Design strategies in mineralized biological materials

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Organisms have been producing mineralized skeletons for the past 550 million years. They have evolved many different strategies for improving these materials at almost all hierarchical levels from Ångstroms to millimetres. Key components of biological materials are the macromolecules, which are intimately involved in controlling nucleation, growth, shaping and adapting mechanical properties of the mineral phase to function. One interesting tendency that we have noted is that organisms have developed several strategies to produce materials that have more isotropic properties. Much can still be learned from studying the principles of structure–function relations of biological materials. Some of this information may also provide new ideas for improved design of synthetic materials.

Many biological materials are known to have unusual mechanical properties, some of which are surprisingly advantageous, especially when taking into account the fact that they are formed at ambient temperatures and pressures.[1–3] This observation has inspired many studies of these materials over the last several decades aimed at discovering some of their structural 'secrets'. The mineralized biological materials represent an interesting subgroup within this vast world. Clearly the presence of the mineral phase and the manner in which the mineral and the organic material are organized are among the key factors that contribute to their unique mechanical properties.

Understanding just how this occurs is, however, not a trivial task, as the scale of ordered structures can vary from Ångstroms to millimetres. Furthermore, one level of structural organization is often nested into another larger-scaled structural type, to produce a very complicated overall structure.[4] In order to reveal the design strategies of these natural materials we not only have to understand their structural features in great detail, but we also need to identify the specific benefits that a particular aspect of the structure contributes to the bulk material. With all these difficulties, it is therefore not surprising that we still really know very little about the strategies used by organisms to form their superior materials.

In this review we will address key issues relating to mineralized biological material formation and function at different dimensional scales. We will start at the Ångstrom level and work our way up to the millimetre level. Our strategy will be to illustrate the concepts presented by focusing mainly on one family of important biogenic minerals, the calcium carbonates, and through them identify more general principles of biological materials design. We also discuss non-carbonate containing materials, such as bone, where appropriate. We will begin, however, with more general information about mineralized biological materials.

Materials with a history

Many endo- and exo-skeletons are composed of mineralized materials. They have one unusual advantage over other biological and synthetic materials—they have a history! Mineralized biological materials are preserved as fossils far more frequently than their unmineralized counterparts. In fact, until the early 1950s it was thought that the fossil record began with the advent of the Cambrian era (about 550 million years ago) when mineralized exoskeletons evolved. We now know that there are infrequent occurrences of preserved non-mineralized fossils in much older rocks.[5] The Cambrian fossil record, however, does provide us with good documentation of the very beginnings of skeletal evolution, and the remaining 500 million years can be viewed, from our perspective, as an 'extensive' product testing period. The evolution of biomineralization processes has been reviewed by Lowenstam and Weiner.[6]

Members of many different phyla began forming mineralized exoskeletons within several millions of years (at the base of the Cambrian).[6,7] This followed what was probably a very major extinction,[8] and in fact the phylogenetic groups that subsequently mineralized were themselves, for the most part, newly evolved. From this we can infer that there was probably some external ecological pressure on these organisms to develop mineralized materials (protection against predators is a favourite explanation),[9] and that the genetic 'backgrounds' of the mineralizers were still rather flexible and hence amenable to novel experiments. We also know that today all the more sophisticated mineralizers use similar families of proteins for controlling mineral nucleation and growth, implying that underlying mechanisms common to many taxonomic groups do exist.[10] We do not, however, know if this capability was divergently inherited from a common ancestor or convergently arrived at independently by each group.

These first skeletal mineralizers did not all use the same mineral. About a third opted for a calcium phosphate mineral (carbonated apatite) and most for a calcium carbonate mineral. Of the latter, almost all chose calcite from among the family of $CaCO_3$ polymorphs. One phylum deposited amorphous silica (also known as opal) and one group of bacteria, magnetite.[6,11] Presumably other minerals were also used, but these have not yet been discovered or were not preserved. So environmental conditions at the time, such as the sea water chemistry, did not apparently affect their options. Nor is there any reason to believe that the capabilities of these early mineralizers were limited. They formed an incredible array of morphologically varied structures, with a variety of minerals and presumably macromolecules as well.[12]

One enigmatic observation emanating from the Cambrian fossil record is that the microstructures of many of the mineralized skeletons of the Cambrian ancestors were remarkably similar to their living counterparts.[12] For example, the skeletons of the Echinodermata have very characteristic sponge-like structures made out of relatively large single crystals of calcite. The earliest Cambrian fossils of this phylum have the same

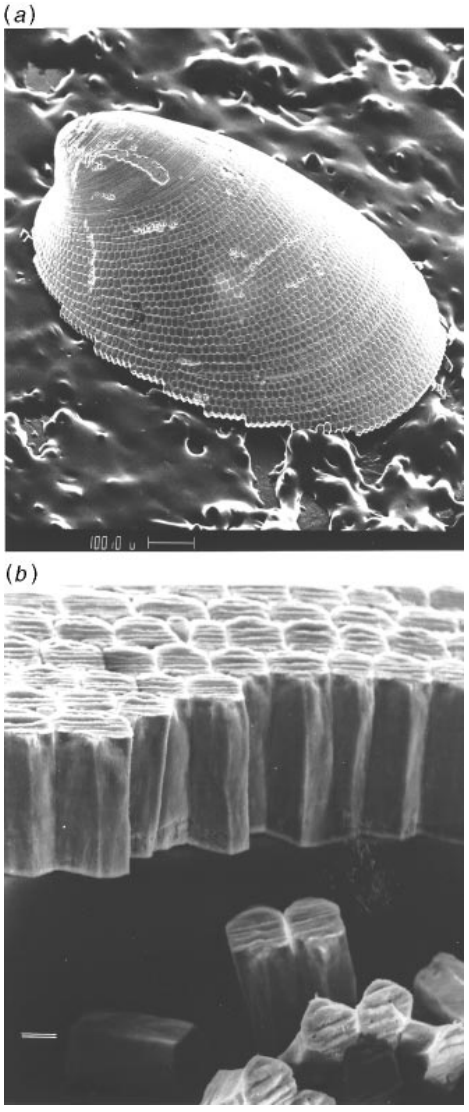

Fig. 1 (*a*) Shell of the primitive mollusc *Neopilina hyalina* showing the prismatic structure of the adult shell. (*b*) High magnification view of the aragonitic prisms. Scale bar: 10 μm.

basic structures. Similarly, some of the earliest known mollusc shells were composed of prisms;[12] a structural motif still common to this phylum today (Fig. 1).[13] Even the best preserved fossils are altered during the passage of time, and we cannot be sure that macroscopic morphological conservation is also indicative of microscopic and molecular structural preservation.

Studies of modern mineralized tissues show that organisms use many different minerals and macromolecules, and these are organized into innumerable structural motifs. Bearing in mind that many of the minerals are actually rather poor building materials, we can guess that with the constant competition to survive, biological materials were continuously put to the test and modified to meet the challenges; hence their enormous diversity. Identifying the 'solutions' they found to these challenges makes their study so special—a theme we will pursue in this review.

Components of mineralized biological materials

Minerals, macromolecules and water are the major components of these materials. The vast majority of biological materials contain only one mineral type. Where two or more minerals are present, they are usually in different locations, such as the inner and outer layers of mollusc shells. More than 60 different minerals are known to be formed biologically, but only a small subset of these are common components of endo- and exo-skeletons.[6,14] These include the two polymorphs of calcium carbonate, calcite and aragonite, and amorphous silica. Less commonly used minerals are vaterite, amorphous calcium carbonate and phosphate, and crystalline carbonated apatite. The latter is used almost exclusively by the vertebrates and a few invertebrate groups. Quantitatively they do not constitute a significant portion of the biogenic minerals formed today.[6,14]

Among the biological materials that are formed under relatively controlled conditions, we differentiate three types based on the organization of their mineral constituents. The one type is composed of multicrystalline arrays, in which the individual crystals are generally all aligned at least in one direction, and often in all three directions. The best known examples of such materials are bones, teeth and shells of various types. In the second type, a single crystal or a limited array of relatively large crystals constitutes the entire structure. The echinoderms are best known for forming such large single crystal skeletal structures of calcite. Many spicules that are used to stiffen organic structures[15] are also composed of single crystals, and again usually calcite. The third group produce biological materials containing an amorphous mineral, the most common being amorphous silica. These structures can vary enormously in size and particularly in shape. Fig. 2 shows an example of each type.

The components that perhaps most distinguish biological materials from synthetic materials are the biological macromolecules. They form an intimate mix or composite with the mineral phase at all different hierarchical levels, starting at the scale of nanometres. In all the mineralized tissues in which the macromolecules have been even partially characterized, they are found to be very diverse and heterogeneous. In fact, initially it was thought that the macromolecular constituents were more or less unique to each mineralized material. This impression changed substantially more than a decade ago when it was recognized that many of these macromolecules have common chemical attributes—they are rich in carboxylate groups.[10] These may be constituents of the protein moieties and/or the polysaccharide moieties. Many of this class of macromolecules also have, in addition to the carboxylate groups, phosphate and/or sulfate groups. The presence of all these charged groups makes these macromolecules excellent candidates for interacting with the mineral ions in solution or with the surfaces of the solid phase.[16–18] For convenience, we will refer to the members of this class as 'control' macromolecules.

