DALTON PERSPECTIVE

Biomineralization: the form(id)able part of bioinorganic chemistry! *

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Early in 1993,[1] I wrote a Dalton Perspective entitled *Biomineralization: the Hard Part of Bioinorganic Chemistry!* which highlighted current developments in the study of biomineralization and how these advances were closely associated with an eclectic but integrated view of modern inorganic chemistry. The article illustrated a new vision of the biology–chemistry interface and addressed fundamental challenges for the future. Now, four years later and in the spirit of the Dalton Discussion Meeting on 'Bioinorganic Chemistry', it seems timely to review the current status of biomineralization research.

In my opinion, there has been a world-wide realization that biomineralization, and by implication, bioinorganic chemistry, encompasses concepts and archetypes that are of fundamental importance for the developing field of materials chemistry. A hint of this can be caught in my previous Perspective but I did not foresee the rapid pace at which this connection would be established (see refs. 2–6 for details). Thus, in this Dalton Perspective I wish to extend the focus on the interconnection between biomineralization and materials chemistry by highlighting a new theme that is currently being investigated by my research group.[7] Our interest lies in understanding how organized inorganic materials with complex morphological form can be produced by biomineralization processes (morphogenesis), and how such complexity can be reproducibly synthesized in biomimetic systems (morphosynthesis). Many biomineralized structures have spiral, convoluted, twisted, fenestrated or reticulated architectures that are fashioned from inorganic crystals. To my mind, these shapes challenge the notion that inorganic form is usually limited to the macroscopic expression of the geometric rules encompassed in the space groups of unit cells. Indeed, morphology is intriguing because it represents the consequence of the dynamic interaction between the internal structure-based force field of a growing crystal and the perturbing influence of the surrounding external environment. Whether this environment ('reaction field') is passive, resistive, directive, interactive or reconstructive will make a significant difference to the morphological signature transcribed in the inorganic phase. Biology, which excels at connecting form to function, has evolved processes for integrating these competing fields so that useful crystal chemical properties (optical axes, fracture planes *etc.*) are incorporated into complex morphologies that reflect higher-order cellular (morphogenetic) processes. The elucidation of this synergy is likely to be a pivotal aspect in designing synthetic routes to inorganic materials with complex form.

Growth and Form of Biominerals

It was D′arcy Thompson in his pivotal book, *On Growth and Form*,[8] who first described in detail the complex nature of inorganic materials formed in association with living organisms. The spiral shape of mollusc shells, and the integrative functional morphology of vertebrate skeletons, contradict the commonplace view of inorganic minerals as rigid, inert, immutable materials of limited form and fabric. Such complexity is not only expressed at the macroscale but resides in the nanoscopic, mesoscopic and microscopic organization of biomineralized structures. Indeed, different length scales bring into operation different controlling forces and hence hierarchical orders of construction. At the macroscopic level, the development of extracellular structures such as skeletal bone is influenced by global force fields, such as gravity and mechanical loading. The microstructure, in contrast, is influenced by the positioning and local activity of bone cells under hormonal regulation, and at the mesoscopic and nanometre scale, collagen fibres and calcium phosphate crystals are assembled by supramolecular and interfacial processes under chemical and biochemical control. For unicellular organisms, the mineralization patterns expressed in intracellular structures, such as radiolarian micro-skeletons, coccolith scales and diatom frustules, are commensurate in size with individual cells or assemblages of vesicles. Such patterns extend to the microscale and are dominated by the interplay of chemical and cellular processes operating within the constraints established by time-dependent localized fields.

At the present time, there is evidence that molecular recognition[9] and molecular tectonics[10] are important aspects of biomineralization but the genetic basis for the diversity and evolution of biomineral patterns remains unknown. The interplay between the equilibrium-driven processes of crystallization from supersaturated solution, and the local energy minimum structures of biological organization gives rise to the reproduction of complex morphologies over geological time-scales. Indeed, biomineralized morphologies are often the basis of phylogenetic classification.[11] If one investigates such materials closely, however, it is clear that even between individual organisms of the same species the complex forms are *similar* but not identical. For example, individual calcite crystals comprising the coccolith plates of *Emiliania huxleyi* are all shaped with a remarkable hammer-headed, stirrup-like morphology but the dimensions and form of each unit are slightly different.[12] Moreover, each plate itself does not contain exactly the same number of crystals even though they all retain an unusual ellipitical geometry. This morphological similarity (equivalence) reflects the tension between predetermined genetic mechanisms, such as the formation of collagen fibrils, nucleation templates, vesicle synthesis, *etc.* and the indeterminacy of fluctuations in the surrounding chemical and physical environment during the lifetime of the organism. A similar dynamic is exploited on much longer time-scales in the evolutionary adaptation of new biomineralized structures to sustained changes in the biological niche.

