMER COMPOSITE: NACRE

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ABSTRACT

The structure-mechanical property relationships were studied in nacre, a laminated ceramic-polymer biocomposite found in seashell. Four-point bending strength and three-point bend fracture toughness tests were performed, and the results averaged 180 ± 30 MPa and 9 ± 3 MPa·m$^{1/2}$*, respectively, indicating that the composite is many orders of magnitude stronger and tougher than monolithic* $CaCO_3$*, which is the primary component of nacre. Fractographic studies conducted with a scanning electron microscope identified two significant toughening mechanisms in the well-known "brick and mortar" microstructure of nacre: (i) sliding of the aragonite platelets and (ii) ligament formation in the organic matrix. These toughening mechanisms allow for high energy absorption and damage tolerance and thereby prevent catastrophic failure of the composite. The structure of the organic matrix and the interfacial structure between the organic and inorganic components were studied with transmission electron microscopy by using both ion milled and ultramicrotomed sections with and without the intact aragonite platelets. We found that the organic matrix is indeed a multilayered composite at the nanometer scale but is thinner (about 100 Å) than reported in the literature. The morphology of the interfacial region between the organic and the inorganic layers suggests the presence of a structural "transitory" region that interlocks the two dissimilar phases.*

1. INTRODUCTION

In designing and processing materials for technological applications, valuable lessons can be learned from biological soft and hard tissues.[1-3] Biologically formed materials almost exclusively have composite structures[4] and the individual phases of these structures are often arranged in complex and highly ordered units to form hierarchically organized architectures.[2,4,5] The design of biological structures developed over time in order to meet specific materials requirements for the organism. Since biological systems have a limited supply of raw materials at their disposal, they must use the available raw materials in the most efficient manner possible to achieve the structures and the properties that are required for survival in their habitat.[6] The formation of biological materials systems, and the relationships between the structures and their resulting properties, can provide important information for the development of synthetic materials.[7]

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We chose to study the property-structure relationship in the nacre of the red abalone, *Halitois rufescens*, because it has been shown to display a higher strength and toughness than other mollusk shell structures.[8-11] Nacre in mollusk shells is a unique biocomposite similar in some respects to synthetic laminated composites.[10] Mollusk shells are composed of various crystallographic and morphological forms of $CaCO_3$ in conjunction with a variety of macromolecules and can be laid up to form many different architectures.[2,4,6,12] Nacre is just one part of the whole structure and usually is the inside portion of the shell. Nacre is found in the shells of various molluskan species including bivalves, gastropods, and cephalopods and is often referred to as mother-of-pearl,[13] which has become fashionable in jewelry because of its opalescence and brilliant coloring. As is now well known, the nacreous structure consists of thin $CaCO_3$ crystals and an organic matrix arranged in a "brick and mortar" structure[13] with the organic matrix serving as the mortar. The $CaCO_3$ platelets take the form of crystalline aragonite and make up 95 to 98% of the composite.[8-10] The aragonite platelets are approximately 5 to 10 μm in diameter and 0.25 to 0.5 μm thick. An example of the nacre structure as viewed in the edge-on orientation, i.e., in a direction perpendicular to the *c* axis of the aragonite platelets, is shown in Figure 1, which was taken from a *Pinctada margaritifera* shell (the so-called pearl oyster).[1] The figure displays relatively thicker aragonite platelets, about 0.5 μm, compared to those of the *Haliotis rufescens*. Although the red abalone platelets are thinner, they have a similar architectural design but a different mode of growth.[2,10]

