Mineralized biological materials: A perspective on interfaces and interphases designed over millions of years

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The one feature that distinguishes mineralized biological from synthetic materials is the presence in mineralized biological materials of a complex assemblage of macromolecules. This assemblage usually acts as the host to the guest mineral particles, a well known phenomenon in composite materials. The macromolecules can, surprisingly, also be the guests inside a host crystal, to form a sort of reverse composite material. Their presence both between and within crystals creates diverse interfaces and interphases, and these have marked effects on the mechanical properties of the material. Such "novelties" are the products of the evolutionary process, which by trial and error over long periods of time arrives at a solution that works. This may not be the most elegant solution or the most economical solution, and is therefore often unexpected. It is thus the possibility of discovering the unexpected that makes the investigation of biological materials so exciting. In some cases the biological solution to a problem may also be useful for improving synthetic materials, an added benefit. In this context, we examine here several interfaces and interphases in mineralized biological materials.

One of the most extraordinary mineralized materials produced in biology, is the sea urchin tooth.[1] Sea urchins use their five teeth to grind down rock surfaces and extract adhering biological material. The rocks they grind are often limestones composed of calcite and the teeth that do the job are also composed of calcite. The trick is built into the design features of the tooth.[2] The grinding surface resembles common synthetic composites with needle-shaped stiff particles embedded in a pliant organic matrix. In the sea urchin tooth, however, the matrix is also composed of the mineral phase, calcite. This calcite is unique in the biomineralization world. It contains in some cases around 45 mole % magnesium, but still maintains its calcitic atomic lattice.[3] The small size of the crystals probably gives this mineral phase unusual mechanical properties. The needle-shaped stiff particles are single crystals of calcite with relatively small amounts of Mg and a diameter of around a micrometer. In fact they are just the ends of long fibers that taper down from some 15–20 μm diameter to less than 1 μm. Following Griffiths, the reduction in size minimizes the chance of containing a critical defect and effectively increases the strength.[4] These are thus two materials with very different properties working together at the tooth tip. A materials scientist would therefore expect a "gasket" to be present at the interface between the two mineral phases, and indeed there is. It is a thin organic membrane, whose composition and structure unfortunately are not yet known.[2]

During the formation of most mineralized biological materials, the cells first produce a framework composed mainly of macromolecules, and then induce the formation of the mineral phase inside the framework.[5,6] In mollusk shells the core of this framework is β-chitin, which has a highly ordered crystalline structure.[7,8] We suspect that the space between chitin sheets is initially filled by a hydrogel composed of silk fibroin.[9] At some point in time nucleation of the crystal occurs. The nucleation, growth and cessation of growth of the aragonitic crystals in nacre are modulated by the matrix surface, the future interface with the crystal. In nacre the surface beneath a single aragonite tablet-shaped crystal can be laterally differentiated into four different functional zones.[10] The center of the polygonal imprint is carboxylate rich and this zone is surrounded by a ring of sulfate-rich presumably glycoproteins (Fig. 1). This is also the site where proteins capable of nucleating aragonite are located. The nucleation site thus has a laterally differentiated surface structure that is reminiscent of the cooperativity model proposed by Addadi and Weiner.[11] The model was deduced from *in vitro* experiments in which oriented calcite crystal nucleation occurred on polyaspartic acid polymers adhering to sulfonated polystyrene surfaces. In this model the sulfonate groups attract calcium ions to the surface and the ordered carboxylate groups of aspartic acid induce oriented nucleation of calcite. The third zone over which the crystal grows appears to be the surface of the chitin itself or the chitin coated with carboxylate containing proteins. The fourth zone is where the crystals stop growing laterally when adjacent crystals meet. This zone contains a variety of macromolecules possibly pushed ahead of the growing crystal.[12] It has also been observed that the mineral phase on the surfaces of mature crystals is disordered.[13] This amorphous calcium carbonate containing layer may possibly be mixed in with the fourth zone macromolecules. Thus one hallmark of the nacreous matrix-mineral interface is that it is laterally differentiated into functional zones. It is also rich in charged groups and can thus form tight electrostatically dominated bonds between the crystal and the matrix.

A very different type of interface is present in another mollusk mineralized tissue, the tooth of the limpet. These snails, like the sea urchins, use their teeth to grind the rocky substrate in order to extract nutrients. Here the matrix frame-

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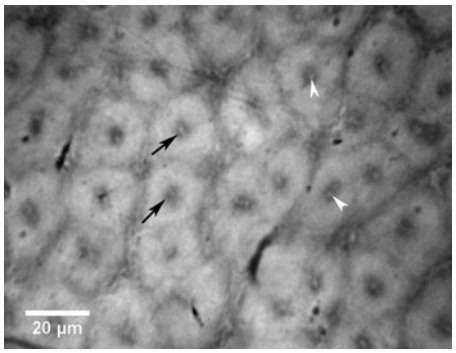

FIG. 1. Light microscopy image of the decalcified interlamellar sheet of *Nautilus pompilius* nacre. Black arrows indicate the stained central area of the imprints, showing the presence of sulfate groups in these areas, and white arrowheads indicate the nonstained core.

work is chitin, but in the α form. Cryo-transmission electron microscopy (TEM) sections show that the chitin fibrils are initially closely packed. When the first crystals of goethite, an iron hydroxide mineral, form they appear as needle-like objects aligned with the chitin fibrils (Fig. 2). Presumably the crystals push aside the matrix as they grow. The crystals also tend to express stable crystallographic faces and are seen in some cases to envelope the matrix.[14] We therefore deduce from these observations that here the interactions at the interface between matrix and mineral may influence crystal nucleation but not crystal growth, as the crystal morphology is not affected in any obvious way by the matrix surface. The space between crystals is eventually filled up with a second mineral, amorphous silica.