Here I wish to emphasize that the interplay between the intrinsic molecular forces of inorganic precipitation and extrinsic fields arising from longer-range cellular activity and organization is pivotal in explaining the extraordinary complex form of biominerals. A key aspect of these biological processes

* Based on the presentation given at Dalton Discussion No. 2, 2nd–5th September 1997, University of East Anglia, UK.

Table 1 Patterning of biominerals within membrane-bound vesicles

Scaffold	Directing agents	Form	Architecture	Example
Internal cell wall	Microtubules	Curved SiO_2 rods	Woven mesh	Choanoflagellates
	?	Shaped Fe_3O_4 crystals	Linear array	Magnetotactic bacteria
	Microtubules	Curved SiO_2 shell	Hollow cyst	Chrysophytes
	Microtubules/ areolar vesicles	Perforated SiO_2	Hollow shells	Diatoms/radiolarians
Endoplasmic reticulum	Microtubules	Curved SiO_2 sheet	Interlinked scales	Chrysophytes
Nuclear envelope	Microtubules?	Shaped $CaCO_3$ crystals	Scales/coccosphere	Coccolithophorids
Cytoplasmic sheath	Microtubules	Reticulated $SiO_2/SrSO_4$	Microskeleton	Radiolarians/acantharians
Cell assemblages	Syncytium/microtubules	Curved/shaped $CaCO_3$	Spicules/hollow shell	Holothurians/sea urchins
Cellular organization (no vesicles)	Biopolymers/force fields	Hybrid composites	Macroskeleton	Vertebrates/molluscs

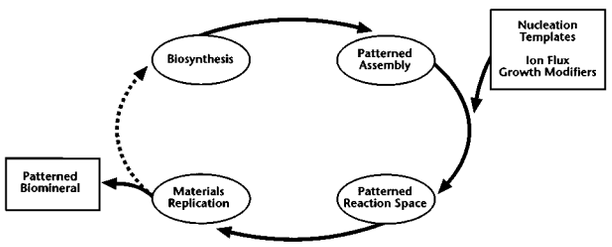

Fig. 1 Generalized scheme for pattern formation in biomineralization. Biosynthesis results in the patterned assembly of organic supramolecular architectures that are functionalized for mineralization by incorporation of proteins and other macromolecules involved in nucleation and growth. Mineralization within the patterned space produces an inorganic 'replica' of the preorganized organic assembly. This may be a one-step 'cast in a mould' transcriptive process, or involve a series of synergistic steps (dashed line) resulting in morphogenesis

is that patterned organic structures are effectively 'replicated in stone' by inorganic mineralization, what could be called a 'chemical medusa' principle (Fig. 1). As organisms grow, so must the minerals within them if their structural functions are to be maintained. But the limitations placed on the symmetry of crystal growth appears at first sight to be fundamentally in conflict with the plasticity of biological development. We might therefore expect to see a predominance of complex forms assembled from amorphous minerals such as silica, in which the intrinsic isotropy can be readily fashioned into a large variety of architectures. Whilst silica is indeed extensively used in this way, so is crystalline calcium carbonate and phosphate, indicating that in many cases the patterns of biomineralization reflect a compromise between the molecular and cellular force fields operating within biological systems.

I now discuss two generalized strategies by which morphogenetic compatibility can be achieved at the biology–inorganic chemistry interface. In some instances, the form of biominerals is *contingent* on the chemical and spatial modification of growth processes, whilst in many others it is *prescribed* by spatial patterning arising from the precise conformation and arrangement of cells or other preorganized biological structures, such as organelles and vesicles. In reality, a combination of these formative mechanisms probably contributes to the spatial and temporal dependence of the morphological features expressed in a specific biomineralized structure across a range of length scales.