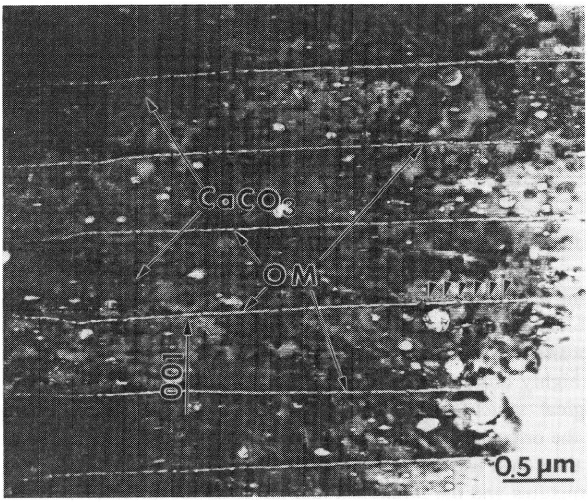

Figure 1. Bright field (BF) TEM image of the nacre section of pearl oyster viewed in the edge-on configuration. The crystallographic c axis, i.e., [001] of the aragonite crystals is shown. The constituent phases, i.e., $CaCO_3$ and organic matrix are indicated by $CaCO_3$ and OM, in the image. The sample was low-temperature ion-milled and the image was recorded with a 300 kV TEM.

The aragonite phase in the nacreous section of the shell has a hierarchical structure, as discussed in the accompanying paper.[14] The organic matrix that surrounds the aragonite crystals also has a laminated hierarchical structure composed of macromolecules, (most likely chitin or other structural polysaccharides), proteins, and acidic macromolecules.[15-17] Furthermore, some shells containing nacre also possess a third level of lamination: in red abalone the aragonite-organic laminates are periodically separated by a relatively thick layer of organic material (Figure 2) called pallial myostracum,[18] which is structurally related to the other organic layers.[1,2,10] This layer is not present in the nacre of bivalves, such as in pearl oyster,[1] and thus may be the possible source of scatter in mechanical properties of red abalone. In the following sections, we summarize the mechanical properties and fracture behavior of nacre. This will be followed by an analysis of the organic matrix and a discussion of its layered structure. Finally, the structure of the interface between the organic and inorganic layers will be analyzed in the context of its possible effect on the mechanical properties of nacre and thus of red abalone.

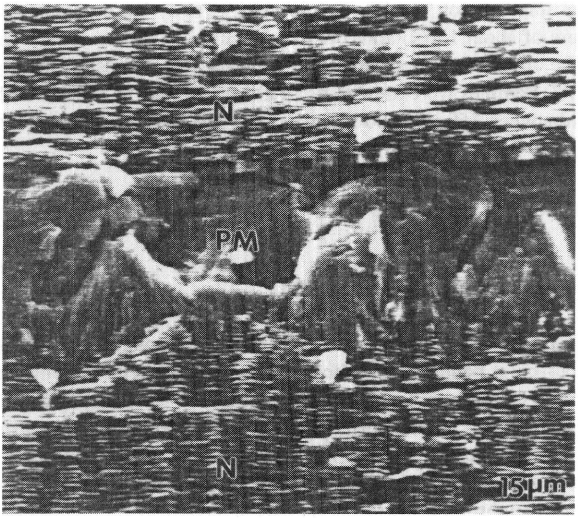

Figure 2. Secondary electron image of the pallial myostracum layer in the nacre of red abalone.

2. EXPERIMENTAL PROCEDURE

2.1 Mechanical Testing

Mechanical tests were performed on *Haliotis rufescens* specimens in which the original nacre portion of the shell was approximately 1 cm thick. Specimens were stored and tested at ambient temperature and humidity.[19] Samples of abalone that were sufficiently thick and large were prepared for notched three-point and four-point bend tests according to the specifications of ASTM standards[20] for fracture toughness and fracture strength, respectively. The specimens were cut with a cooled diamond blade into parallel-sided rectangular blocks and polished prior to testing. Flexural strength and fracture toughness tests were performed using an Instron Universal Testing Instrument. All specimens were mounted in the testing apparatus so that crack propagation would occur across the laminates.

