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Block copolymers for biomimetic composites

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ABSTRACT

Mineralized biological tissues can be regarded as composites where a fine reinforcement is laid down in a very controlled fashion within a tough polymeric matrix. Such materials include bone, antler, tooth enamel, mollusc shell and crustacean shell. We have been exploring ways of forming similar structures by synthetic routes involving precipitation of reinforcing particles directly into a polymeric matrix. Part of this biomimetic approach requires polymer matrices which can exert a high degree of control over the mineralization process.

Polymer gels have been formed from cross-linked methacrylates with various types of functionality within the gel. By incorporating calcium binding groups we have been producing gels which lead to preferential mineralization of the gel when it is incubated in a supersaturated solution of calcium oxalate or calcium carbonate. Similarly we have been incorporating silane groups within the gel in order to promote the deposition of silica in a gel body when it is immersed in a metastable solution of partly hydrolysed silicon alkoxides.

We envisage that such controlled deposition will allow local modification of organic materials so that active minerals such as piezoelectric materials or optical sensors can be incorporated with good control of particle size and orientation to give smart composites.

1. INTRODUCTION

Biological materials such as bone or skin can be regarded as the epitome of intelligent materials. Skin performs a structural function as a tough barrier layer, contains stress and thermal sensors, can respond rapidly to changes in the environment and can be remodelled to resist high levels of abrasion or other forces. Similarly bones are stiff structural elements, which have a complex composite structure with local variations in microstructure and properties. They also can remodel in response to continuous stresses. For example, it is well known that the bones of a tennis players racquet arm will be bigger than those of the other arm. Both materials are also capable of repairing themselves if damaged. In skin the coupling between the sensors and the responding actuators is outside the material in the central nervous system. Hence the intelligence of these "intelligent materials" is actually external. As a result the response can be modified depending on historical or environmental factors. It is not a simple reaction, such as one observes in a heated bimetallic strip, for example.

If the properties of bone are translated into a synthetic context, we could expect to construct a group of composite materials which comprise a polymer matrix, reinforcing fibers, sensors and a method of coupling them to the outside. In addition we would need a method of supplying reagents to remodel the structure in the case of excessive stress or damage. A softer composite could also include mechanism for changing shape or modulus in response to a signal from the sensors.

The processing methods which would be required to produce such materials are essentially those already which exist for building complex devices in silicon, by layerwise processing. These methods would need to be extended to a wider range of hard and soft component materials and also required would be the ability to make large objects as a series of layers.

As part of our effort to reach this goal, we have been studying the ways in which biological mineralization produces complex structures of minerals in organic matrices and have been investigating synthetic methods which

would offer the same controlled deposition of mineral particles in a polymer matrix.

2. BIOLOGICAL MINERALIZATION

A recent book has given a thorough survey of mineralized tissues.¹ Bone is essentially a matrix of collagen, a tough polymer, reinforced with hydroxyapatite, a basic calcium phosphate. The mineralized cuticle of crabs and other crustaceans is a similar structure with calcium carbonate reinforcing chitin. Mammalian tooth is essentially a hydroxyapatite ceramic with a small amount of polymer. Mollusc shell is largely calcium carbonate bound by small amounts of polymer. Each of these tissues forms by extracellular mineralization of an existing organic matrix. The good mechanical properties of these hard tissues, and their resemblance to synthetic composites and ceramics, has lead to much recent interest in duplicating their microstructures for engineering materials.

A second form of mineralization occurs within a confining membrane. Thus the skeleton of a sponge consists of silica rods, spicules, which are formed individually in a vesicle within a cell and are then exported and joined to make a framework. Many bacteria form intracellular particles which grow surrounded by a membrane. Siliceous diatoms are single cells which form a surrounding silica shell that has a very well defined pattern of porosity. These structures show feature sizes of less than one micron and resolutions of better than 0.1 micron. Coccoliths form a rather similar shell from crystalline calcium carbonate, which demonstrates that complex shapes on a scale of a few microns can be formed from single crystals.²

In extracellular mineralization, the growing composite structure must be controlled in ways that should be understandable in terms of normal crystal growth. This understanding should allow us to grow sensing structures embedded in a tough polymer as part of a synthetic composite.

