

# **Biomimetic mineralization and biomineralization**

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## INTRODUCTION

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### INTRODUCTION

Minerals in biology provide stiffness and compressive strength. Proteins and polysaccharides can form excellent strong fibers and, like the keratin of a horse's hoof, can form tough solids. By adding minerals to these polymers, biology can increase stiffness, hardness and wear resistance at a low cost in terms of the energy input needed to form the material. Insects work well with un-mineralized exoskeletons but larger animals depend on mineralized bones and shells for support and mineralized teeth for good wear-resistance.

While the synthetic world uses many hard materials, the range of minerals in biology is limited: hydroxyapatite, calcium carbonate and silica account for the most biological mineral. Evolution has apparently compensated for the limited range of minerals by developing very sophisticated microstructures which seem to provide optimum combinations of properties for the particular circumstances of the organism. "Seem to" must be emphasized here because it has proved to be very difficult to define an optimum for any biological system where survival under fluctuating conditions is the real requirement [1]. Despite this caution, it is clear that we can learn much that would help us improve the properties of synthetic composite materials if we could understand and reproduce the structures found in biology.

We need to understand how the biological structures give rise to such good properties. Much of the emphasis in this area of biomimetics has been in understanding the ability of bones and shells to combine stiffness and toughness but it has more recently become clear that we can also learn from biological optical structures, sensors, sound detectors and probably many others. Having decided what structure we would like to make, we should also study the mechanism by which biology makes it. There has been much progress in this area in the last few years.

As we understand mineralized tissues, we want to reproduce these structures and use them. For the present the uses fall into two separate categories. Biomedical applications require materials that will be compatible with a wet, salty and oxidizing environment. Some applications may require resorbable materials, while others may require a long life with no degradation or wear. Biocompatibility is more important than mechanical performance while price is only a secondary consideration.

In contrast, materials in the synthetic world are driven mainly by a balance between cost and mechanical performance. Biomimetic structures will be employed if the clever use of microstructure allows cheap starting materials to replace more expensive ones without dramatically increasing processing costs. In considering this it should be kept in mind that the applications are often not the obvious ones. A great effort went into improving structural ceramics for engines, largely without success. Meanwhile, a large and unexpected new ceramics business was built in semiconductor packaging, capacitors and other electronic components.

Between the soft, wet biomedical applications and the hard, dry synthetic applications there is also the possibility of a future family of soft, wet machines. Biomimetic materials may find application in biomimetic machines that are more animal-like, with integrated environmental sensing and intelligence.

This article will follow the preceding discussion with a modified order. Growth and structure of mineralized tissues, properties of mineralized tissues, applications in medicine and biomimetic applications will be discussed. The field is currently in a state of flux as new understanding is replacing earlier ideas of simple nucleation and growth with mineralization that occurs through an amorphous phase and through aggregation. The situation is quite unclear but this will open new possibilities for making biomimetic materials that could lead to wider applications. This article has focussed on two of the best-studied materials, nacre and bone in order to illustrate how much our thinking has changed in the last few years. Many other puzzles will become resolved as this new picture is filled out.

### MECHANISMS OF BIOMINERALIZATION

The key fact in biomineralization is that structures are formed from dilute aqueous solution at constant temperature. Where synthetic solids are often formed by a temperature change, pH is the only general environmental variable that can be locally controlled to induce precipitation. In addition supersaturation can be locally increased by binding one or more species with a ligand that can then be broken down enzymatically after the complex has been transported to the precipitation site. In the synthetic world, physical processing such as by heat or evaporation is preferred to processing by chemical reaction. Thus, in forming composites from pre-

impregnated sheets, it is notoriously difficult to achieve reproducible properties because the system is so sensitive to small changes in temperature or impurities. Biology makes high quality materials using chemical reaction so we should also expect very complex control mechanisms on the process to ensure good uniformity.