Vickers and Knoop microhardness tests were performed on polished sections of nacre from *Haliotis rufescens* and *Pinctada margaritifera* using a Shimadzu Type M Micro Hardness Tester. The nacre layers were oriented both at an acute angle and perpendicular to the surface of indentation.[19]

2.2 Electron Microscopy

The bulk specimens of seashell were coated with gold-palladium and observed in a Philips 515 scanning electron microscope (SEM) operating at 25 kV. Thin sections of the shell were prepared for transmission electron microscopy (TEM) by cryogenic ion beam milling (sample temperature of about 130K) and room-temperature ultramicrotomy. Uranyl acetate was used on some of the ultramicrotomed samples to enhance the contrast of the organic component for TEM analysis.[19] Samples were viewed in a Philips EM 300 at 100 kV and in a Philips EM 430T operating at between 150 and 300 kV using a liquid nitrogen holder (sample temperature of about 150K).

3. RESULTS

3.1 Mechanical Property-Structure Correlations

The three-point bend tests showed an average fracture toughness of approximately 9 ± 3 $\mathrm{MPa \cdot m^{1/2}}$. The four-point bend tests resulted in a measured fracture strength of approximately 180 ± 30 MPa. Most of the samples failed in mixed mode behavior.[10,19] Due to the inherent curvature of the layers within the test specimens, slightly wider scatter was observed in the data. As mentioned earlier, the nacre of *Haliotis rufescens* contains a relatively thick periodic layer of organic material which runs parallel to the long axis of the aragonite platelets (Figure 2). This thick organic layer, thought to be related to the yearly growth front, acts as a weak region, and samples often fail by shear within this region.[2,17]

As discussed in earlier publications,[7] the relative values of the mechanical properties of the composite shell compared to those of the individual components which make up the shell are of interest from an engineering standpoint with regard to the possible mimicking of nacre for technological applications. Considering that the shell is 95 to 98% $CaCO_3$, the mechanical properties of the composite structure would be expected to be near those of $CaCO_3$. It is clear, however, that the incorporation of an organic component and the highly ordered lamination of the nacre structure produce properties which are substantially enhanced with respect to those of synthetic and geological aragonite.[5,8-10]

We also investigated the response of the nacre structure to microhardness indentations with SEM to determine the process(es) by which cracks travel through the composite. The most

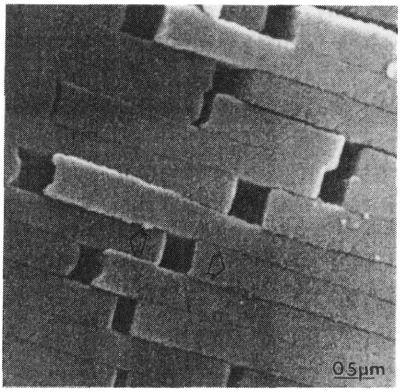

Figure 3. Secondary electron image of the nacre structure near a microhardness indentation displaying the sliding of the aragonite platelets in red abalone.

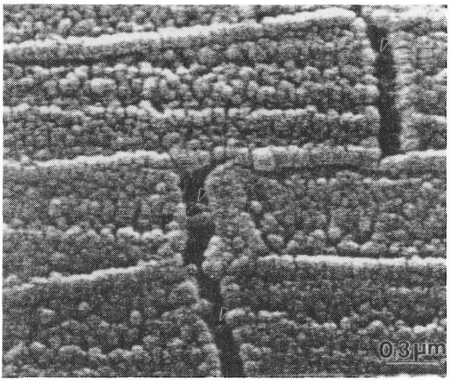

Figure 4. A close-up secondary electron image of the deformed region of the sample showing ligament formation by the organic matrix extending across the edges of platelets.