A simple form of extra-cellular mineralization is observed in coral but the mechanism is still not clear. Constanz³ has shown that aragonite grows on nuclei produced by the coral. These nucleating bodies do contain calcite crystals on which the aragonite seems to form. Constanz suggests that this is the main control mechanism with no controlled modulation of local solute concentrations or secretion of growth-modifying polymers. However, the process of crystallization is faster in the light as photosynthesis by algae removes carbon dioxide from the sea water and shifts the bicarbonate/carbonate equilibrium.

The calcite crystals (otoconia) found in the balance organs of mammals are a similar case of controlled extracellular crystallization.⁴ What appears to be a single crystal several microns long, actually has an organic core covered with nucleation sites. The nucleating core is extruded from a cell. Oriented nucleation on the core gives rise to radially growing crystals which then merge to form a single unit.

2.1. Bone

Bone mineralization may be seen as a more advanced form of coral formation. The precipitation is also extracellular but now occurs in a matrix which has a structure that can guide the mineralization.¹ Bone formation is very complex and not well understood despite an enormous body of research.

Bone mineral is hydroxyapatite, a basic calcium phosphate $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with 4-6% carbonate substitution and some monohydrogen phosphate. Much of the non-stoichiometry may be accounted for by surface sites. The crystals have the form of thin plates about $50\text{nm} \times 25\text{nm} \times 3\text{nm}$. The crystals show central dark lines in electron microscopy which may be a layer of octacalcium phosphate and the initial precipitate.

The earliest evidence of bone crystals in new bone is often associated with matrix vesicles. These vesicles are buds that develop from cell membranes. They are rich in phosphatase enzymes and the hydroxyapatite crystals first form in the vesicle membrane. When the bulk of the mineralization takes place these first randomly-arranged crystals seem to disappear and their significance is unclear.

Subsequent crystallization takes place in association with collagen fibrils. There is discussion about whether amorphous calcium phosphate is formed before hydroxyapatite and also about whether octacalcium phosphate is the initial precipitate as mentioned above. It has long been believed that collagen is responsible for nucleating hydroxyapatite. Collagen fibrils are composed of rod-like triple helices grouped into bundles and laid end-to-end with a 36nm gap between ends. The triple helix of collagen is 300nm long and adjacent fibrils are arranged with a 1/4 stagger. As a result the fibrils have gap regions every 68nm where a group of helices ends. This 64nm spacing becomes very marked in the early stages of mineralization, suggesting that collections of acidic groups near the ends of the helices may form a nucleating site. The hydroxyapatite plates ultimately form a parallel stack within a collagen fibril.

At a more macroscopic level, the long bones of the new born are cartilage which contains some mineral. This is invaded by bone-forming cells which propagate mineralization towards the bone ends. A residual plane of cartilage is left near the bone ends. In this zone new matrix continues to form and calcify as the youth matures and the bones lengthen. The bones thicken by deposition at a membrane surrounding the outer surface.

Bone is not static but is continually resorbed and redeposited. This allows remodelling to account for changing stress distributions. The mechanism of stress sensing which allows the appropriate growth patterns is not known but piezoelectric effects have been suggested.

Normal body fluids and tissue are supersaturated for hydroxyapatite but mineralization is inhibited. Pyrophosphate ($P_2O_7^{4-}$) may be responsible for growth inhibition, it turns over very rapidly in the body due to enzymatic action and is a strong inhibitor of crystallization. The presence of phosphatase in matrix vesicles may be due to their role in removing pyrophosphate in the mineralization zones.

To summarize the "processing" of bone we can derive the following points:

Organic matrix is first laid down then mineralized.

Long bones grow in length by adding new material in a transverse layer, the growth plate.

The details of nucleation and growth are not understood.

Growth inhibitors are important for preventing unwanted mineralization.

Very small crystals are formed with precise shape as a result of the composition of inorganic salts present.

These crystals are laid down parallel under the influence of the collagen fibril structure.

The body has many types of collagen but many of them do not mineralize.

2.2. Mollusc shell

One form of shell which has attracted much interest amongst materials scientists is nacre or mother of pearl, which is found on the inner surface of the shells of many bivalve molluscs including mussels⁵. Aragonite crystals are stacked like bricks in a wall. Each aragonite crystal is about 0.5 μm thick by several microns in width and length. Each crystal is separated from its neighbors by a thin layer of polymer which provides considerable toughness during fracture. The crystals all have the c-axis along the vertical, short, direction. Crystals in successive layers can have similar a,b orientations and an epitaxial relationship to the underlying polymer. how this structure grows is not known.