Our studies of the control macromolecules of calcium carbonate-containing biological materials from several phyla suggest that this class can be somewhat arbitrarily subdivided into several groups. One is the aspartic acid-rich proteins and glycoproteins, which tend to be associated with the crystalline mineral phases. A second group is the glutamic acid (and/or glutamine)- and serine-rich glycoproteins, which are the major components of the several amorphous $CaCO_3$-containing mineralized tissues we have examined recently from two widely diverging taxa.[19] A third group is characterized by being relatively rich in polysaccharides, with proteins containing fairly average (*ca.* 10 mol%) amounts of Glx, Asx and Ser. These macromolecules are the major components of echinoderm skeletons and are minor components of mollusc shells.[20]

The control macromolecules are usually the quantitatively minor macromolecular components of a biological material. The major components are more hydrophobic, often cross-linked and are hence relatively insoluble in mild acids or at neutral pH. They can vary considerably from tissue to tissue and in many cases they are indeed tissue specific.[6] Unlike the control macromolecules which are difficult to extract or degrade without dissolving the mineral, these macromolecules can often be extracted or degraded chemically in the presence of the mineral, implying that they are less intimately associated

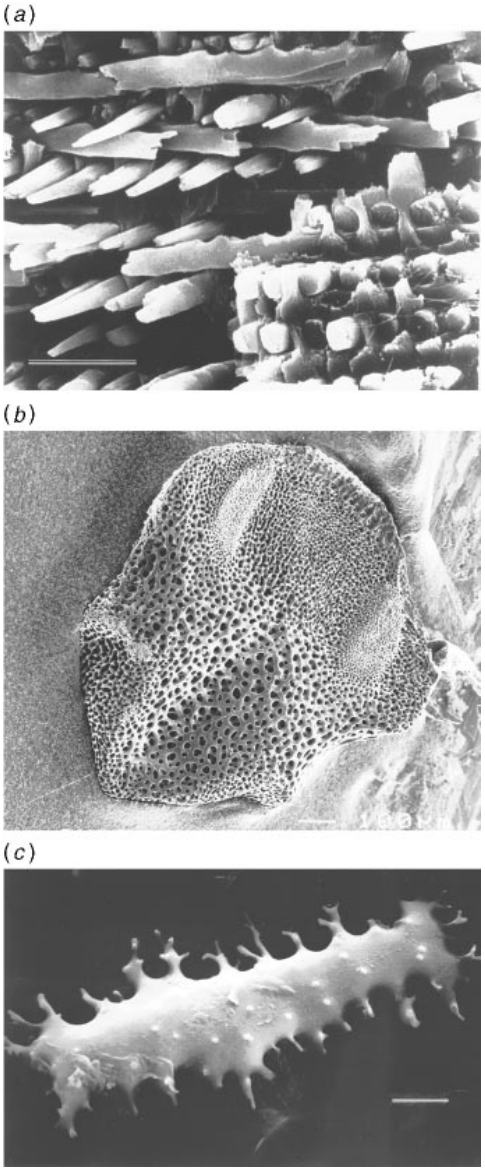

Fig. 2 (*a*) Tooth enamel of the incisor of a rat. Each elongated rod is composed of hundreds of spaghetti-shaped crystals of carbonated apatite. Scale bar: 10 μm. (*b*) A ventral plate from the arm of the brittle star *Ophiocoma wendti* (Echinodermata). The whole structure is one single crystal of calcite. Note also the spongy stereom structure changes in texture in different parts. (*c*) Amorphous silica deposit in the cell walls of the wheat plant *Triticum aestivum*. Scale bar: 10 μm.

with the mineral phase. They have been referred to as 'framework macromolecules', a term which alludes to their major conceived function, namely providing a three-dimensional matrix in which the mineral phase forms, and a substrate from which some of the control proteins interact with the mineral phase.[10] Common examples of framework macromolecules are Gly- and Ala-rich proteins (structurally similar to silk-fibroin) in mollusc shells, type I collagen in bone and tooth dentin, amelogenin in tooth enamel, α-chitin in crustaceans and β-chitin in mollusc shells.[6]

There are some interesting cases, such as tooth enamel, where the framework proteins are broken down enzymatically and removed during mineral formation.[21] This is presumably to allow the crystals to grow larger and form a very dense and mechanically resistant outer layer for vertebrate teeth. In vertebrate bone, certain mollusc shells and echinoderm skeletons, the originally formed mineralized composite material may be locally removed to remodel the material as growth alters its functional requirements, or to replace, in the case of bone, older more mineralized and probably mechanically weaker bone with new stronger bone.[22] This type of control, like all control, is exercised through the cells directly associated with the tissue. These cells communicate with other cells in order to orchestrate the complex processes of tissue formation. So in a very real sense, the study of the design features of biological materials in general, reveals the 'intelligence' and often amazing capabilities of living cells.[14] It is therefore unlikely that we can mimic these processes synthetically. We can, however, try to elucidate the design principles and use them to improve our synthetic materials.

We will now examine control processes, starting at the Ångstrom level, and progress through to the millimetre level.

Control at the level of Ångstroms: from the dissolved molecules to the crystal

All mineralization first involves Ångstrom level processes that start with a solid phase forming from solution. The test case that we choose to follow in detail is calcium carbonate precipitation, either in the crystalline form or as an amorphous hydrated phase. Calcium carbonate crystallizes in five different polymorphs, and in addition, an amorphous form. Calcite, aragonite and vaterite are stable under appropriate conditions, while calcium carbonate mono- and hexa-hydrate and amorphous calcium carbonate are very unstable and hence rare in non-biological environments.[23] In the biological world, there are very few examples of the monohydrated form,[6,24] and no known example of the hexahydrated form. Vaterite is present in some ascidian spicules,[25] in a variety of gravity receptors[6] and in the egg shells of some gastropods,[26] but is, as a whole, also quite rare in biomineralization. The highly unstable amorphous calcium carbonate[27] is produced and stabilized biologically, and in fact may be much more abundant in biomineralization than is currently believed. By far the most abundant forms of calcium carbonate produced biologically are calcite and aragonite. It is thus appropriate to consider these two structures in some detail.

Calcite and aragonite crystal structures

Aragonite and calcite have very similar crystal structures and thermodynamic stabilities.[23] The former is slightly less stable than the latter at ambient temperatures and pressures, but is very common in biomineralization.[6] Both calcite and aragonite crystal structures are composed of alternating layers of calcium ions and carbonate ions perpendicular to the *c* axis (in the *ab* plane) (Fig. 3).[23] The calcium ions occupy almost the same lattice positions in this plane, and in both structures the carbonate ions lie with their molecular planes parallel to the *ab* layer. In aragonite, however, some of the carbonate ions are raised in the *c* direction to form two layers separated by 0.96 Å, and their orientations in the two layers are different.

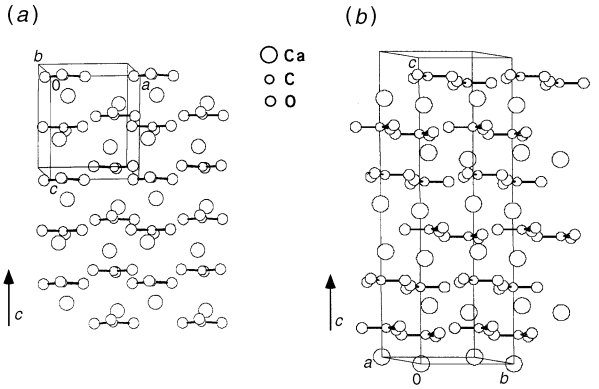

Fig. 3 Crystal structures of (*a*) aragonite and (*b*) calcite. Note that the *c* axis has been tilted out of plane by 5° to improve perspective.

This shift is the basis for the very different properties of these two phases.

The optimization of the interactions in aragonite allows better packing, and consequently the density of this phase is higher than that of calcite. In aragonite growth is preferred along the *c* axis, relative to the other crystallographic directions. Thus under conditions of normal temperature and pressure, aragonite forms as thin needles (acicular crystals) that do not generally grow into large crystals. Even when they appear to do so in some biogenic crystals, the large crystals are in reality highly twinned, *i.e.* formed of polycrystalline domains.[28–30] Synthetic calcite, in contrast, grows as almost isotropic rhombohedra delimited by a set of equivalent oblique faces; {10.4}† in the hexagonal notation. The stability of these faces is easily understood from the closely packed arrangement of calcium and carbonate ions along the layer. A layer of high stability (large layer energy that holds the ions together within the layer) is, however, always accompanied by a proportionally low attachment energy (the energy that holds parallel layers together). We note that the sum of the layer energy and attachment energy is constant, because it corresponds to the bulk energy of the crystal.[31] Thus, the stability of the {10.4} layers in calcite is also the reason for its mechanical weakness, and hence the cause of extreme brittleness. The calcite crystal cleaves easily along its {10.4} planes, called 'cleavage rhombohedron' planes, where a crack can propagate along a minimum-energy pathway (with minimum dispersion of energy).[32] In contrast, there is no such plane of easy cleavage in aragonite.[33]

In biology the two polymorphs are used widely as building materials and the choice of polymorph used is almost always under strict genetic control. It would appear, therefore, that one polymorph offers some advantages over the other, even though both have very similar lattice energies and the same composition. Aragonite has the advantage of not having cleavage planes, but has the disadvantage of its small size and needle-like morphology. It also has a strong tendency to form spherulitic clusters of crystals with high porosity. Calcite, on the other hand, tends to form larger crystals, but these are very brittle. An examination of the distribution of aragonite and calcite among mineralized biological materials does not produce any simple or clear-cut answers as to the reason for polymorph selection by organisms.