Contingent form

Many biominerals, such as the intricately banded calcareous otoliths ('ear-stones') of bony fish[13] or the rounded irregular or branched Mg calcite concretions (spicules) of horny corals[14] [Fig. 2(*a*)] are polycrystalline composites that are deposited in delineated biological compartments. The unusual morphological forms appear to be determined primarily by local perturbations in the crystal growth and aggregation mechanisms operating at the fluid–solid interface. Fluctuations in time and position of the concentrations of surface-active macromolecules, supersaturation levels, viscosity and hydrodynamical mixing can give rise to diffusion and chemical gradients which induce curvature and complexity in the mineral growth front. These processes are not necessarily under genetic control so that even within a single species the biomineral may vary considerably in shape and size about a recognisable common pattern. Thus, the external form appears to be very much contingent on the precise chemical and structural conditions of particular biological milieu (vacuoles, intercellular spaces, vesicles) which may significantly change during the mineralization process. Such perturbations in the growth conditions also impact on the internal microstructure of these materials, which can vary significantly between different parts of the same biomineral.[15]

Prescriptive assembly

The majority of biominerals produced by unicellular eukaryotes, such as the delicate micro-skeletons of radiolarians, elaborately patterned scales of coccolithophorids and diatoms, or the denser more robust shells of foraminifera, have complex but regular forms that cannot be explained by contingent modification of inorganic crystallization. Similarly, the fluidity of form expressed in higher organisms such as echinoderms (sea urchin tests), molluscs (shells) and vertebrates (skeletons) is dependent on highly controlled processes of mineral patterning. In general, the fashioning of these biominerals is prescribed by confining the mineralization process within vesicles, vacuoles or polymeric matrices that are assembled and organized using structural scaffolds and associated directing agents (Fig. 3 and Table 1). The chemistry of these localized sites is regulated by ion and molecular transport into and out of the shaped reaction environments such that mineralization and organic pattern formation are effectively coupled in space and time. In some systems, the deposition of the inorganic mineral occurs after the patterning process has terminated, a process similar to casting an inorganic replica in a static organic mould. In others, mineralization and vesicle shaping proceed in concert, with the mineralization front remaining some distance behind the developing organic structure. Under these circumstances, there is the possibility that synergistic interactions between the mineral and vesicle contribute to the developing morphogenetic field through coupling of the inorganic and organic assembly processes. For example, as the mineral begins to dominate the replicated morphology, there may no longer be a requirement for directing structures such as microtubules.

Under equilibrium conditions, the surface energy of a bilayer vesicle is minimized by adopting a spherical shape. Because intracellular space is criss-crossed with micro-skeletal networks and associated stress fields, an isotropic vesicle can be readily shaped by mechanical and structural forces operating locally and at a distance on the fluidity of the enclosed bilayer membrane. Empirically, it appears that the shaping of a vesicle can be directed by two perturbing force fields acting either