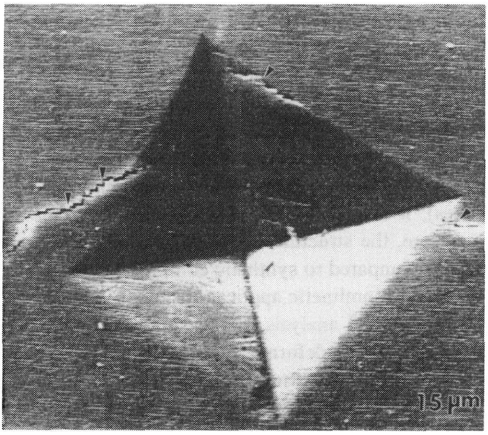

Figure 5. An SEM image of the microhardness indentation in an edge-on configuration in the nacre of abalone. Note that the indentation involves considerable deformation in the composite without extensive formation of cracks, indicating ductile behavior, as opposed to brittle cracks that usually from at the tips of the indentation.

obvious fracture feature is that crack propagation generally occurs in a tortuous manner across the laminates, with the weakest region being the organic matrix. The tortuosity increases the surface area of the crack and, thus, the amount of energy absorbed during fracture. However, measurements of tortuosity show that increased fracture surface (up to 50%) is not sufficient by itself to account for the toughness value of the composite.

In many instances, aragonite platelets slide over the organic layers, leading to overall deformation of the composite without causing total failure (Figure 3).[19] Through this deformation process, a large amount of energy is absorbed within the organic matrix and at the organic-inorganic interface. In addition, during deformation, the organic matrix was observed to bridge the crack between layers of aragonite platelets by the formation and extension of ligaments. This was shown earlier to take place within the matrix between the broad faces of the aragonite platelets, i.e., in the transverse direction.[10] Figure 4 shows that bridging also takes place in the longitudinal direction (i.e., in the direction parallel to the plane of the platelets). The formation and extension of organic ligaments slows or prevents the propagation of the crack within the organic matrix by absorbing the energy that would have propagated the crack. Although other toughening mechanisms are usually encountered in brittle materials,[21] such as microcrack formation, crack blunting and branching, it is the sliding of platelets that provides the two major toughening mechanisms most effective in the prevention of catastrophic failure: the deformation of the overall composite and the formation of ligaments to bridge cracks. These mechanisms can be seen in Figure 5, which shows the deformation, rather than brittle failure, around a microhardness indentation made on a sample with the aragonite crystals at an acute angle to the polished surface.

The fact that aragonite platelets slide over the organic matrix while, at the same time, the organic matrix forms ligaments, each under appropriate applied stress conditions, suggests that both the structure of the interface between the macromolecules and the aragonite platelets, as well as the structure of the organic matrix, play significant roles in allowing deformation and thereby preventing catastrophic failure of the composite. Both of the processes, i.e., the sliding of the aragonite platelets over the organic matrix and organic ligament formation between the platelets, seem to indicate that the interface between these highly dissimilar ceramic and organic materials might be strong enough not to fail in either mode of deformation. Sliding may either happen between the platelets and the top layer of the organic matrix or within the layers of organic matrix. On the other hand, ligament formation clearly takes place within the organic matrix, although it is not yet known which component of the organic matrix plays the most significant role during the deformation process. It is obvious that the ligaments are anchored to the aragonite platelets, suggesting the presence of a strong interface between the organic matrix and $CaCO_3$ platelets. A strong interface would allow the transfer of stresses between the rigid ceramic crystals and the pliant organic matrix, in addition to allowing the deformation of the overall composite to take place. With synthetic composites such as cermets (ceramic-metal composites) and cerpolys (ceramic-polymer composites), it is usually difficult to achieve strong interfaces between such dissimilar phases.[22] Therefore, the structure of the interface in nacre might possibly play a part in its superior properties as compared to synthetic composites. Interface design may be an important design criteria for future biomimetic applications.

In the following section, we provide a more detailed analysis of the structures at the interface region in nacre in order to gain further insight into the deformation processes that take place during the structural response of the composite material to stress. First, we discuss the structure of the organic matrix and then the structure of the interface region between the two phases.