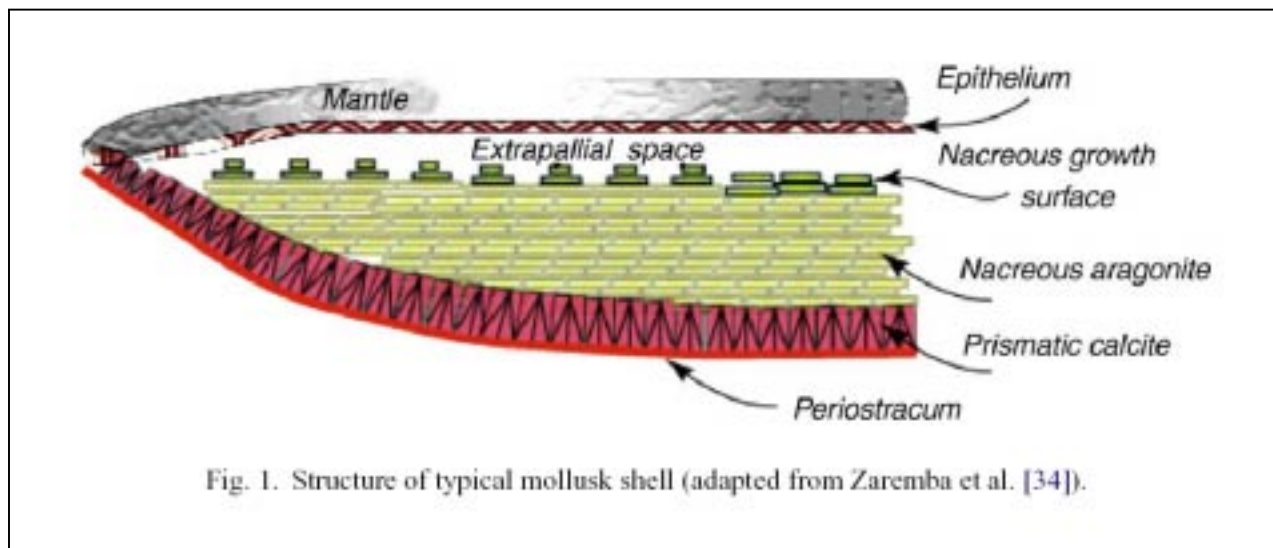
Initially it was believed that biomineralization was controlled by increased supersaturation of the precipitating ions and provision of a suitable nucleating surface on which the mineral grew by addition of single ions. We now are discovering that this is not generally true and may never be true. Since biology is not required to follow particular principles, it may also be that we have to regard every mineralization process as a special case until we understand it better.

### Growth and structure of nacre

Mollusc shells have many different structures, all comprising calcium carbonate as calcite or aragonite with a small organic content. One would expect that each shell structure allows the mollusc better to exploit a slightly different lifestyle or habitat. Certainly swimming scallops have a light, strong shell compared to sedentary oysters. Several different shell types have been characterized mechanically but detailed studies of the growth mechanism have largely focussed on nacre. Nacre or "mother of pearl" is a layered structure of aragonite platelets with a thin organic sheet separating the layers and a an organic film at the boundaries between platelets.