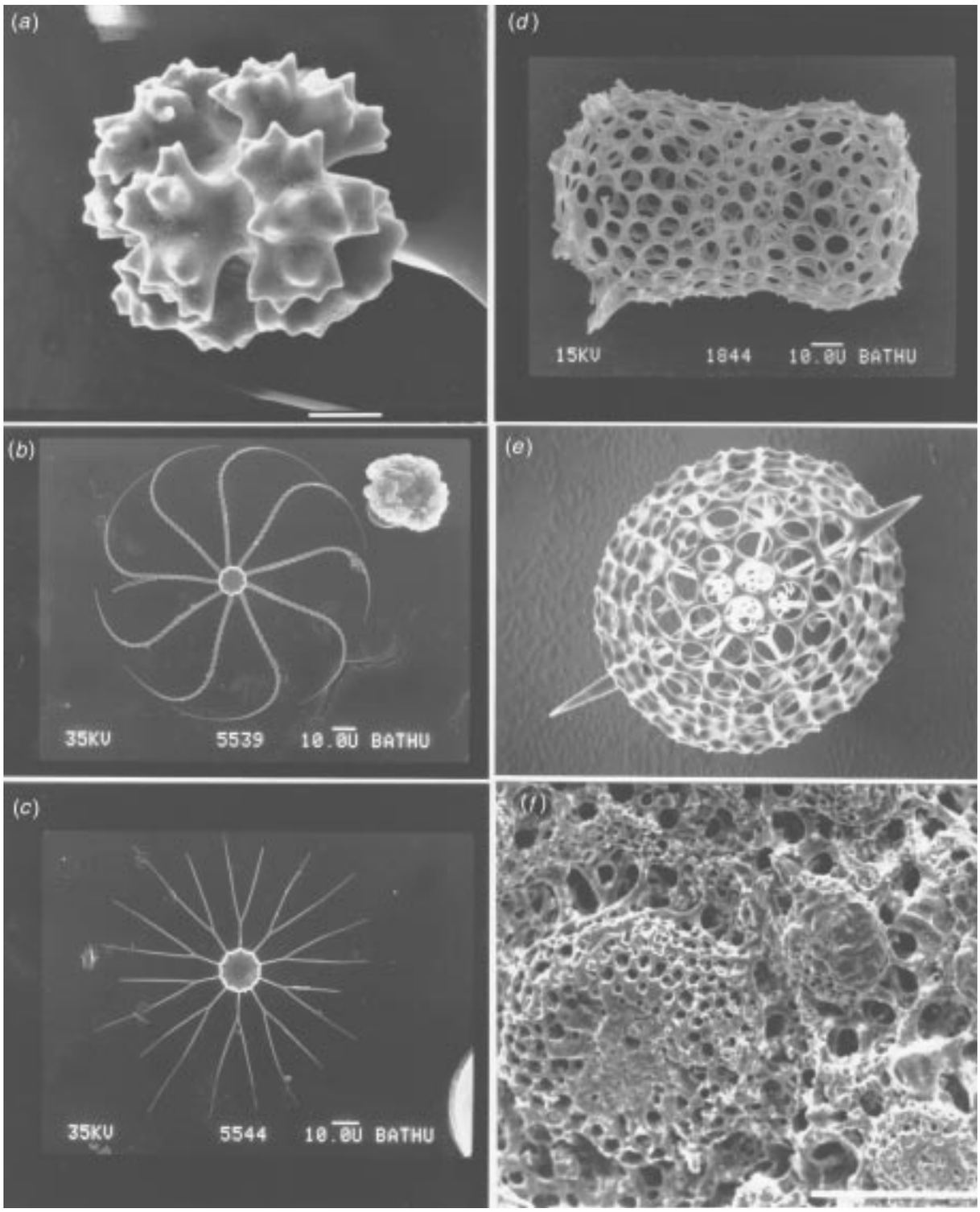

Fig. 2 (*a*) Magnesium calcite polycrystalline concretion from the red coral *Corallium rubrum* showing irregular surfaces protuberances, scale bar 10 μm. (*b*) and (*c*), examples of intracellular morphogenetic fields, scale bar 10 μm; in (*b*) the biomineral is patterned by radial and tangential constraints to give the wheel-like architecture, whereas radially-directed processes alone control the spoke-like structure of (*c*). (*d*) Radiolarian micro-skeleton consisting of a continuous spheroidal framework of amorphous silica, scale bar 10 μm. (*e*) Radiolarian micro-skeleton showing how the hollow porous silica microshell is structurally connected to an internal set of radially-directed mineralized spicules; scale same as in (*d*). (*f*) Labyrinthine micro-skeleton of a sea urchin showing complex porous architecture formed from a single Mg calcite crystal, scale bar = 100 μm [(*b*) to (*e*) courtesy of J. Forsdyke, University of Bath]

tangentially along the surface of the cytoplasmic wall or an internal organelle, or radially along tubulin-based filaments [Fig. 2(*b*) and (*c*)]. Independently, or in combination, these fields can generate a wide range of intravesicular mineralized patterns.

Cell wall scaffolds. The internal surface of cell walls and their associated structural polymers, such as spectrin, can be utilised as organic scaffolds for the patterning of intracellular vesicles and associated inorganic minerals. For example, curved rods of silica are formed by certain species of choanoflagellates due to mineral growth within preformed elongated vesicles that are shaped against the cytoplasmic membrane by two stress filaments.[16] Addition of microtubule inhibitors, such as colchicine, to cultures of these organisms results in the

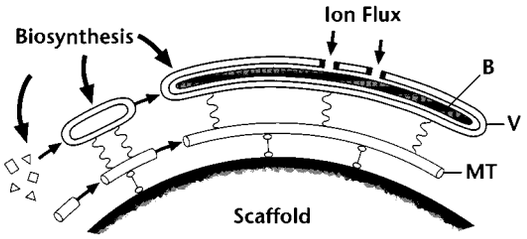

Fig. 3 Illustration of the general features of prescribed assembly in biomineralization. Cell walls, intracellular organelles and cellular assemblages can act as scaffolds for the assembly of microtubules (MT) which in turn are used as directing agents for the patterning of vesicles (V) involved in biomineralization (B)

formation of bent, mis-shaped mineralized rods,[16] consistent with a fundamental role for structural scaffolding in the development of the curved biomineralized form.