It does appear to be true that when large single crystals of calcium carbonate are used as skeletal parts, such as in echinoderm spines and tests and in sponge spicules, they are normally composed of calcite. The large prisms of the prismatic layer of mollusc shells are also usually built out of calcite. Some molluscs do, however, produce aragonitic prismatic layers. There is no obvious advantage or reason for this choice. In contrast, the molluscan nacreous tablets are always composed of aragonite, although very similar structures are produced by some bryozoans out of calcite.[34]

We know that organisms are able to circumvent the problems arising from calcite brittleness (see the section on control at the nanometre level). It is, however, not at all clear whether organisms 'relate' to the calcite–aragonite dichotomy with the same simplistic mechanical analysis as we would deduce from their basic properties. Whatever the reason behind the choice of one polymorph rather than the other, the key step in polymorph determination must be crystal nucleation. Polymorph control during nucleation is thus the next subject to be considered.

† The notation {*h*,*k*,*l*} indicates the family of symmetry-related faces or planes. (*h*,*k*,*l*) indicates only one member of the family. [*h*,*k*,*l*] indicates the direction of the vector perpendicular to the plane. When the notation (*hk*.*l*) is used *e.g.* (10.4), the period is in the plane of the fourth index, *i*, in the hexagonal system.

Calcite–aragonite nucleation

An easily conceived way of inducing nucleation of an ionic crystal is from a cationic plane. This only requires initial concentration and complexation of ions from solution onto a matrix substrate with negative charge. Such substrates are very abundant in biology. This mechanism was shown to operate in artificial systems, when crystallization was induced from various monolayers of long chain fatty acids deposited at the air/water interface. The first reported example involved the oriented nucleation of sodium chloride crystals from the homocharged (111) plane under monolayers of stearic acid.[35] Mann and co-workers[36,37] subsequently performed analogous experiments on supersaturated calcium carbonate solution subphases, whereby oriented crystallization of calcite and vaterite from homocharged cation layers was obtained. The oriented nucleation of calcite from polystyrene surfaces decorated with sulfonate and carboxylate moieties was studied in our group as a model for the nucleation process occurring in mollusc shell formation.[38] A similar mechanism was also shown to operate when acidic proteins extracted from the mollusc shells themselves were adsorbed on rigid plastic substrates.[39]

Nucleation only by concentration of charge should be, at first approximation, non-specific. No repulsion is created between the cationic crystal layer and the anionic matrix layer, even if the positions of the ions in the two layers do not match perfectly. Calcite has two homocharged calcium planes, (001) and {01.2}, whereas aragonite has one, (001). Furthermore, in calcite and aragonite the calcium ion positions on the (001) plane are, as noted, practically identical. If the only driving force for nucleation was the recruitment of positive ions on a negatively charged surface, the most stable polymorph should always be formed. Indeed, only calcite was nucleated from the (001) plane on acidic macromolecules adsorbed on plastic, irrespective of whether the nucleating macromolecules had been extracted from calcitic or aragonitic mollusc shell layers.[39] It is therefore difficult to conceive that only nucleation of this type can be responsible for polymorph control.

Mollusc shells are among the best studied $CaCO_3$-containing biological materials. They are composed of either calcite or aragonite. In some cases both polymorphs are present, but are always separated in different layers (Fig. 4).[13] Both calcite and aragonite crystallize from their (001) planes. The same organism always produces the same polymorph at the same site. One conceivable strategy could be the involvement of an inhibitor of the stable polymorph in solution, while the

Fig. 4 Fracture surface through the shell of the bivalve mollusc, *Mytilus californianus*, showing the outer calcitic prismatic layer (top) and the inner aragonitic nacreous layer (bottom). Scale bar: 10 μm.

substrate proteins are responsible only for nucleation and orientation of the crystal. The obvious candidate in biological mineralization was considered to be magnesium, which is known to favour aragonite formation by inhibiting calcite growth.[40–42] In fact aragonite precipitates out of evaporating sea water because of its high concentration of magnesium. Whatever the controlling element, chemical or structural, it must be present selectively in the microenvironment where the crystal forms. The microenvironment of nucleation is thus the key to understanding the process.

Mollusc shell nacre is the best studied tissue in this respect. Crystallization occurs inside a pre-deposited matrix,[43] composed of thin layers of β-chitin sandwiched between two thicker layers of silk fibroin-like proteins, onto which acidic macromolecules are adsorbed.[44,45] The fibre axis of the chitin and silk proteins are perpendicular to each other, and aligned with the *a* and *b* axes of the aragonite tablets, respectively (Fig. 5).[46,47] This well defined spatial relation between substrate and overgrowth phase suggests an epitaxial mechanism of nucleation.

Surprisingly, the same mollusc shell acidic macromolecules that exclusively induced calcite formation when adsorbed on plastic substrates, were shown to retain polymorph specificity in an appropriately assembled artificial microenvironment, designed to match roughly the biological one.[48] The acidic glycoproteins associated with calcitic prismatic and aragonitic nacreous layers of various mollusc shells were adsorbed on an artificial assembly of β-chitin (from squid pen) and silk (from silkworm cocoons). Neither of these matrix components is calcified in the original tissue. Once adsorbed on this scaffold, the macromolecules extracted from aragonitic mollusc shell layers induced aragonite formation, while those extracted from calcitic layers induced calcite formation, with total fidelity. When no acidic macromolecules were introduced, only vaterite spherulites formed on the chitin surface layers. The orientation of the nucleated crystals relative to the inducing proteins is not yet known. If these crystals are nucleated from the (001) plane, similar to their orientation *in vivo* and *in vitro* after adsorption on plastic, it would be tempting to conclude that a three-dimensional nucleation site fixes the carbonate positions, in addition to those of the calcium ions. The structural requirements for such a nucleation site, however, appear to be prohibitively stringent. Another possibility is a combined nucleation–inhibition mechanism, but in this case the inhibition and nucleation must involve one or more proteins. It certainly does not involve magnesium which was absent in the experiment. At present we do not understand the mechanisms involved *in vitro*, and certainly not *in vivo*.

The resulting calcite- or aragonite-impregnated chitin, although not as well organized as in mollusc shells, possesses some features of composite materials at the nanometre scale (Fig. 6). This is the scale at which the chitin fibres are intergrown intimately with the crystallites. Interestingly, in the artificial assembly the single crystalline domains within the polycrystalline spherulites of calcite preserve the size and morphology typical of the mineral. Calcite crystallites range up to 500 nm in size and develop well defined {100.4} cleavage rhombohedron morphologies, while the aragonite crystallites achieve a maximum size of 150 nm and have ill-defined elliptical shapes.

Belcher *et al.* studied a different *in vitro* system, using as a nucleating matrix the so-called 'green layer' sheet isolated from abalone shells.[49] They also observed aragonite crystallization when proteins extracted from abalone shell aragonitic phase were added to the green layer, and calcite crystallization when calcite-extracted proteins were added.

Nucleation of calcite and aragonite from the (001) plane is common in biomineralization. Well studied examples are, in addition to mollusc shells, coralline algae,[50] calcareous sponge spicules[51] and sea urchin larval spicules.[52] There is also evidence of oriented nucleation of calcite from the homocharged (01.2) layer. This occurs in certain scimitar-shaped calcareous sponge spicules.[53] The calcite crystal forming the spicule is oriented such that the [01.2] direction is always along the spicule axis. In addition, the *c* axis direction is uniquely fixed such that the positive end always points out of the convex part of the spicule. The combination of the asymmetric spicule morphology and its uniquely defined relationship to the crystal axes orientation can only be explained if the nucleation surface structure is totally controlled. This includes distinguishing

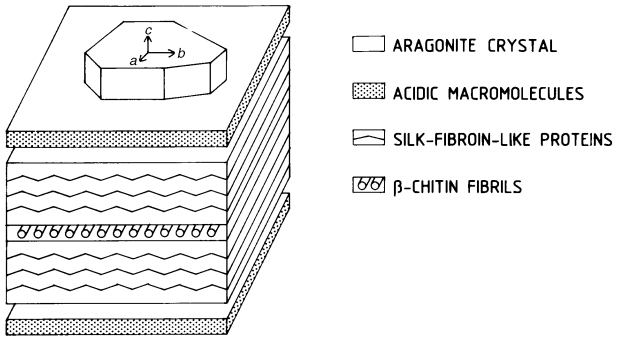

Fig. 5 Schematic block diagram showing the spatial relations between the crystallographic axes of an aragonitic nacreous polygon and the underlying organic matrix. [From *On Biomineralization* by H. A. Lowenstam and S. Weiner. Copyright © 1989 by Heinz Lowenstam and Stephen Weiner. Used by permission of Oxford University Press, Inc. (ref. 6 of this work)].