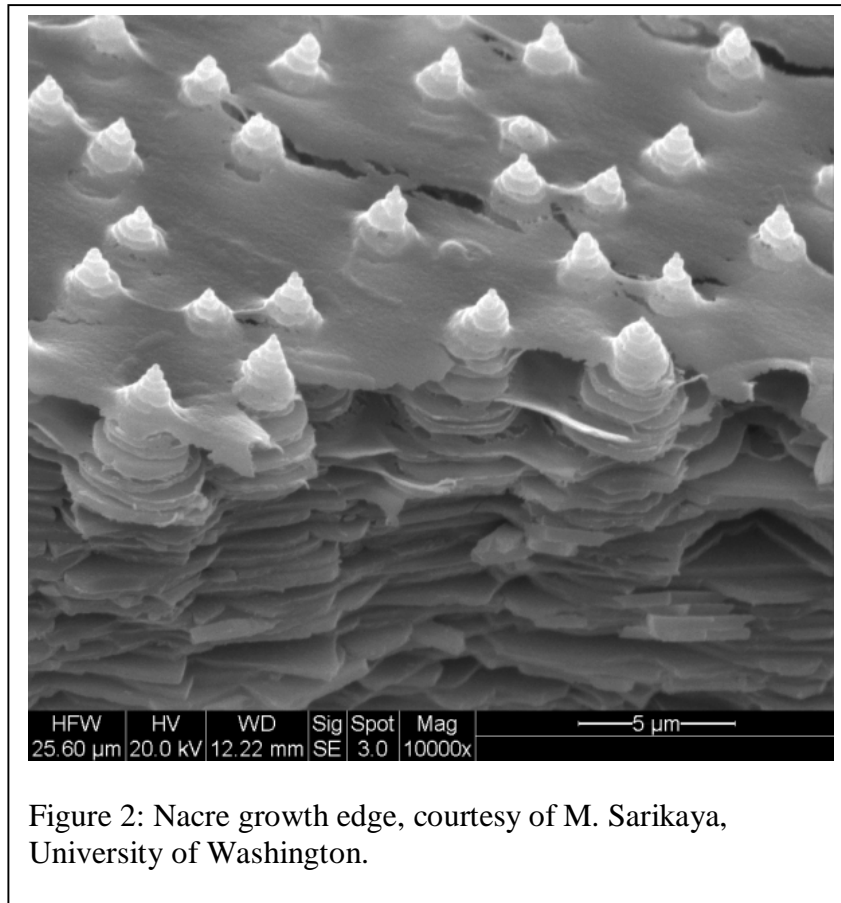
Abalone shell and many other nacreous shells have an inner layer of nacre and an outer layer of prismatic calcite with an outer protein coating, the periostracum (Figure 1). Shell is formed by the mantle, a tongue-like organ that can be pushed out over the outer lip of the shell. Inside the

fold of the mantle, the edge of the shell is extended as the creature increases in size. By looking at the edge of the shell, we can see in what order the deposition occurs. The periostracum is deposited first and then the prismatic and nacreous layers are built in turn. Thus the mantle must nucleate oriented prisms of calcite on the protein layer, then nucleate aragonite plates on the calcite and sequentially build layers of platelets with intervening organic sheets. There are many aspects of this process that we do not understand and some where our understanding has been improving greatly in recent years.



The best information on this sequence of events comes from "flat pearl" experiments, where a glass coverslip is placed under the mantle [2-4]. The sequential deposition of material on the glass can be followed by removing the slip at different times. Three sequential steps of protein deposition, growth of calcite prisms and deposition of nacre can be observed as can the production of different acidic proteins corresponding to the different mineralization stages.

Presumably the mantle can control the calcium, carbonate and pH levels in the space immediately adjacent to the growing shell and so can control the supersaturation of calcium



carbonate. Images of nacre at the growth edge (figure 2) show pillars of partly formed plates ("Christmas trees") with the organic sheets already in place. This implies that new layers of aragonite are seeded at a number of sites on existing layers and then these seeds and the intervening spaces are covered with an organic sheet. Alternatively, [5], the layer thickness may be limited by specific growth inhibitors for the c-face of aragonite coupled with sporadic nucleation events. Another possibility is that the organic sheets could be deposited as a pairs of sheets that are then pushed apart by the growing aragonite.

One question that has been thoroughly discussed is the mechanism that controls the orientation of plates in successive layers. The aragonite deposits with the c-axis perpendicular to the organic sheet. The a- and b-axis orientation varies within a layer but there is good alignment between

any platelet and those immediately above and below it. It was originally thought that this was due to structure within the organic layer. This layer has surfaces of acidic proteins such as are often found associated with mineralization. At least three components have been identified. The inner core may be silk-like proteins or chitin polysaccharides [6, 7]. It was originally thought that the silk-like protein could provide the directional structure to give an epitaxial relationship with the growing aragonite, although it would have to function in the presence of adsorbed layers of other proteins [8]. It now seems that the silk-like protein is in the form of a soluble gel attached to the chitin [9]. More recently holes have been found through the organic layer and these could allow the aragonite to simply grow through and initiate the next layer through direct connections [10]. currently, holes have been seen in isolated sheets of interlamellar polymer but mineral bridges have not been directly seen and it is not clear how growth through the holes would occur if there are also proteins present which inhibit growth of aragonite in the c-direction.