This idea can be developed to account for more complex mineralized architectures. If the development of a single vesicle against the cytoplasmic wall is so extensive that it produces a large bilayer sphere, then a complete mineralized shell can be fabricated by growth along the internal rim of the constraining membrane wall. This is observed during cyst formation in chrysophytes (golden brown algae) in which 10 to 15 μm diameter hollow silica spheroids are produced to house the cellular material.[17] Similarly, porous spherical frameworks can be generated from assemblages of vesicles that are organized against the cell wall. For example, the perforated silica shells of diatoms and many radiolarians are derived from close-packed arrays of large 'areolar' vesicles that are secreted and attached to the membrane wall (plasmalemma) of the cell prior to mineralization.[18,19] The arrangement of the vesicles into a thin polygonal foam provides organized interstitial boundary spaces that can be replicated in the form of delicate porous frameworks of amorphous silica [Fig. 2(*d*)]. Although many of the resulting patterns can be considered to arise from geometrical deviations in close-packing of vesicles against a curved surface, the process is not controlled by surface tension, as previously postulated,[8] but is the consequence of cellular organization within the interstitial spaces.[18,19] In the diatom *Coscinodiscus*, for example, a thin tubular membrane system is secreted from the golgi apparatus and assembled along with microtubules in the gaps between the areolar vesicles.[19] Silica deposition is then confined tangentially to this tubular vesicle system such that an open geometric mesh of mesopores is established. Whilst this architecture often represents the final micro-skeletal form exhibited by radiolarians, many diatoms such as *Coscinodiscus* continue to process the areolar spaces with a fine delicate pattern of silica. This is achieved by detachment and withdrawal of the areolar vesicles from the plasmalemma and infiltration of the new interface with cytoplasm containing silica deposition vesicles and small unmineralized golgi vesicles. Together these vesicles self-assemble to produce a species-specific nanoporous pattern of silica within the preformed mesopores.

Intracellular scaffolds. Spheroidal surfaces of intracellular organelles can also be recruited as structural templates for scale formation. For example, thin silica plates are deposited within flattened membrane-bound vesicles that are closely appressed to the surface of the endoplasmic reticulum in cells of the chrysophyte *Synura*.[20] A similar mechanism is involved in the formation of coccolith scales against the nuclear envelope in cells of *Emiliania huxley*.[21] In both cases, the organelle surface provides a substrate for the tangential development of the plate-like mineralized architecture, but other directing agents are required to produce the three-dimensional morphology. This is often achieved by orchestrating the growth away from the organelle surface through the use of cytoskeletal structures that are organized radially towards the periphery of the cell.

The combination of radial and tangential growth gives rise to some extraordinary intracellular architectures. In the radiolarian *Actinomma* concentric shells of reticulated silica dominate the skeletal morphology suggesting that the apposition of vesicles against a series of curved surfaces at successive levels within the cell is of key constructional importance. Close inspection of these structures indicates that structural connectivity is established between the different mineralized shells by radiating arrays of small silica spicules [Fig. 2(*e*)]. By comparison, the primary structural units in the acantharian exoskeletons are large spicules of strontium sulfate ($SrSO_4$) which emanate outwards from the centre of the cell.[22] When the spicules come into contact with the cytoplasmic membrane they begin to grow tangentially back along the surface of the cell to form a thin spherical frame that interconnects with the radial struts. The basis for the architecture seems to be dependent on the spicules and their associated vesicles which are arranged with high point symmetry (D_{4h}). It is as if the cytoskeletal framework is assembled along axes corresponding to the poles of a geometric solid, almost as if the vesicle network is suspended on the supporting threads of an external polyhedral cage. Similar observations can be made about the relatively simple internal silica skeletons of silicoflagellates[23] such as *Dictyoca,* in which the central reticulated framework is connected to six spines that radiate outwards. The completed structure could be achieved by the structural support of a central cluster of a small number of vesicles within an external organic frame of pseudo-hexagonal symmetry.