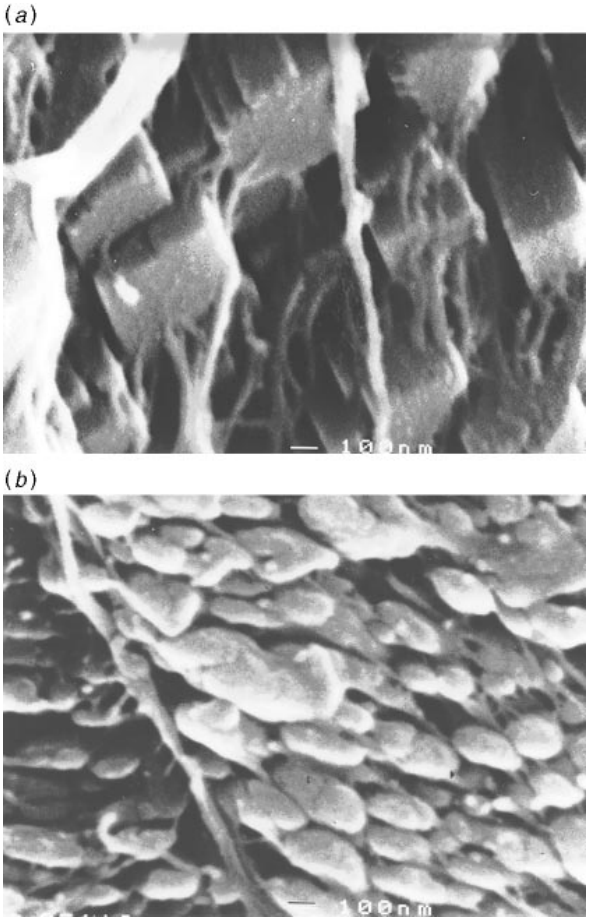

Fig. 6 Synthetic composite materials produced *in vitro* containing (*a*) calcite and (*b*) aragonite crystals in a matrix. The matrix is composed of β-chitin and silk fibroin, as well as soluble proteins from the calcitic shell of *Atrina serrata* in the case of (*a*) and from the aragonitic shell of *Elliptio* sp. in the case of (*b*).

between the positive and negative surfaces of the (01.2) layer. This is equivalent to saying that the nucleated surface is chiral and defined in three dimensions. Note that the calcite structure is not chiral. Mann and Sparks pointed out an analogous case in coccoliths,[54] where the morphology of the single crystals is asymmetric and uniquely defined, suggesting chiral recognition at the nucleation stage. Furthermore, Berman *et al.*[55] recently studied the nucleation of calcite under monolayers of polydiacetylene carboxylates. They observed nucleation from the (01.2) plane, as well as orientation within the plane relative to the polymer backbone direction. This implies that almost complete control over the nucleation site geometry may be achieved under artificial conditions. Whether there is any advantage in such a high level of nucleation control in biology is not clear. What is clear is the enormous intrinsic controlling power of some of the biological nucleation processes.

Control at the nanometre level: crystal growth and morphology

The next step, following crystal nucleation, is the growth of the crystals into desired shapes and sizes. Crystals grow by progressive addition of molecules or ions onto the crystallization nucleus. Growth in the various directions is governed kinetically by rules determined by the crystal structure and symmetry. Molecules will be added faster where the balance of the interactions with the existing crystal is more favourable. In general, adding a molecule within a growing crystal layer is more favourable than creating a new layer. The first molecule of a new layer makes contacts only with molecules of the underlying layer, while a molecule added at a growing step or kink establishes contacts in two or three directions. Thus crystals normally grow in layers, and are delimited by a well defined set of faces. Spherical smooth surfaces are only observed above the so-called roughening transition, where the driving force to growth is so large that adding a molecule in any position does not make, kinetically, any difference.[56]

The growth morphology of crystals is determined by their relative rates of growth in the various directions. For example, if it is much easier to add molecules in one unique crystallographic direction relative to all others, the crystal will develop as a needle. On the other hand, if the energetics involved in adding layers of molecules in all directions are approximately equivalent, the resulting crystal will be roughly isotropic in shape. The slow growing directions are the ones that determine the crystal morphology, with the layers perpendicular to them developing as stable faces (having high layer energy and low attachment energy).[31]

Each crystal thus has a typical growth morphology under a given set of conditions. These include physical parameters such as temperature, pressure and supersaturation, and chemical parameters, such as interactions with the solvent and with co-solutes. In particular, both co-solutes and solvent may act as inhibitors of crystal growth in specific directions.[57] If they are adsorbed on certain crystal planes rather than others, crystal growth will be slowed down in the directions perpendicular to the planes. A set of faces parallel to the plane may consequently develop, or increase in morphological importance, when already present. Macromolecular inhibitors, that structurally match the molecular motif on one set of crystal planes, may interact with these planes from solution in a manner equivalent to the process described for nucleation. This results in modulation of crystal morphology through the above mechanism.[18]

In biology, the microenvironment where crystallization occurs is the key to the control over crystal growth, as well as nucleation. Crystals are generally formed in pre-defined spaces, delimited by extracellular matrices and cell membranes, or inside vesicles.[14,58] Inside these defined spaces the crystals grow under shape, size, concentration and composition constraints imposed directly or indirectly by surrounding specialized cells. In the simplest scenario, crystals grow in a solution containing the component ions, not dissimilar from conventional growth from solution in non-biological environments. The crystals tend thus to assume their regular growth morphology. Growth is stopped only by contact with neighbouring crystals. This is probably the case for calcite crystals in egg shells[59] and aragonitic crystals in scleractinian corals,[60] and fish otoliths[6] that grow as polycrystalline bundles off predisposed nucleation centres (Fig. 7). As orientation is not well controlled during nucleation, a less tightly packed material is formed, which is porous and brittle. The bulk material produced reflects this process, and is thus relatively weak.[1] The properties of the material are therefore controlled to a large extent at the level of nucleation, by the density and the relative geometry of the nucleation sites. In mollusc shell nacre and simple prismatic layers only one well oriented crystal originates from each nucleation site. The lateral growth of both aragonite and calcite crystals is also limited only by meeting the neighbouring growing crystals, resulting in a typical honeycomb structure of irregular polygons (Fig. 8).

There are many examples in biomineralization where single crystals grow as separate entities with well defined individual morphologies and sizes that are very different from their non-biological counterparts. All these crystals grow inside closed

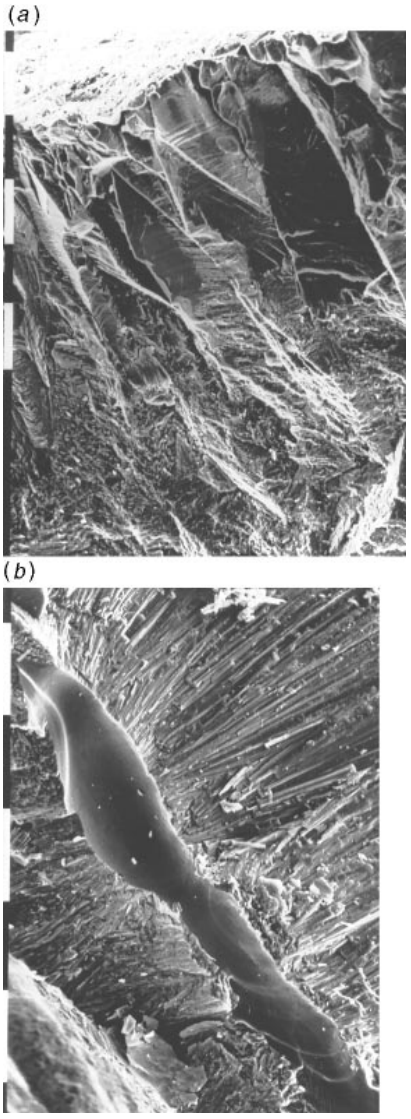

Fig. 7 Fracture surfaces of (*a*) the calcitic egg shell of the domestic hen, and (*b*) an aragonitic otolith from the bony fish *Seriphus politus* (reproduced with permission from ref. 18, p. 158). Scale bars: 0.1 mm.

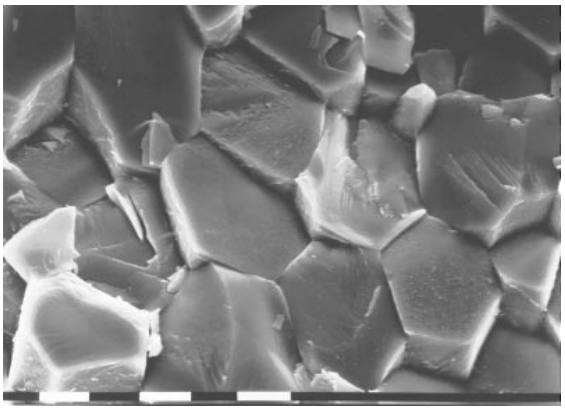

Fig. 8 Fracture surface of the prismatic calcite layer of the shell of the mollusc *Atrina serrata* showing the polygonal crystals. Scale bar: 10 μm.

spaces delimited by lipid bilayer membranes or macromolecular matrices. It could be envisaged that shape is established directly and only by the membrane or matrix, by simple mechanical interference. It is, however, necessary to invoke a mechanism whereby an intrinsically soft and deformable barrier can overcome the forces acting on it by the growing crystal. Other mechanisms are conceivable, that probably operate together with the membrane/matrix to achieve final shape determination. These are induction of growth in controlled directions and active growth inhibition. In the induction scenario, the component ions may be delivered into the crystallization space at specifically controlled sites, such that growth can occur only in certain directions. An example is the sea urchin larva where calcium preferentially enters the vesicle close to the fast growing tips of the spicule.[61] This would imply a close proximity of the ion pumps presumably in a membrane with the growing mineral, at least during the final stages of growth. It has also been observed in sea urchin larval spicules that the just-nucleated crystals display the regular {10.4} faces of calcite.[62] The formed spicules, however, always terminate with smooth and curved surfaces. The formation of such curved surfaces is in itself difficult to understand. It probably requires some other mechanism that keeps all the growth sites on the crystal surface active, similar to the situation occurring above the roughening transition.[56] One possibility, but by no means the only one, would be an inhibition process that, by interfering continuously with the completion of the crystal layers, would generate a surface composed of steps in the nanometre scale. Such inhibitors may even be active during the entire crystal growth process.