3.2 Study of the Microstructure at the Interfacial Region: Results and Discussions

Structural analysis of the interfacial region was performed by TEM as this technique provides the resolution necessary for imaging the details in both the organic and inorganic phases of nacre. Particular attention was given to the preparation and handling of the samples to ensure that they did not deteriorate. Generally, TEM samples, which consist primarily of the aragonitic phase, are prepared by ion-beam milling, as was demonstrated by us[10] (see, for example, Figure 1) and other earlier studies.[23] During a typical milling process, the sample is inadvertently heated, which can lead to structural damage of both the crystalline and the organic components. Structural damage to the samples can be minimized by performing the milling process under cryogenic conditions (about -130° C in our case). However, even with cryogenic ion beam milling, the organic components of the structure are highly sensitive to ion bombardment and, thus, to knock-on and radiolysis degradation. For this reason, the ion milling method of sample preparation was used only when the structure of the organic region was not of great interest. (Even in ion milled samples, most of the organic material, particularly the innermost layer, stayed intact. This permitted limited analysis of the organic matrix, as discussed below.)

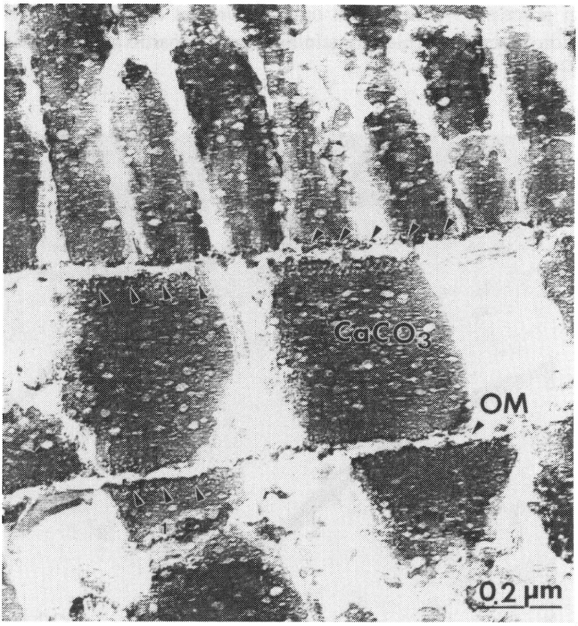

Figure 6. A BF TEM image of the nacre of red abalone prepared by ultramicrotoming. Rough interface between the organic matrix and aragonite crystals and the frothed structure in the aragonite are clearly seen (small arrows).

Ultramicrotoming can be used to preserve both the structure of the crystalline and the organic regions simultaneously (Figure 6) with some sacrifice to the sample.[19] Due to the brittle nature of the crystals and the presence of mechanical instabilities in the experimental set-up, however, the microtomed samples displayed periodic chatter in the aragonite crystals. As shown in Figure 6, the chatters run perpendicular to the aragonite crystals and appear roughly vertical in the image. Even so, local regions of the aragonite crystal-organic matrix interface remain intact (arrows in the image) and leave sufficiently large areas for imaging, diffraction, and high-resolution analysis, as described below.

Uranyl acetate was applied to some microtomed sections to demonstrate that the organic matrix was indeed undisturbed by the microtoming process and that it is composed of a layered structure. Figure 7 shows the structure of the organic matrix in decalcified nacre as observed in an edge-on configuration (note that, due to the application of uranyl acetate staining solution, the aragonite crystals are dissolved and, thus, not present in the image). As indicated by the arrows, the five-layer structure of the organic matrix is clearly visible. This layered, sandwich structure of the organic matrix was proposed earlier in the literature.[15-17,24] According to the hypothesis, the middle layer is thought to be structural polysaccharide, most likely chitin. On either side of the middle layer are structural proteins, such as silk-fibrionlike proteins, which are thought to be stereochemically related to the aragonite matrix, and, therefore, more directly involved in the biomineralization process.[24] Finally, the outer layer that is in contact with the aragonite is assumed to be acidic macromolecules containing mostly aspartic and glutamic acids, its function being secondary.[24]