Extracellular scaffolds. As pointed out by Thompson,[8] the positioning and aggregation of cells can provide geometrical patterns in multicellular biomineralization in much the same way as for vesicles in unicellular organisms. Mineralization within the interstitial spaces and edges of a planar assemblage of three 'founder' cells generates the triradiate symmetry and curved arms of spicules formed in calcareous sponges[24] and the larval sea urchin micro-skeleton.[25] By similar reasoning, a cluster of four cells could account for the tetrahedral arrangement of silica spicules in tetractinellid sponges, whilst larger but discontinuous aggregates may be responsible for the patterned Mg calcite ossicles and spicules of holothurians.[8] Continuous assemblages in the form of foams and rafts of closely packed cells are responsible for much of the patterning of the labyrinthine skeleton of the crystalline shell (test) of adult sea urchins[26] [Fig. 2(*f*)]. It is important to stress that in each of these examples the intercellular boundary edges and interstitial mineralization sites are delineated by membrane-bound structures so that mineralization in the geometrically defined space is under strict biological control.

In higher organisms, the extracellular space is controlled by the secretion, assembly and cross-linking of polymeric frameworks, such as collagen, that are spatially and chemically fine-tuned for crystal nucleation and growth from supersaturated solutions. Pattern formation of extended structures is then determined by the spatial positioning of specific cells, such as epithilial cells in shell mantle,[27] osteoblasts and osteoclasts in bone matrix,[28] or ameloblasts in enamel.[29] In bone, this cellular organization can be significantly influenced by gravitational and mechanical force fields operating at the systems level. This is an interactive process because the matrix and mineral grow together and the intrinsic properties of the latter, such as density, deformation, ion mobility (piezoelectric behaviour) and surface reactivity are not under biological control. Moreover, bone can be remodelled or resorbed by cell-mediated processes that are often different from those involved in primary deposition.