In the inhibition scenario for controlling shape, growth inhibitors are delivered into the solution and are adsorbed actively onto the growing crystals in controlled directions. This mechanism presents an additional attractive possibility, of actively modifying the properties of the crystal bulk, while modulating its shape. Some of the inhibitors adsorbed at the crystal surface are eventually overgrown, and remain occluded inside the crystal, specifically along the planes where they have been adsorbed. If these are sheet-structured macromolecules, and adsorption occurs with a high enough frequency, the final result is a kind of fibre-reinforced reversed composite material.[18] The host crystal constitutes a continuous matrix that is hard and often brittle. The guest macromolecules embedded inside it are the fibres or sheets that endow the crystal with pliancy and increased resistance to brittle fracture. In the case of calcite one may envisage that any type of interference with the propagation of cracks along the cleavage planes would reinforce the crystal against fracture, by both deviating and absorbing the propagating crack energy.[32] This mechanism appears to be exploited as a reinforcement strategy by organisms that choose to build single crystal skeletal elements. Emlet measured the Young's modulus of sea urchin larval spicules and indeed showed that it is quite different from pure calcite.[63] We, however, suspect that this may be also due, in this particular case, to the presence of some amorphous calcium carbonate[79] (see stabilization of amorphous calcium carbonate).

Sea urchin spine

It has been long recognized that sea urchin spines are each composed of one single crystal of calcite (based on polarized light and X-ray diffraction), with the *c* axis of the single crystal oriented along the morphological axis of the spine.[64] The single crystal grows inside a membrane (syncitium) in communication with many cells that provide the ions and all other biological components necessary for crystal growth and shaping.[65,66] The cells populate the meanders of the channels (stereom) running all along the spine in a continuous structure. The spine grows by elongation at the tip and thickens on all the peripheral surfaces. The result is a convoluted spongy element. This is later filled in with mineral, giving rise to a radial structure of full sectors of calcite, connected by spongy septa (Fig. 9). The mature spine still diffracts X-rays as a single crystal. The presence of channels not only provides a means for the cells to populate the whole spine, but also contributes to the mechanical performance of the material. Spongy structures are both lightweight and more elastic than full structures.[1] However, the typical size range of a septum in the stereom, *ca.* 1 μm, is still very large relative to the size of the unit cell of the crystal. Fracture of the septa could thus still easily occur along the cleavage planes of calcite, but in fact does not (Fig. 10). The organism adopts the reversed composite material approach to further reinforce the crystal against fracture.[67,68]

Glycoproteins are trapped inside the spines in amounts of *ca.* 0.02% by mass of mineral. New calcite crystals, grown epitaxially on the cleaned spines develop, in addition to the stable {10.4} faces, a set of unstable faces, slightly inclined to the *c* axis [of index {01,*l*}, with $l \approx 1.5$].[69] The original spine

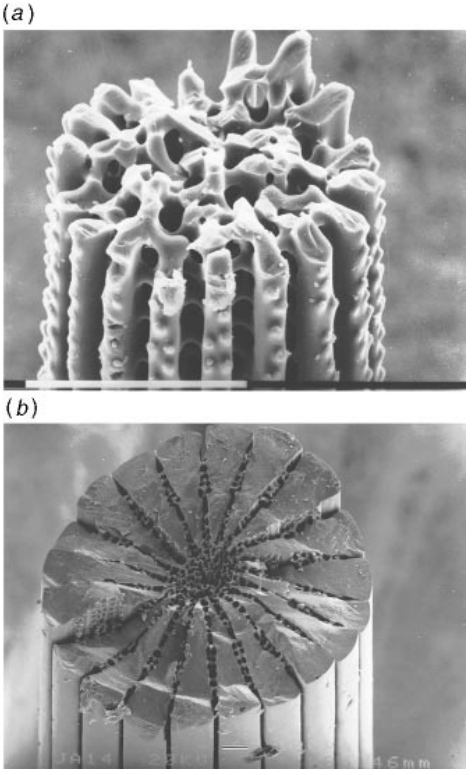

Fig. 9 Fracture surfaces of (*a*) immature and (*b*) mature spines of the sea urchin *Paracentrotus lividus*. Illustration (*a*) is reproduced with permission from ref. 18, p. 159. Scale bars: 0.1 mm.

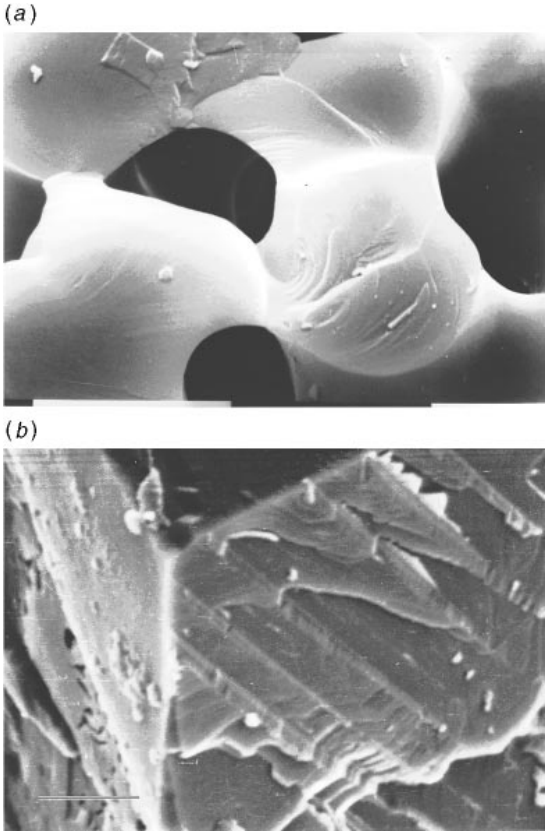

Fig. 10 High magnification views of the fracture surfaces of (*a*) the calcitic sea urchin spine showing conchoidal cleavage, and (*b*) a synthetic calcitic crystal showing the smooth surfaces of the {10.4} cleavage planes. Scale bars: 10 μm.

concomitantly becomes etched, suggesting that glycoproteins leaked out of the etched surface and readsorbed at the growing crystal surfaces along the $\{01, l\}$ planes. In agreement with this interpretation, calcite crystals grown *de novo* from a solution containing the same glycoproteins released from the spines after dissolution, developed the same morphology as the overgrown crystals. The crystals grown in the presence of the glycoproteins are also mechanically more resistant to fracture than pure calcite. They cleave with a conchoidal-type fracture similar to the biogenic spines, and are very different from the pure calcite crystals.[67] The latter shatter easily under an applied force, with the fracture lines always being along the cleavage planes of calcite (Fig. 10). Recently, Albeck *et al.*[20] showed that the key constituents of the glycoproteins that interact with the growing crystals involve oligosaccharide chains linked to the polypeptide chain in tightly structured clusters.

Modulation of crystal texture

To gain more insight into how macromolecules are occluded inside single crystals, we have mapped by X-ray diffraction the microtextures of a series of biogenic single crystals of calcite from various organisms.[70] A macromolecule is far too large to be incorporated into the perfect lattice of a single calcite crystal. Thus, when it is adsorbed and eventually overgrown by the growing crystal, its presence will leave a permanent imprint inside the crystal, in the form of an imperfection. Imperfections always exist even in the most perfect crystals.[71] Their distribution can be characterized by means of the diffraction behaviour of the crystal. Diffraction originates from domains of perfect structure, and the sharpness of the diffraction peaks is inversely proportional to the size of the perfect domains. Imperfections intercalated along certain crystallographic planes limit the size of the domains to the distances that separate contiguous imperfections (the coherence lengths). They affect maximally the width of the diffraction peaks from a set of planes containing the imperfections, but not from planes forming a wide angle with them.

Three-dimensional mapping of the distribution of imperfections in ten sets of biogenic calcite single crystals of very different shapes (sea urchin spines and larval spicules, five different kinds of calcareous sponge spicules, single prisms from mollusc shells and two kinds of foraminifera shells) showed in seven cases out of nine a striking correspondence with macroscopic crystal shape.[53,70,72] There is thus a link between textural properties at the nanometre level and crystal shape at the sub-millimetre level. One possibility we proposed is that the macromolecules shape the growing crystal by specific adsorption onto some crystal faces and not others. One exception is the so-called slender monaxon spicule from the calcareous sponge *Sycon*, that does not contain any occluded protein. Its texture is isotropic, as is the texture of pure calcite. The second exception is the prisms from the shell of the mollusc *Atrina*. The prisms are elongated along the *c* axis, but the coherence length is shorter along *c*, indicating higher protein intercalation in that direction. This is, however, also the only case we studied of a single crystal taken from a polycrystalline assembly (the prismatic layer), where crystal growth occurs in a preformed organic matrix. Growth in the lateral directions is stopped by the matrix and/or by the adjacent crystals. Furthermore, we have independent proof that the main components of the intracrystalline macromolecules, proteins rich in aspartate, are indeed intercalated along the (001) planes. We thus conclude that in *Atrina* a different mechanism is operating in the determination of crystal morphology.