The conversion from growing calcite prisms to growing aragonite layers has been shown to be controlled by acidic proteins. Evidence comes both from the flat pearl experiments and from studies in which calcite crystals started to form aragonite overgrowths when the soluble proteins derived from abalone shell were changed [3, 11, 12]. These proteins could act either by inhibiting calcite or by nucleating aragonite, the exact mode of action is unclear. However there have been a series of AFM studies on the action of growth inhibitors on the surface of growing crystals. The proteins tend to bind to steps sites, which are the site of ion addition and sweep across the surface as the crystal grows [13-15]. What we learn from these studies is that growth modification is not just a question of protein binding to a crystal surface but of exactly which

surface, which sites on that surface and how strong is the binding. Many of these proteins do also get entrapped within the crystal structure.

Thus current studies of nacre growth leave us with a number of pieces but no complete picture. There is evidence for soluble proteins which can speed up or slow down the growth of specific crystal surfaces and so can modify the crystal shape or phase. There is less evidence for soluble proteins that can enhance nucleation and it is not clear whether new nucleation occurs in each layer. The interlamellar layer may act to control structure, as a toughening layer, or both.

#### Growth and structure of other shells

Although the structures of many shells have been characterized, detailed studies of growth mechanisms have been very focussed on nacre in a small number of species including abalone, some bivalves such as mussels and oysters [16]. "Flat pearl" studies have also been carried out on Conch shell which has a crossed-lamellar structure [4].

#### Amorphous Calcium Carbonate

A number of recent observations from different sources are suggesting a major role for nanoparticle or amorphous calcium carbonate in biomineralization.

Amorphous has long been known as resulting from precipitation at high supersaturation of calcium and carbonate. It contains a varying water content as well as OH<sup>-</sup> or other ions [17]. It occurs in plants and crystallizes on moistening [18]. It occurs as an outer layer in some sponge spicules over a calcite core, where it is stabilized by a high content of acidic macromolecules

[19] and in sea urchin larvae [20]. It is now clear that amorphous calcium carbonate is widespread in biology and is often a precursor to crystalline calcite [21, 22]. The amorphous phase can be stabilized by a range of additives, including magnesium, phosphates, acidic and basic macromolecules.

Gower has extensively studied the formation and growth of liquid amorphous calcium carbonates, stabilized with acidic macromolecules [23, 24]. Metastable liquid drops form in solution and crystallize with a spherulitic morphology after accumulation on a substrate. Recent work has revealed a layer of amorphous calcium carbonate on the surface of the aragonite tablets in nacre [25]. This layer may be stabilized by included acidic protein as with other amorphous carbonates.

This work on amorphous carbonate has led to the suggestion that some of the elegant shapes of carbonate skeletons, at least, in coccoliths and spicules arise by addition of amorphous particles to existing crystal surfaces rather than by addition of ions as in conventional crystal growth [26, 27, 70]. The evidence for this is that many of these materials have a nanocrystalline substructure while being apparently single crystalline at the micron scale [28].

Much earlier Matijevic had grown very regular monosized particles of oxides that were apparently aggregates of nanoscale particles [29, 30]. Many of the structures seen in these biominerals and in biomimetic mineralization in the presence of acidic polymers are also very reminiscent of spherulites and multilayer single crystals formed by polymers [31]. The

spherulitic structure is also characteristic of mineral and organic crystals growing in impure viscous melts where the impurities accumulate at the surface of the growing crystal.

We thus see that amorphous phases commonly precede biomineralization and that the final mineral often shows a nanoscale substructure. This may lead to a wholesale rethinking of biomineralization processes. It still leaves open the question of whether the process occurs via dissolution of the amorphous phase and reprecipitation of crystals, by formation of nanocrystals followed by aggregation or by deposition of amorphous particles on the crystal surface followed by phase conversion. Since biology is not limited to a single method, different tissues may grow by different processes.

#### Growth and structure of bone and tooth

The main structural unit of bone is a collagen fibril with embedded hydroxyapatite platelets. The rigid collagen triple helices are 280 nm long with a quarter-stagger arrangement so that there are gaps corresponding to molecule ends every 67nm. Mineral seems to nucleate at the gaps where collagen triple helices end. The resulting composite fiber is about 50% mineral by volume.