Morphosynthesis of biomimetic forms

Recently,[30] Ozin and I attempted to establish a conceptual

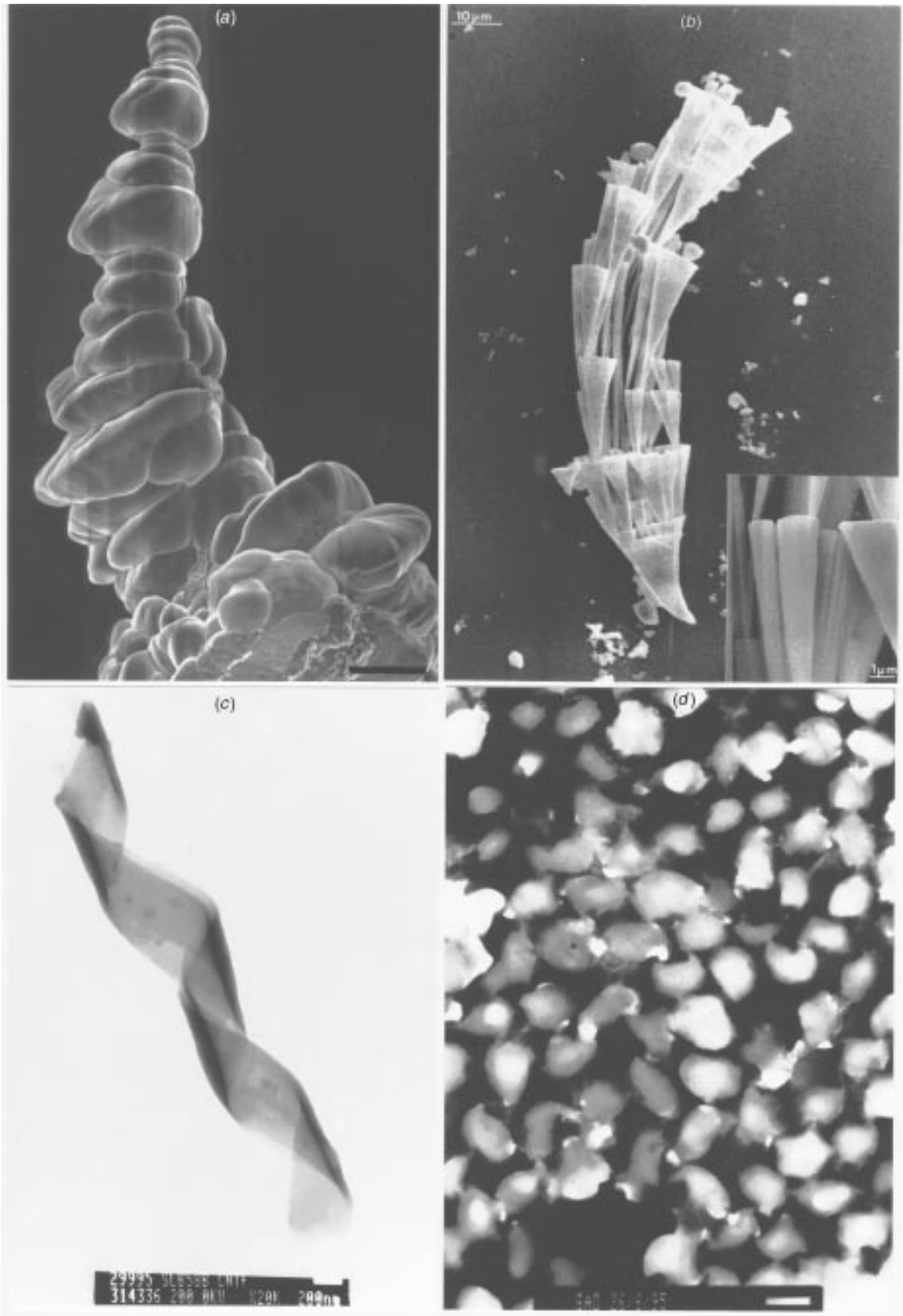

Fig. 4 (*a*) Spiral outgrowth of calcium carbonate formed by growing crystals in the presence of 10 mg dm^{-3} of a linear poly α,β-aspartate of M_r 7100, scale bar 100 μm. (*b*) Hierarchical morphology of $BaSO_4$ crystals formed in a 0.5 mM aqueous solution of polyacrylate of M_r 5100; scale bar 10 μm. The cone-shaped units develop on the rim of pre-existing cones, and each cone consists of myriad $BaSO_4$ nanofilaments (inset, scale bar 1 μm). (*c*) Self-assembled helical ribbon of a silica-phospholipid biphase, scale bar 200 μm. (*d*) Thin section showing a continuous silica framework produced by bacterial templating. The porous channels (white circles) are viewed end-on and are approximately 500 nm in width, scale bar 500 nm