The correspondence between coherence length distribution and shape is particularly striking for the curved monaxon spicules and asymmetric triradiate spicules from the calcareous sponge *Sycon*.[72] As noted, the scimitar-shaped curved monaxon is elongated in the general direction [01.2]. The circular section of the spicule contains many non-equivalent crystallographic directions, and correspondingly the coherence lengths (l_c) are almost identical, $l_c \approx 1500$ Å. On the other hand, of the three equivalent {01.2} reflections, the one along the morphological axis of the spicule has $l_c \approx 8000$ Å. The other two, inclined to the morphological axis by 60°, have $l_c \approx 2000$ Å (Fig. 11). This phenomenon can only be explained by assuming an accurate, nanometre-scale controlled delivery of the proteins

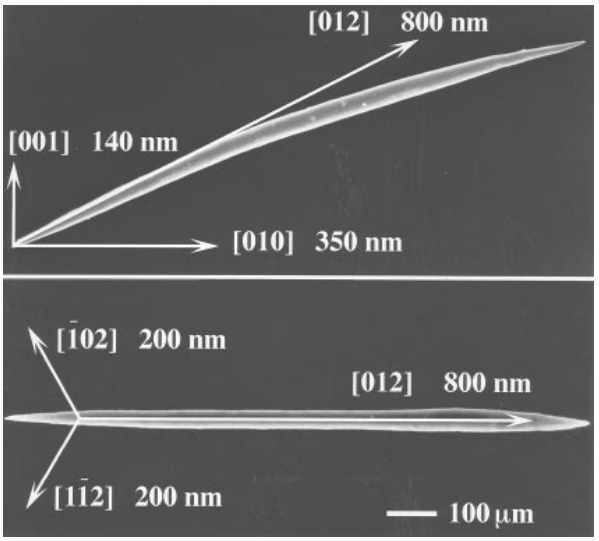

Fig. 11 Two views of the calcitic curved monaxon spicules from the calcareous sponge *Sycon* sp. The lengths of the superimposed arrows are proportional to the coherence lengths in the crystallographic directions indicated.

onto the growing crystals. Interestingly, protein intercalation is mirrored by the mechanical properties. Microindentation performed on polished longitudinal sections of the spicules results in anisotropic crack propagation along the spicule, in the same unique direction where proteins are not intercalated.[73]

It would thus appear that protein intercalation serves the double purpose of modulating shape and mechanical properties. When the crystal morphology matches the crystal symmetry, it may be sufficient to exploit the recognition capabilities of the (glyco)proteins for specific crystal motifs. When, however, the single crystal morphology does not respect the crystal symmetry, the targeting strategy can overcome the intrinsic anisotropy of the crystal, and of the protein–crystal interactions as well. This raises the intriguing question of whether or not this biological 'override' of the inherent nature of the crystal–protein interactions has the functional purpose of producing a more isotropic material in terms of defect distributions.

Another interesting illustration of this strategy was observed recently in sea urchin spines. The diffraction data indicate that the anisotropy in crystal texture (*c vs. ab*) is larger in mature secondary spines, where the stereom is already filled with mineral sectors, than in immature spines that had only developed the spongy stereom. Etching of broken stereom sections of immature spines show curved, onion-like mineral deposition lines, transverse to the septa, irrespective of their direction (Fig. 12).[74] These lines do not appear in the filled sectors, suggesting a different, possibly less controlled, mechanism of crystal growth during the filling stage. Interestingly, synthetic calcite crystals grown from solution in the presence of the proteins extracted from the spines have even higher textural anisotropy. This is true both for growth along *c* relative to the *ab* plane, and, within the *ab* plane, between the directions [10.0], where protein intercalation occurs, and [11.0]. The mechanism of growth during the filling stage in the spine is thus closer to that of the protein-containing synthetic crystals, where no control over the microenvironment is exercised.[75]

The 'strive for isotropy' may thus be a more widespread strategy in the construction of single crystal skeletal elements.

Stabilization of amorphous calcium carbonate

If the achievement of isotropy in mechanical performance is an important issue, the best construction material should in itself be intrinsically isotropic. This property is shared by amorphous minerals. Amorphous silica is indeed used by a wide range of organisms, from the complex beautifully sculpted diatoms to siliceous sponge spicules and plant phytoliths.[76] In terms of quantities formed worldwide, silica is one of the three most abundant biogenic minerals (together with calcite and aragonite). It therefore appears to offer important benefits as a component of biological materials. In addition to being isotropic, silica has the obvious advantage of being stable under ambient conditions, and therefore minimal energy is required for this aspect of its formation. This contrasts with the other fairly commonly used amorphous minerals, which do need to be stabilized. Amorphous calcium phosphate is often used for temporary storage of ions, because its solubility is higher than that of the crystalline materials. It is also used, however, for skeletal strengthening purposes, for example in some ascidian spicules and the gizzard plates of some gastropods.[6] Amorphous hydrous iron(III) phosphate is the mineral used in sternal shields of certain annelids[6] (Fig. 13). Amorphous calcium carbonate is also formed by several organisms in widely divergent taxa.[6] It is most abundant in some plants, where it presumably functions as a temporary storage site for ions.[77] It is used for structural purposes, such as in the spicules of ascidians of some Pyuridae[78] (Fig. 14), in the spicules of the sponge *Clathrina*,[19] and as a precursor phase of calcite in sea urchin larval spicules.[79] We elaborate briefly on the case of amorphous calcium carbonate, not because it is so abundant in the field of biomineralization, but because it presents such intriguing paradoxes.

The use of amorphous calcium carbonate is puzzling. The mineral is very unstable, and its transformation into one of the crystalline polymorphs is extremely fast in solution under normal conditions.[27] Organisms must invest a lot of energy to stabilize this phase, and hence presumably derive considerable benefit from using this unusual mineral. The strategy used for stabilization of amorphous calcium carbonate again involves specialized macromolecules. Recently, glycoproteins have been isolated from within the amorphous mineral of both *Pyura* antler spicules and *Clathrina* triradiate spicules. Their amino acid compositions, rich in glycine, serine and glutamic acid, are very similar. They both have associated oligosaccharides. When introduced into supersaturated solutions of calcium carbonate, both prevent crystallization completely, and the

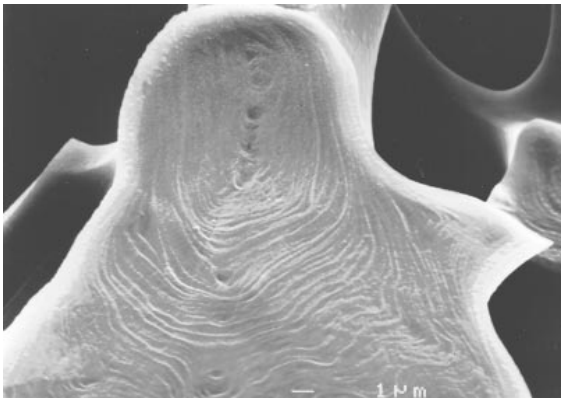

Fig. 12 EDTA-etched fractured surface of an immature sea urchin spine showing the mineral deposition lines

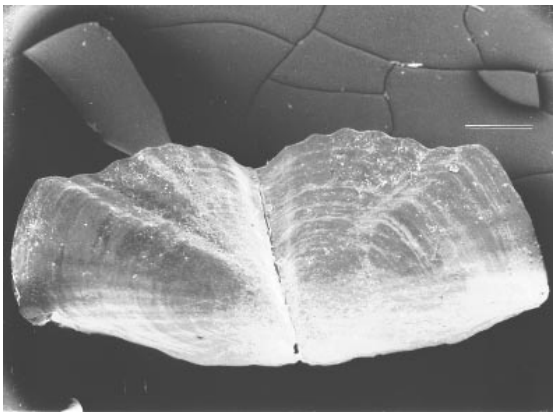

Fig. 13 Sternal shields of the marine annelid *Sternaspis* sp. composed of an amorphous hydrous iron(III) phosphate. Scale bar: 0.5 mm.

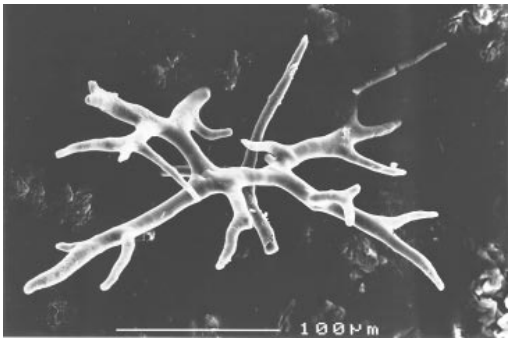

Fig. 14 Antler-shaped spicules of the marine ascidian *Pyura pachydermatina* composed of amorphous calcium carbonate

amorphous precipitate that is consequently formed is stable over long periods of time.[19]

Control at the micron level: the intimate involvement of cells

Cells form biological materials. Their involvement can be direct, with the mineralization structures forming in specialized vesicles within the cells, or in close association with cell walls. It can also be indirect in that the cells synthesize and release macromolecules to the extracellular environment. Here they self-assemble into a three-dimensional framework or matrix in which the mineral subsequently forms. Whatever the process used, one level of structural organization of a mineralized biological material can frequently be related to the size of the cells that form the structure. In general such cells tend to be elongated and range in size from a few microns to ten microns in cross-section and can be tens of microns long. It is the cross-sectional plane which usually interfaces with the extracellular environment. This length scale may constitute a 'structural benchmark' of cellular activity, and is often a key element in the structural organization of a biological material.

Cellular controlled organization at sub-micron levels can be imposed by the scale of the spaces in the three-dimensional extracellular matrix, or by the formation of the mineralized building blocks in vesicles within a cell, followed by assembly outside the cell. Examples of the latter are the marine calcareous plants Coccolithophoridae[80] and the marine protozoans belonging to the group of the miliolid foraminifera.[81] One phylum which consistently forms single crystals that are much larger than the size of normal cells is the Echinodermata. Their strategy is to have a whole team of cells fuse their membranes to form a giant vesicle or syncitium.[6] A single calcite crystal is nucleated within the syncitium and in some cases can grow to even centimetre size (see Sea urchin spine). Here we will examine the product of cellular activity on the higher order structural organizational patterns of two well studied mineralized materials, the mollusc shell nacreous layer and bone.