Mineralization processes vary between initial mineralization of infant bone, mineralization of tendon, repair of broken bone, remodeling of adult bone and growth [32]. During bone growth a layer of osteoblasts initially deposits a highly ionic proteoglycan matrix, then collagen forms in this matrix and the collagen then mineralizes. As seen in figure 3, this occurs as a series of stages at successive distance from the cell layer. One puzzle of this process is that

hydroxyapatite seems initially to form randomly in the collagen matrix and later appears both within the collagen fibrils and on the outside of the fibrils [33]. In vitro studies have found that

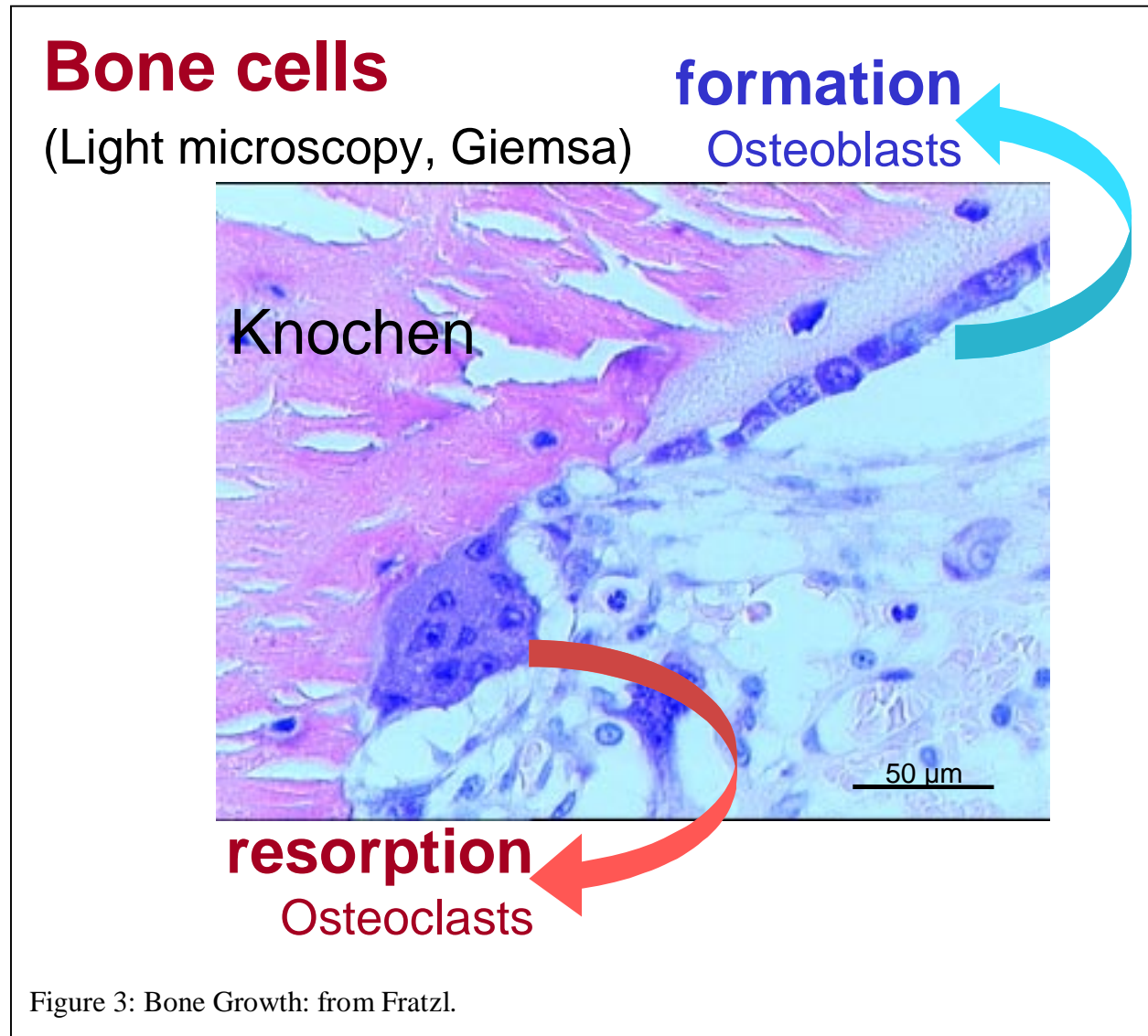


Figure 3: Bone Growth: from Fratzl.

collagen can readily nucleate hydroxyapatite crystals that are parallel to the fibrils. However, it seems that the mineral is always outside the fibril not inside as is found in bone [34-36].