3. IN VITRO STUDIES OF MINERALIZATION

Extracts of soluble protein from mollusc shell have high levels of acidic amino acids, especially aspartic acid. These proteins are very efficient inhibitors of calcium carbonate crystallization⁶. Interestingly they are also very efficient inhibitors of hydroxyapatite mineralization, suggesting that this may not be a very specific process.

Various workers have adsorbed these proteins onto surfaces and have shown that they can enhance the nucleation of calcite from solution and control the orientation of the growing crystals on the surface. Addadi has proposed that polymers which are good growth inhibitors in solution are also good nucleating agents when bound to a surface.⁷ However none of these treated surfaces prove to be very efficient, nucleation densities are

still low.

Various have studied various modifications of polymer surfaces to enhance calcite nucleation. Rieke has found that sulfonation of polystyrene increases the number of surface-bound crystals but the effect is again relatively small.⁸ Addadi obtained similar results.⁹ Wheeler has proposed that this effect may be due to adsorption of nuclei from solution onto the surface rather than surface-induced nucleation.

In the case of hydroxyapatite, it has been shown that phosphinate treatment of polymer surfaces can lead to abundant nucleation and growth which does not occur on untreated surfaces.¹⁰

Tarasevich and Rieke have shown that treatment of sulfation of polystyrene surfaces or silicon surfaces with phosphate functions can give formation of iron oxide films from iron salt solutions under conditions where no oxide forms on untreated surfaces or in solution.¹¹ This suggests that the treated surface may be catalytic for the hydrolysis of the iron in solution.

Mineralizing bone contains a host of polymers and simple compounds which influence the process of crystallization. Pyrophosphate is a known inhibitor of hydroxyapatite crystallization as are the related and more stable organophosphonates. It has been widely believed that collagen is a specific nucleating agent for hydroxyapatite. Mineralization starts in the "gap" regions, which contain many acidic amino acids. This suggests that these regions contain a cluster of groups which bind calcium in the correct spacing to nucleate the mineral. In fact extracted collagen has proved to be a poor nucleating agent for hydroxyapatite in vitro¹².

3.1 Inhibition of oxalate crystallization

It has been proposed that the inhibition of crystallization by dissolved polymer depends on sequences of surface-active groups. To test this we prepared a number of butylmethacrylate-methacrylic acid copolymers. The methacrylic acid units are expected to bind to the crystal surface and so limit attachment by oxalate ions while the butylmethacrylate units should have no effect. Polymers were made as both block and random copolymers with different ratios of acid to neutral units.

Block copolymers were prepared by group transfer polymerization. If sequences of acid groups are important, the block polymers should be much more effective than the random polymers.

Calcium oxalate was chosen for nucleation studies because it is more controllable than carbonate which is vulnerable to loss of carbon dioxide from solution. Crystallization was followed by monitoring pH and conductivity of supersaturated solutions. The initial rate of crystallization was monitored. Solutions containing $2.3 \times 10^{-4} \text{M}$ oxalate and $2.3 \times 10^{-4} \text{M}$ calcium were allowed to crystallize at 30°C . Figure 1 shows the effect on crystallization time of added polymer of molecular weight 2000-4000 at a concentration of 1.25 ppm.

From these data it can be seen that more acid polymers are more effective but there is little difference between block and random polymers. Higher molecular weights are more effective.

3.2 Modelling of surface interactions

It would be expected that different polymer subunits would have different efficiencies for growth inhibition. Since the calcium ion is doubly positive charged, while the carboxylate ion is singly charged negative, two monomer units should cooperate to bind a calcium ion. In principle it should be possible to model this process.

The Hyperchem molecular modelling program has been used to calculate the strength of calcium binding by a series of dimers. The energy of a methacrylic acid dimer (figure 2) is minimized with the carboxylates ionized, in a medium with the dielectric constant of water and with a calcium ion at a large distance. The calcium ion is then allowed to approach the dimer and the system is re-minimized and the energy recalculated. The energy

change reflects the strength of binding between the dimer and the cation. As shown in table 1, the binding is stronger for the acrylate ion than for methacrylate. This reflects our observation that polyacrylic acid is a much stronger inhibitor of calcium oxalate crystallization than is polymethacrylic acid. The component of mollusc shell soluble proteins which is believed to be responsible for mineralization control is aspartic acid. Polyaspartic acid has a binding energy similar to that for polymethacrylic acid.