Mollusc shell nacreous layer

The cells that form the nacreous layer are located on the side of the shell-forming tissue (the mantle) that faces the inner surface of the shell. They are usually close-packed and hence polygonal in cross-section.[82] These cells form an extracellular matrix in which the aragonitic crystals grow. The dominant matrix structural feature is a series of sheets regularly spaced at distances of a half to one and a half microns from each other (Fig. 4). The resultant mineralized structure is composed of polygonal-shaped flat tablets of aragonitic crystals, with each layer of crystals separated by a matrix sheet. Although it has still not been demonstrated directly that each polygonal crystal is formed by one mantle cell, the observed correspondence in size between crystals and cells in different species suggests that this is the case.[83]

The sheets of matrix formed by the mantle cells are composed of no less than five different layers, following the model proposed by Weiner and Traub.[45] Each cell probably makes its own three-dimensionally ordered 'patch' of matrix and mineral. Atomic force microscope[84] and electron diffraction studies[85] of the vertical orientations of nacreous crystals from several bivalves show that stacks of four or five layers of crystals may be very well oriented. This could be the result of each stack being nucleated once and a single crystal growing through the matrix sheets. Alternatively, it could be the product of the synchronized activity of a single mantle cell forming a highly ordered matrix–mineral structure. X-Ray diffraction studies of the lateral orientations of the *a* and *b* crystallographic axes of an assemblage of aragonitic tablets extending for

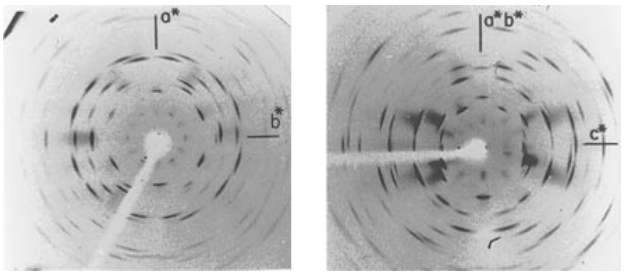

Fig. 15 X-Ray diffraction patterns of the aragonitic nacreous layer of the bivalve *Neotrigonia margaratifera*. Hundreds of crystals are in the diffracting volume. The patterns in two orthogonal directions show that they are all relatively well oriented in three directions.

hundreds of microns have shown that in gastropods there is no lateral preferred orientation whatsoever. In the shelled cephalopod, *Nautilus*, there is some degree of preferred orientation, as is also the case in many bivalves.[86,87] For example, in the bivalve *Neotrigonia margaratifera* the extent of organization can be rather good in all three directions[45,87] (Fig. 15). These observations show that the cell influences the size and orientations of the crystals in two dimensions. The third dimension (layer thickness) is presumably determined by the properties of the self-assembled matrix. These studies also show that the cells determine the orientations of the crystallographic axes indirectly through the matrix substrate. The fact that the degree of orientation of whole areas of polygonal crystals is genetically controlled suggests that there may be some selected mechanical advantage for random crystal organization in one case *vs.* preferred orientation in others.

The nacreous layer functions mechanically as a classic composite material rather than a ceramic, despite the fact that the organic component usually constitutes only *ca.* 1% by mass of the material. It is also a platelet-reinforced composite, as opposed to the more common fibre-reinforced composites of the synthetic world.[88] Mechanical studies demonstrate well the rather remarkable bulk materials properties of nacre both under compression and under tension.[89,90] Observations of fracture planes show clearly the tortuous route followed by the crack as it progresses along the matrix sheets or in the perpendicular direction as it traverses across the crystal layer between tablets. At this structural level, the nacre is deduced to derive its unusual mechanical properties directly from its highly ordered layered structure, prompting the conclusion that no really novel mechanisms are involved in achieving its mechanical properties.[88] We suspect, however, that this may not be the case. We note the unique plywood-like structure of the matrix itself, the fact that it is composed of two very different polymers (chitin and silk fibroin-like protein), the very real possibility that macromolecules are also occluded inside the aragonitic crystals where they may alter the bulk properties of the mineral phase, and the well designed interface between matrix and mineral inferred from the documented specific spatial relations between them. All or some of these features may indeed constitute 'novel' design strategies that contribute to the unique mechanical properties of nacre.

In general molluscs offer a wide variety of opportunities to investigate structural design features. The commonly formed crossed-lamellar structure comprises a three-dimensional array of closely packed aragonitic crystals. The structure is in itself fascinating, and the few mechanical studies performed to date point to interesting bulk properties.[91] One enigmatic observation is that the hardness of the aragonitic shell is greater than inorganic aragonite. The matrix component of these shells is *ca.* 0.5% by mass.[91]

Bone

The basic building block of bone (and tooth dentin) is the mineralized collagen fibril.[92] In the world of biomineralization

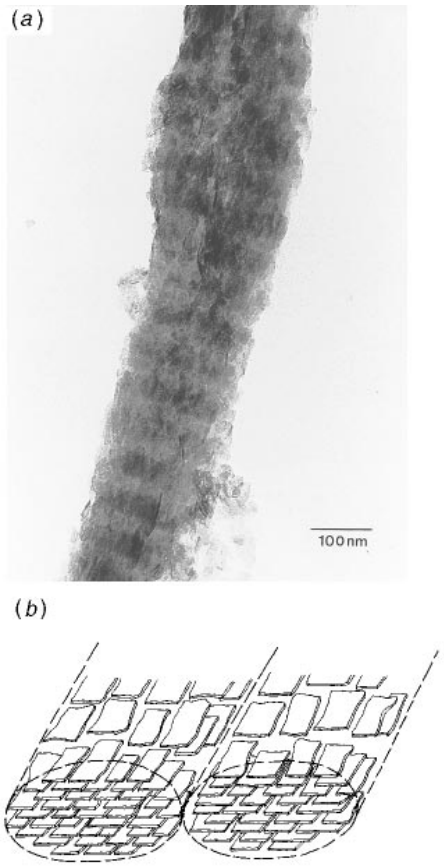

Fig. 16 (*a*) Transmission electron micrograph of an unstained mineralized collagen fibril from calcified turkey leg tendon. Most of the plate-shaped crystals of carbonated apatite are viewed face-on. The characteristic 67 nm banding of collagen is also apparent. (*b*) Schematic illustration of the organization of the crystals in layers in the collagen fibril. Reproduced with permission from ref. 112.

this is a most unusual matrix–mineral composite in that the carbonated apatite crystals are among the smallest, if not the smallest, biologically produced crystals known. They are on the average $50\,\text{nm} \times 25\,\text{nm} \times 2\,\text{nm}$.[6,93] Most of these plate-shaped crystals are located inside grooves or channels within the type I collagen fibril (Fig. 16) to form a layered structure across the fibril.[94] Thus the mineralized collagen fibril is itself crystalline and highly anisotropic.

Cells synthesize the collagen polypeptides and these assemble into small fibrils in vesicles within the cell. These are then packaged for secretion. Further assembly occurs into bundles in the extracellular environment, presumably in such a way that the three-dimensional orientation of the fibrils is well controlled.[95] Mineralization takes place in the extracellular environment.

In some fast-forming tissues the first crystals form inside very small vesicles.[96] These crystals have no preferred orientation. As these mineral-filled vesicles have also been observed in the proximity of the sites of ordered nucleation that occurs within the collagen fibril,[97] it is conceivable that they function as a supplier of ions for intrafibrillar mineralization.[98] The crystals that form within the fibrils nucleate at a very specific location within the fibril,[99,100] and then grow rapidly along their *c* axes. The latter are well aligned with the collagen fibril axis. At this initial stage the crystals are needle-shaped. They soon, however, grow into plates filling the collagen fibril channels.[101] The plate-shaped crystals finally push their way out of the fibril channels into the overlap zone between layers of triple-helical molecules.[102] Thus the collagen fibril seems to fulfil a matrix framework function by defining the nucleation site location and controlling initial crystal growth. At a later stage the crystal growth dynamics dominate, and 'push' the collagen molecules aside.

In mineralized tendons and parallel-fibred bone the extruded fibrils are arranged into long parallel arrays, with the fibril axes all in the same direction.[103] In dentin the extruded fibrils are all in the same plane, but are not well oriented with respect to each other.[104] The most complex form of bone is lamellar bone. Here the cells extrude the fibrils such that all the fibrils that constitute one newly formed layer are aligned in one direction in a plane. The next fibril layer is rotated by some degree such that a plywood-like structure is formed. The cells control not only fibril orientation, but also the azimuthal orientation of the crystal layers around the fibril axis. These too are rotated with each additional layer. The cells form a complex structured unit 2 to 3 μm thick, and then begin the whole process again.[105,106] The resulting so-called 'rotated plywood' structure is thus a highly complex composite material (Fig. 17).

A detailed study of the microhardness properties of parallel-fibred bone[107] by indentation clearly reflected the anisotropic nature of the array of aligned mineralized collagen fibrils. The lowest values are obtained when the indenting direction is perpendicular to the alternating layers of triple helical molecules and crystals (P). It is highest when the crystals are indented edge-on in the direction parallel to the long axis of the bone (T) (Fig. 18). When the microhardness properties of the lamellar bone structure were probed, they revealed the well known general tendency for the bone to be somewhat harder in directions parallel to the bone long axis as compared to directions perpendicular to the long axis. The differences were, however, gradual when the structure was probed in many different directions, and relatively small compared to parallel-fibred bone. It thus appears that the design motif of lamellar bone is to form a mineralized structure that tends towards isotropy, even though the building block used is highly anisotropic. This is achieved by the formation of complex higher ordered structures.

Conclusions: towards the millimetre scale and beyond

Measurements of the mechanical properties of biological materials, millimetres in size or larger, can be made relatively easily.