There has also been a long discussion about the possible role of amorphous calcium phosphate in bone mineralization. Many studies seem to show the transient presence of amorphous or poorly-

crystalline calcium phosphate during mineralization and the new information on amorphous carbonate phases may lead to reconsideration of the role of amorphous phosphates.

Recent studies on enamel growth have shown that amelogenin is present in large amounts at the growth site and does interact with specific faces of the hydroxyapatite crystals [37-39]. The tip of the growing crystal is in contact with the membranes of the cells, the ameloblasts but lateral growth of the crystals continues many microns back from the tip.

### Mineralization On And Around Implants

Mineralization in medical devices places two contradictory roles. In osteopedic implants such as titanium hip and knee joints, titanium tooth implants and bone repair, it is desirable that bone attaches to the implant in order to provide a strong mechanical bond. Coating of these implants with a calcium phosphate layer does enhance the rate and degree of attachment of bone to the titanium [40]. The addition of bone morphogenic protein also seems to enhance attachment [41]. In the longer term bone may resorb due to stress shielding in the vicinity of the stiffer metal.

Titanium is osteoconductive in the sense that neighboring bone will attach. In areas where there has been considerable bone loss, to a cancer for instance, there is a need for osteoinductive materials that will cause initially detached bone to form [42]. Calcium phosphate glasses have also been shown to be osteoinductive [43, 44].

The work of Kokubo [45-47] has led many groups to identify various materials as bone compatible on the basis of their ability to form a coating of hydroxyapatite when exposed to a supersaturated solution ("SBF", synthetic body fluid). Many of the materials so identified contain silica and an important step may often be the formation of soluble calcium phosphosilicate polymers or colloidal particles which might be responsible for the initial nucleation of hydroxyapatite. Silica is known to be present in raised concentration during bone mineralization. DeGuire has shown that synthetic hydroxyapatite growth on a surface can be attributed to adsorption of nuclei or nanocrystals from solution [48, 49]. Given the complexity of such simple synthetic mineralization processes, it is premature to worry about what occurs at the surface of an actual implant.

#### MECHANICAL PROPERTIES OF NATURAL MINERALIZED MATERIALS

One early motivation for the study of mineralized tissues was that the toughness of shells and teeth is obviously superior to that of synthetic ceramics [50]. Likewise bone has a much better combination of stiffness and toughness than equivalent short-fiber reinforced polymer composites. After many studies of the fracture of nacre and other shell structures, we really do not have a firm understanding of where this toughness comes from.

The organic content of nacre is about 5% and it is difficult to attribute the toughness increase to the toughness of the polymer alone, even though it is obviously capable of drawing out into long fibrils that bridge cracks in the structure [51]. The most widely accepted model for nacre toughness is that the process resembles fiber pullout in synthetic composites. As the material cracks perpendicular to the platelets, they pull out across the crack and energy goes into shearing

the interlamellar polymer layers [52]. Other workers have pointed to the possible importance of interlocking bumps and holes in the lamellae [53, 54] or to the deformability of the aragonite platelets [55].

The crossed-lamellar structure of conch shell has an even lower organic content, less than 1%, but the crystals are divided into very fine platelets [56, 57]. It does have high toughness which could be attributed to the fine fibers, as is the toughness of minerals such as the jade family [58]. However, drying conch shell does greatly reduce the toughness, suggesting that the organic content is still important [59].

An important factor in mechanical properties, that has been stressed by Weiner in recent writings, is that biological microstructures are oriented in order to provide toughness or strength in the major stress directions [60]. While the majority of synthetic material are isotropic, the majority of biological materials have properties which are very dependent on direction. Biological microstructures then have to be designed to provide good properties along one axis, in a plane or occasionally in three dimensions. This approach is rare in the use of synthetic materials but it clearly would be possible to tune local microstructure to local stress state in a composite material.