Table 1: Calculated binding energies of calcium ions to model polymers

Calcium binding anion	Calcium binding energy /kcal mol ⁻¹
Isotactic polymethacrylic acid	4.85
Syndiotactic polymethacrylic acid	4.95
Isotactic polyacrylic acid	5.80
Syndiotactic polyacrylic acid	5.17
β -sheet polyaspartic acid	4.61
t-butylacetate	3.21

3.3. Nucleation by bound acids

Copolymers containing 56 mol% methacrylic acid and 44 mol% n-butylmethacrylate were produced by free radical polymerization and cast from solution as films. The films were treated with 1M $\text{Ca}(\text{NO}_3)_2$ solution to form calcium methacrylate as a starting point for mineralization. They were exposed to calcium oxalate at $4.5 \cdot 10^{-4}\text{M}$ for 7 days, 37°C , to observe mineralization. The films picked up small numbers of 1-5 micron crystals on the surface, especially associated with the clamping points where the films were held.

Polymer gels were made in an effort to imitate the structure of collagen. 45 mol% butylmethacrylate, 49 mol% methacrylic acid and 6 mol% triethyleneglycoldimethacrylate were polymerized to cross-linked gels in dioxane. They were washed with water for 3 hours to exchange the dioxane and were then treated with 10^{-2}M $\text{Ca}(\text{NO}_3)_2$, 10^{-3}M NaOH for three hours to produce a calcium methacrylate gel. These gels were then treated with calcium oxalate solution for 7 days. A scattering of crystals was observed throughout the gel with more near the surface.

These studies are continuing using conductivity and pH to monitor the progress of crystallization of oxalate in the presence of the gels.

3.4. Oxide precipitation in gels

A second interest in this system is to imitate the ability of diatoms to control silica precipitation by setting up a compartment system. In principle a layer of polymer gel could be attached to a surface, patterned lithographically and used to deposit layers of metal oxides. To test the feasibility of this approach we have studied methods for impregnating polymer gels with silica via a sol-gel reaction. The aim is to develop methods by which the gel is catalytic and becomes impregnated with silica without any precipitation occurring in the surrounding solution.

We have previously shown that aminated polyethylene films can be silica impregnated by exposure to an acid sol-gel silica solution.¹³ The basicity of the polymer allows rapid condensation of the silicic acid within the swollen polymer film. The polyethylene is a commercial product which cannot be readily prepared in modified forms for laboratory studies.

Cross-linked butyl methacrylate gels were prepared with different fractions of 3-trimethoxysilylpropylmethacrylate by polymerization in tetrahydrofuran. The expectation was that the bonded siloxane units would convert to hydrated silica clusters and provide nuclei for condensation of silica. These gels were then added to acidic silica precursor solutions prepared by the acid hydrolysis of tetraethoxysilane (TEOS) in tetrahydrofuran (THF). Silica formation in the gel was monitored by ashing the gel and weighing the residue and by energy dispersive x-ray analysis (EDS) of sections.

Without the treatment with silica-forming solutions, the original gels contained up to 1% silica residue from the siloxymethacrylate. After treatment with the precursor solution or with a solution of TEOS on THF, the gels gave an inorganic residue of up to 20%. EDS showed high ratios of silicon to carbon, in terms of x-ray peak area, and more silica at the surface of the sample than in the center.

4. CONCLUSIONS

In our view, a central surprising fact about mineralization is that it is very easy to devise additives to inhibit crystal growth but very hard to find surfaces to nucleate it. In the case of biological and synthetic precipitation of calcium salts, many anionic polymers are effective growth inhibitors.

The studies outlined here are aimed at forming polymer layers which will provide a medium for controlled mineral deposition from solution. The model systems chosen are silica and calcium oxalate. In both cases we have some evidence for mineralization of synthetic gels but not yet at high concentration levels or with sufficient rapidity.

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Figure 1: Effect of acid content of poly n-butylmethacrylate-methacrylic acid copolymers on the relative crystallization rate of calcium oxalate expressed as a ratio with polymer/without polymer.

Figure 2: Dimer of syndiotactic polymethacrylic acid showing calcium ion (large sphere) bound to a carboxylate (dark spheres).