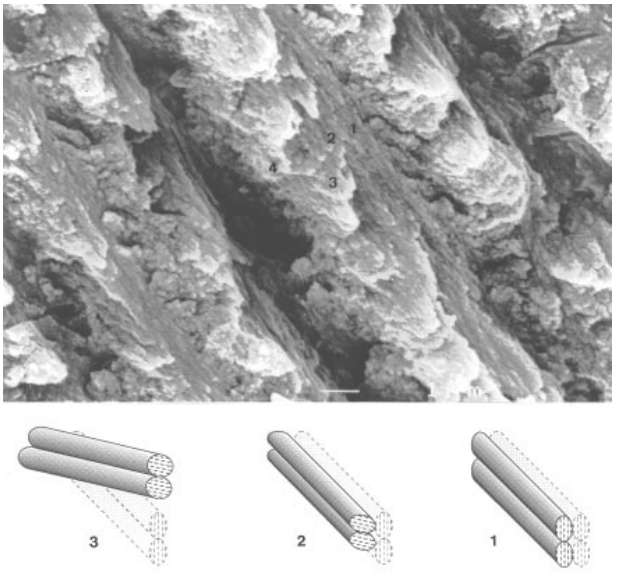

Fig. 17 Fracture surface of lamellar bone from the midshaft of a rat tibia showing several individual lamellar units (top). Schematic illustration of the orientations of the collagen fibrils (cylinders) and the crystal planes inside them at three different locations within a single lamella (bottom). The structure in area 4 is unclear.

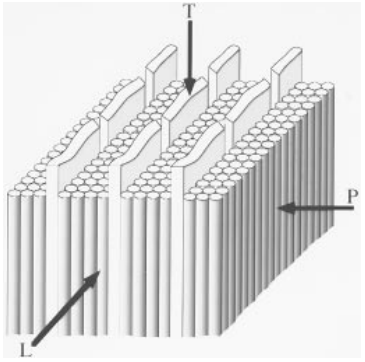

Fig. 18 Schematic illustration of the nanometre-scale structure of the mineralized collagen fibril showing the triple helical molecules of collagen (cylinders) and the plate-shaped crystals. The arrows show the three directions of indentation. (Reproduced by permission of the publisher from V. Ziv, H. D. Wagner and S. Weiner, *Bone*, 1996, **18**, 417 (ref. 107 of this work). Copyright 1996 by Elsevier Science Inc.)

Their interpretations in terms of structure, mechanical behaviour and function, however, are difficult, because they incorporate the contributions of different hierarchical structural levels. Many bulk measurements of biological materials have been made and analysed in terms of known mechanical engineering properties. Indeed these are the studies that have shown just how mechanically interesting many biological materials are, especially when compared to analogous synthetic composite or ceramic materials.

By analysing the structural properties of biological materials at different length scales, it is clear that organisms have evolved a variety of interesting strategies to improve the mechanical properties. This is particularly impressive when bearing in mind the many disadvantageous properties of the starting mineral components. It is particularly helpful to be able to measure directly the mechanical properties at the appropriate length scale of the structural feature of interest, and in particular the key properties that are important for the organism. Unfortunately in many cases, the appropriate tools for making such measurements are not available, and we do not know for sure what the important parameters are. It is also often tacitly assumed or implied that the biological materials are well, or even perfectly, adapted to the needs of the organisms that produce them. This is in reality almost impossible to demonstrate.

A different conceptual approach to the analysis of structure–mechanical function relations in biological materials millimetres or larger in size, is to differentiate, if possible, between those materials that are used for many purposes, the 'concretes' of the biological world, and those that are structurally designed for specific tasks. The latter tend to have bulk structures and architectures that vary within a given phylum even at relatively low taxonomic levels. A good example is the mollusc shell. Molluscs produce seven major structural materials for their shell layers. These, however, vary from group to group (about 50 variants are known) and different structural types are often combined in one shell.[108] The overall impression is that each shell type has evolved to fulfil specific functional requirements. A good example of an 'all-purpose' type material is the echinoderm stereom structure. The same calcitic material, which has sponge-like microarchitecture, is used by almost all members of this phylum for a wide variety of purposes.[6] Another example of a more generally functional material is the chitinous exoskeleton of the arthropods. It has a complex lamellar structure with a well defined plywood motif. In crustaceans it is also mineralized with calcite. This basic skeletal material is used by almost all members of this huge phylum.[6] A third example of such a material, in our opinion, is lamellar bone. It is used by many mammals and in particular relatively active mammals, suggesting that it is able to withstand a wide variety of mechanical challenges. The study by Ziv *et al.*[106] of the microstructure–microhardness relations in lamellar and parallel-fibred bone, showed that the former tends to be significantly more isotropic than the latter at the tens of micrometres scale. This observation raised the interesting question of whether other multifunctional biological materials are also structured in such a way as to emphasize isotropic properties. We have noted that this might well be the case for the echinoderm stereom structure at the nanometre scale (see Modulation of crystal texture, earlier).

The structure of the shell plates of a most unusual marine barnacle, *Ibla*, is interesting in this respect. The barnacles are members of the Arthropoda, and generally have mineralized calcitic exoskeletons. *Ibla* is the exception. It produces a shell plate mineralized with carbonated apatite, the same mineral present in bone. The framework constituent of the matrix is α-chitin, like all other arthropods. A detailed study of the shell plate structure revealed remarkable similarities to lamellar bone, right down to the nanometre level[109] (Fig. 19). This appears to be an example of convergent evolution producing a very similar, in this case probably more generally functional, material in two quite different phyla.

Isotropy in a material has obvious advantages. Macromolecules that constitute the matrix in biological materials are always highly anisotropic, as are the crystalline mineral components. The substitution of a crystalline mineral

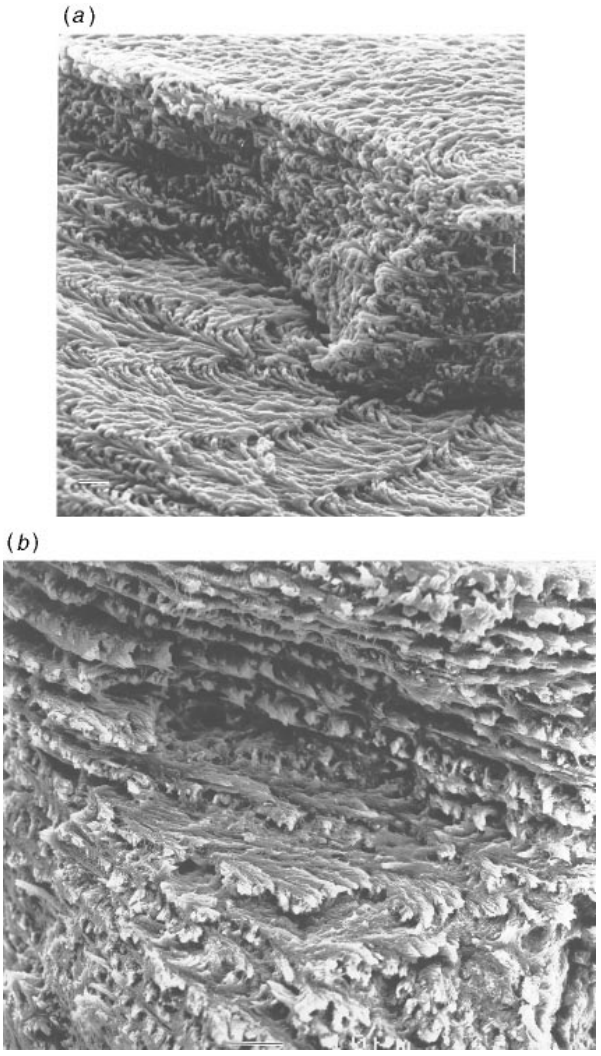

Fig. 19 Fracture surfaces of (*a*) the shell plate of the marine invertebrate barnacle, *Ibla*, and (*b*) lamellar bone from the tibia of a rat. Note the remarkable similarity in lamellar structure.

for an amorphous mineral should contribute significantly towards improving the overall isotropic properties of a biological material. Biology does indeed make use of a variety of amorphous minerals for structural purposes (see Stabilization of amorphous calcium carbonate, earlier). The full extent of this phenomenon is still probably grossly underestimated, as the presence of amorphous minerals is often difficult to detect, especially when crystalline material is also present.

Silica is the most common and quantitatively most abundantly formed biogenic amorphous mineral. The sizes of these biogenic siliceous products vary from several microns to tens of centimetres in the case of some sponges.[76] Macromolecules are associated intimately with these siliceous biological materials, both within the mineral phase and as framework structures that order the spherical mineral particles into higher order structures.[110] The probable reasons why silica is so widely used biologically are that it is very insoluble at neutral or close to neutral pH, it is relatively abundant in a soluble form in ground water and in sea water (except where diatoms have used almost all of it), and it polymerizes rather easily into a solid phase under a variety of conditions. It is not nearly as obvious why the two other fairly common biologically formed amorphous minerals, amorphous calcium phosphate and calcium carbonate, are used for structural purposes. Both these phases need to be stabilized, and they are relatively soft compared to their crystalline counterparts. One possibility is that organisms benefit from their isotropic properties.

The strive towards isotropy may be a common theme in the design strategies of many biological materials, and in particular those that are required to fulfil more general functions. The advantages of constructing materials that are more isotropic have also been recognized by the designers of synthetic composite materials.[111] We believe that one potentially promising avenue of research in materials science is to reveal some of the strategies used by organisms to produce more isotropic composite materials out of highly anisotropic building blocks. Organisms appear to have had to solve this and many other problems relating to their structural materials during the 550 million years of on-the-job testing. Some of the strategies used and solutions derived may well have practical applications in the world of synthetic composite materials.

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