In the case of bone, extensive microcracking precedes fracture but the detailed source of the cracking and how it contributes to toughness is unclear [61]. One suggestion is that it depends on the breaking and reformation of cross-links between collagen molecules [62]. This has been

discussed in terms of damage mechanics [63]. It is really not clear how partial or local damage should be treated in relation to the toughness of useful materials.

Many synthetic layered ceramic structures have been built to mimic nacre. Most show "graceful failure" in bending, in the sense of an initial fracture followed by a series of downward steps in stress before final fracture [64-66]. The energy to break is greatly increased. However, given the large thickness of the layers in these composites, the initial fracture leave the part severely damaged. It is not clear whether we would achieve significantly more damage tolerant or damage resistant materials if these structures were built on a much finer scale. For metal-ceramic layered composites, the volume fraction of metal that is needed is sufficient to really detract from the high-temperature resistance and high modulus of the ceramic [67].

#### MEDICAL APPLICATIONS OF HARD BIOMIMETIC MATERIALS

Orthopedic implants have major successes in the form of total hip and knee replacements for which there are many alternative designs in titanium and ceramic. A long term problem of wear of the ultra-high molecular weight polyethylene bearing surface is being improved by cross-linking processes. This leaves the long term bone loss due to stress shielding as a major problem. Given the major penalties associated with joints that fail after several years in use, there is little incentive to introduce new materials.

Biomimetic coatings for titanium implants have been commercialized [68]. They apparently offer a more soluble source of phosphate to promote bone growth and attachment.

More immediate needs for medical devices include good replacements for the smaller joints, methods to repair or replace damaged cartilage, better methods to repair damaged intervertebral discs, replacement for lost jaw bone and better systems to repair fractured or eroded bones at other sites. The current trend towards "keyhole" surgery also puts an emphasis on devices that can be introduced through a small incision with less trauma and less exposure to infection. In many cases the loads to be carried are low, compared to those in the hip or knee. This means that new medical devices will tend to be based on injectable cements, soft materials that later harden or expanding metal mesh. Ideally the device can be shaped in the operating room to suit the needed shape and size. Biomimetic materials can certainly have a role here.

The question of whether implanted materials should be permanent or resorbable is open. A material which undergoes wear or degradation to leave debris is bad and a device which requires a second removal operation after healing is bad.

There is great interest in the use of tissue engineering to grow, in the laboratory, organs for transplantation. Currently only skin has proved successful. One would assume that other organs would generally be grown on soft or plastic skeletons. However, a rigid, resorbable framework might have advantages in providing more support without occupying much space.

### SYNTHETIC APPLICATIONS OF BIOMIMETIC CERAMICS AND COMPOSITES

As mentioned above, there is a real need for short-fiber composites and ceramics with increased toughness. Mineralized tissues show that this should be possible. For high temperature applications of ceramics, such as turbine blades, a polymer component would be

unsuitable but there are many applications for ceramics at room temperature, including bearings, armor and cutting tools. At present there are no demonstration synthetic materials that offer properties good enough to make them highly desirable outside of medicine and no processing strategies that show how such materials could be made economically.

A composite that could be made economically with the mechanical properties of bone would find many applications as a substitute for metal parts. Likewise, a significant improvement over sheet molding compound would find applications in automobile bodies. The prospect of repetitive cycles of slow growth from aqueous solution makes the biomimetic approach unpromising. However, as Gower has implied [24], the new paradigm involving accretion of amorphous precursor particles suggests that structures could be built more rapidly, at least where sheets, rods or small parts were needed.

One other approach that is related to biomimetics and has proved successful is the formation of composites of clay nanoparticles with polymers. These offer some enhancement of stiffness with little cost in toughness, when compared to polymer alone. However they do also show good flame retardance, lower permeability and heat resistance at low cost [69].

## CONCLUSIONS

Biological materials show us that it would be possible to build superior properties into synthetic materials if we can control the composite structure on a fine scale. There are just a few examples where such a biomimetic approach has actually been adopted because methods for forming them have looked uneconomical. The newly-developing understanding of biological processing opens

the possibility of new biomimetic processing methods. In addition, technological trends towards soft electronics and the needs for more soft tissue implants in medicine both open new areas of application for biomimetic materials.

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