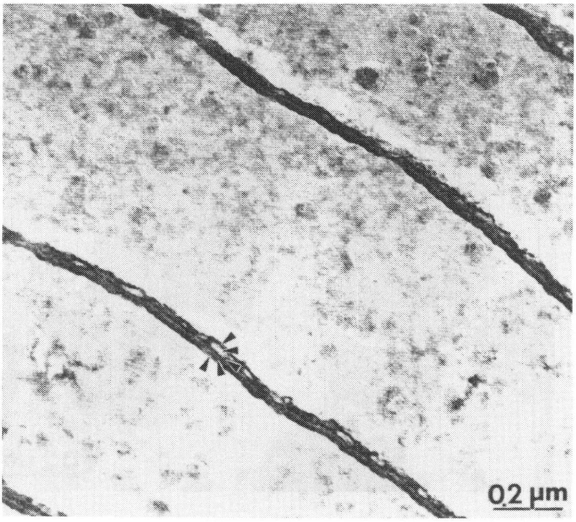

Figure 7. A TEM image of the organic matrix displaying a five-layer sandwich structure of the composite. This section was stained with uranyl acetate and the aragonite crystals were dissolved by the staining solution.

Despite extensive research, neither the composition nor the structure of each of the organic layers have been quantitatively analyzed.[4] Both the structural interrelationship of the organic matrix and the aragonite crystals in nacre and its effect on the overall mechanical properties of the composite structure require an understanding of the structure and composition of the organic matrix in detail at the nanometer scale. Such knowledge is also essential for a fundamental understanding of the control of the organic matrix over the biocrystallization process in this and other similar hard tissues. (Along with efforts by other groups working in this area,[25] a part of our current research focuses on the analysis of each of these layers by protein purification and antibody generation and decoration procedures.[26])

In addition to the organic matrix, it is clear that the detailed structure of the interface between the aragonite crystals and the organic matrix has to be investigated in order to describe the mechanical properties of nacre and its fractographic features. Obtaining this information on the interface structure has not yet been possible mainly because of the difficulties in the simultaneous imaging of both the aragonite platelets and the organic matrix with TEM. Despite these difficulties, however, our TEM investigation indicates periodic contrast fluctuations that run parallel to the aragonite/organic matrix interface both in the ion milled and the microtomed thin sections, as displayed in Figures 1 and 6, respectively. These images indicate that there is a string of small pores approximately 8 to 12 nm in diameter and spaced 20 to 32 nm apart (measuring center to center) parallel to the organic matrix. The nature of these pores has not yet been clarified, but it is assumed that they contain either water or organic material or both.[19] Because these features are present in samples prepared by both techniques, they are considered to be representative of the structure and are not an artifact developed during sample preparation.

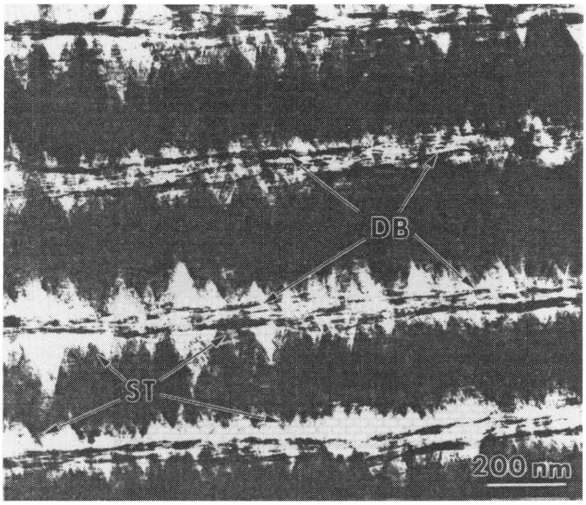

Figure 8. TEM image of a gold citrate stained section of nacre which was originally ion-beam milled. Two major features of interest are dark bands (DB) within the organic layer and the preferential etching characteristics of the aragonite crystals (ST) which give a saw-tooth angle of about 46° in this projection.

These features were observed both in *Haliotis rufescens* and *Pinctada margaritifera*,[19] and they are similar to those that were reported in bivalve mollusks.[23] Therefore, they appear to be a common feature, characteristic of the nacre structure in general and not specific to a single molluskan species.