A similar situation appears to occur in the mineralization of vertebrate bone, where the carbonate apatite crystals adopt the stable plate-like morphology, and as they grow they push aside the associated collagenous matrix.[15] One difference from the limpet tooth is that the collagenous preformed matrix does initially contain spaces, and the first-formed crystals nucleate inside these spaces (known as gaps or holes) at a specific location.[16,17] They subsequently grow out of the gaps and push the collagen fibrils aside.[18] The crystals at this stage essentially "straightjacket" the collagenous organic framework.[19]

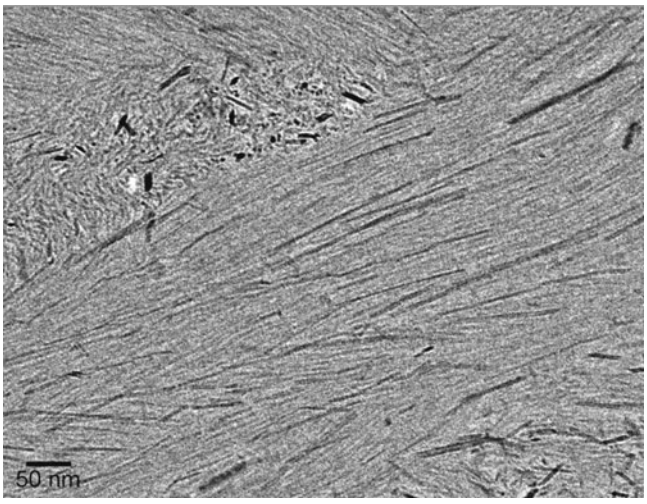

FIG. 2. Cryo-TEM micrograph of early-formed goethite crystals in an ultrathin section of teeth of the limpet *Patella caerulea*. The crystals appear as needle-like objects aligned with the chitin fibrils in the central portion of the micrograph, where the section is longitudinal to the fibrils. Note that the section is unstained and tissue is embedded in a thin film of vitreous ice.

One of the most thoroughly investigated interfaces in biomineralization is the so-called "junction" between the hard outer enamel layer and the inner more pliant dentin in vertebrate teeth. This has long been recognized to be more than just a two-dimensional interface. It has a scalloped shape, presumably to prevent shearing.[20] The collagen fibrils from the dentin penetrate into the enamel. In fact in human teeth, a zone some 200 μm thick is less mineralized compared to the bulk dentin and has a different structure.[21–23] Strain mapping of human premolar tooth slices loaded under compression shows that this 200-μm-thick zone actually takes up most of the strain.[21,24] The compressive modulus of this zone has been determined using speckle interferometry, based on a change in the gradient of displacements over about 200 μm. The stiffness is much lower than that of bulk dentin and the measured moduli are significantly different on the outer and inner sides of the tooth.[22] There is clearly built-in asymmetry. An interface as such only exists between this zone and the overlying enamel, while deeper in, a graded transition is observed in the structure and properties, and this forms an interphase. In fact graded materials properties are the hallmark of the whole tooth.[25] Hardness profiles through the enamel show a marked decrease towards the inner dentin, followed by a precipitous drop into the "soft" zone, and finally a gradual increase and then decrease within the dentin.[26,27] The tooth is a totally graded structure with the enamel and dentin securely bound together. A similar interphase also exists in cementum, the tissue that binds the tooth to the mandible.[28]

Interfaces and interphases are omnipresent in mineralized biologically formed materials. The few examples described above serve to show that these are very diverse. Part of the diversity is a direct function of the scale at which they are described. At the atomic scale, the interface between mineral and matrix can be more electrostatic to form a tight junction (for example, the mollusk shell), or more hydrophobic to allow interplay between the growing crystal and the matrix (examples are the limpet tooth and bone). At higher length scales, graded structures can be introduced between materials with different properties (dentin-enamel junction). Graded structures can also exist laterally along the interface, as demonstrated by the mollusk shell example above. Interfaces and interphases are probably responsible for most of the unique properties of biological materials.

In the early 1990's many materials scientists turned to the study of biological materials in order to acquire new ideas for improving the properties of synthetic materials. Bearing in mind that everything that ends up in the bulk of the material has to pass through the interface, it can be anticipated that significant advancements in our understanding of biological materials will come about as a result of studies of the

interface. To do this for biological materials, less energetic and hence less destructive analytical tools will need to be used. One possibility could be x-ray photoelectron emission spectromicroscopy (X-PEEM). The ever improving capabilities of both transmission and scanning electron microscopes offer some of the most powerful means of investigating biological interfaces, especially under conditions in which the water component is still present. In the last few years the use of highly resolving ion probes has provided important new insights into distributions of various elements in mineralized skeletons. Most recently the application of time-of-flight secondary ion mass spectrometry to the study of interphases and interfaces is opening up even more opportunities, as both inorganic ions and amino acids can be co-mapped at very high resolution. There is little doubt that the huge diversity of natural biological materials represents a potentially rich source of new insights into how nature produces and uses interfaces and interphases. The know-how and experience of the surface science community can certainly contribute much to better understanding this most important aspect of mineralized biological materials, and at the same time produce new ideas for the improved design of synthetic materials.

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