A closer examination of the interface between the organic and the aragonite platelets indicates that the interface does not have a flat surface but rather is rough and the edges of the aragonite crystals near the interface are "frothed" in addition to containing the aforementioned "pores." As shown in the ultramicrotomed sample in Figure 7, these features suggest a "transitory region," whose structural characteristics are quite different from the substructure of the aragonite in the interior of the platelets. This transitory region is even more apparent in Figure 8, which was recorded from an ion milled sample in which parts of the aragonite crystals near the interface have been partially dissolved by the colloidal gold solution containing citric acid. As shown in the image, thin dark bands run parallel to the aragonite platelets in the interface region previously occupied by the organic matrix. If these bands are a surviving portion of the organic matrix, or what remains of it after ion beam milling, they are very likely structural polysaccharides, or the hypothesized[16] "chitin" section of the organic sandwich structure.[15-17] In addition, as can be seen in the image, the $CaCO_3$ crystals are etched preferentially, making a 46 ± 1° angle with respect to the plane of the interface which is parallel to the (001) plane of the aragonite lattice. This produces a characteristic "saw-tooth" shaped etching of the transitory region, suggesting that the organic matrix may have infiltrated through the interface into the inorganic crystals. At this stage of our research, this conclusion is only a conjecture and remains to be proven by other means.

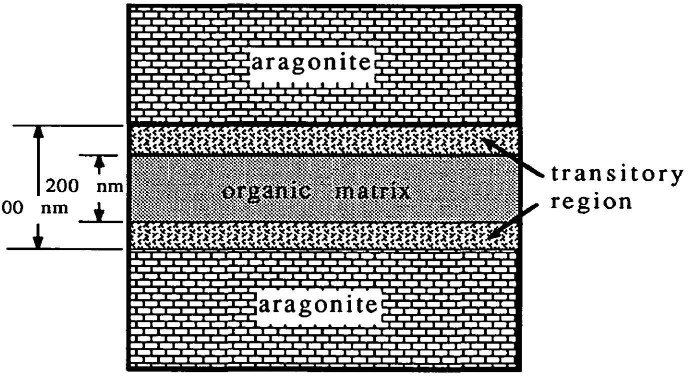

Figure 9. Schematic illustration of the *transitory region* in the $CaCO_3$ crystals near the organic matrix.

Nonetheless, this transitory region in the aragonite phase near the interface (which is effectively a "composite" of $CaCO_3$ and organic material as schematically depicted in Figure 9) may have a significant effect on the mechanical properties of the overall composite. First of all, a spatial interlocking of the organic matrix and the aragonite crystals would be expected based on the assumption that part of the organic matrix is occluded in the aragonite crystals. This would increase the effective surface area and thus the adhesive strength between these dissimilar phases. Our mechanical test results and fractographic analysis have some parallelism with these observations of the detailed structure of the interfacial region.

4. CONCLUSIONS

The nacre portion of mollusk shell has been shown to display greater toughness and strength than $CaCO_3$, which makes up 95 to 98% of the nacre structure. The increase in toughness has been attributed to a variety of fracture mechanisms that increase energy absorption during failure, the main mechanisms being sliding of the inorganic crystals and ligament formation in the organic phase. An essential feature critical to several of these energy-absorbing mechanisms is the presence of a strong interface between the aragonite crystals and the organic matrix. This strong interface is necessary for the transfer of stress from the crystals to the organic matrix and subsequent energy absorption within the organic matrix. Factors leading to higher strength are not yet understood.

We determined that the interface between the organic matrix and the aragonite crystallites is not planar but is what we call a transitory region, which seems to be an interlocking of the organic matrix into the aragonite, as suggested by the structural features. Although the true nature of this region has not yet been clarified, it appears to increase the surface area between the two phases and thereby increases the strength of the bond across the interface, thereby resulting in a composite structure with desirable mechanical properties.

ACKNOWLEDGEMENTS

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