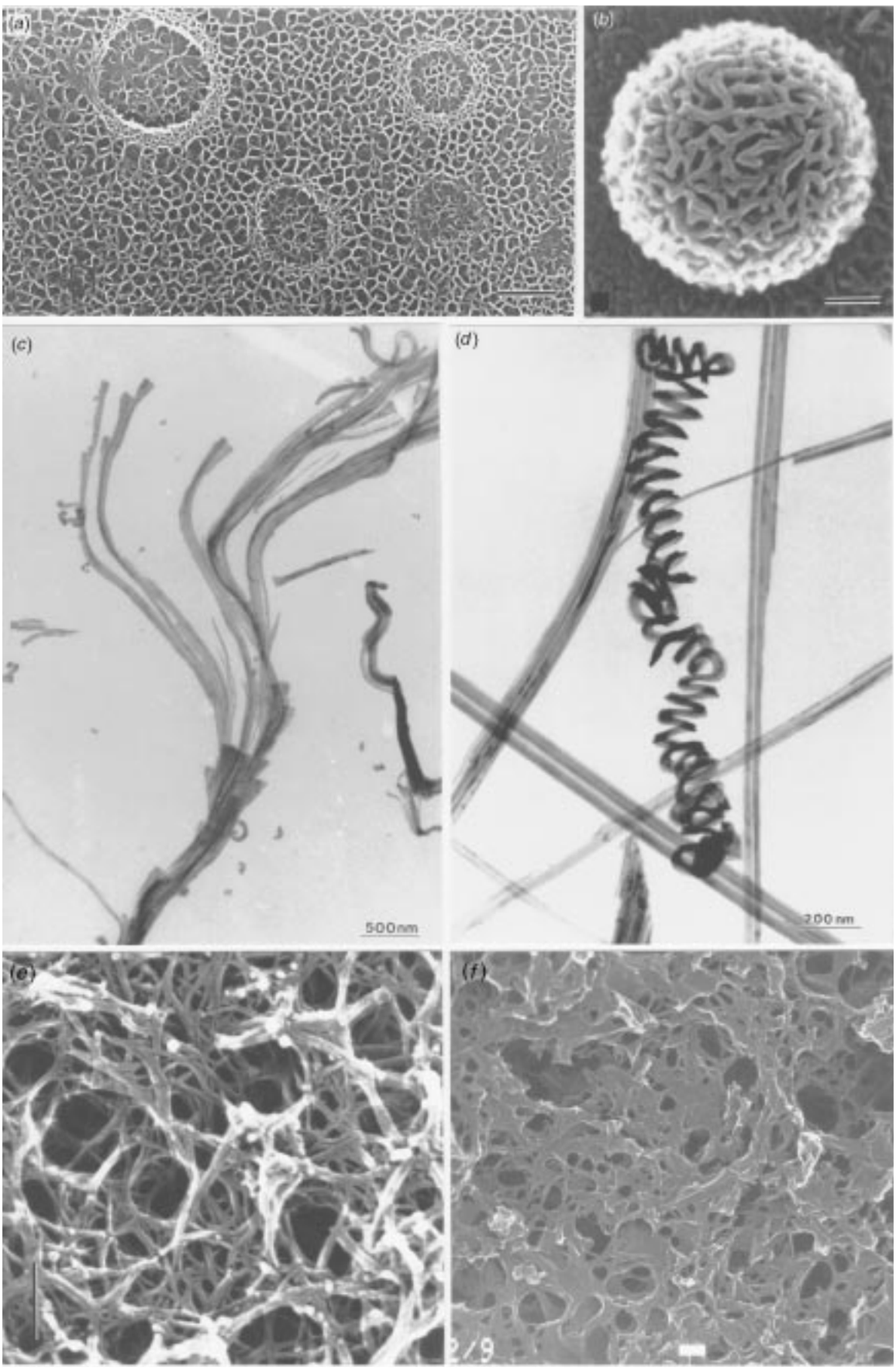

Fig. 5 (*a*) Cellular film of manganese(III/IV) oxide synthesized by reaction field templating in an oil droplet biliquid foam. The framework has cell sizes of 300 nm with continuous mineralized walls, 100 nm in thickness; note the additional higher-order morphological features (circular pits) with micrometre length scales, scale bar = 2 μm. (*b*) Hollow spherical shell of calcium carbonate (aragonite) formed by synthesizing a cellular mineralized film on polymer microspheres, scale bar = 200 nm. (*c*) $BaSO_4$ 'tentacles' formed from coaligned crystalline nanofilaments produced by synthesis in supersaturated microemulsions at room temperature, scale bar = 500 nm. (*d*) Individual $BaSO_4$ nanofilaments and a coiled morphological form synthesized as in (*c*) scale bar = 200 nm. (*e*) Micro-skeletal calcium phosphate synthesized in frozen-oil bicontinuous microemulsions, scale bar = 500 nm. (*f*) Silica microstructure produced by alkoxide condensation reactions in bicontinuous microemulsions, scale bar = 1 μm

framework in response to the question: can an understanding of pattern formation in biomineralization (morphogenesis) be integrated within a synthetic approach to inorganic materials with complex form (morphosynthesis)? Some key connections are immediately apparent. First, unusual morphological forms can be produced by fluctuating chemical processes involving local perturbations in the fluid–solid interface during mineralization. Secondly, many bioinorganic forms are directed along, around and inbetween the external surfaces of organic structures and assemblages that act as scaffold-like templates. Thirdly, biominerals can be shaped within patterned organic compartments (vesicles) which may be static, interactive or reconstructive. These generalizations are embodied, respectively, in three approaches to the synthesis of complex inorganic form; *viz.* fluid–solid patterning, template-directed assembly and reaction-field replication.

Fluid–solid patterning of mineral surfaces

Synthetic analogues of biomineralized forms produced by contingent interactions at inorganic–organic interfaces have been known for many years, being the basis of extensive investigation in the late nineteenth century. Many of these are generated from viscous crystallization solutions or gels in which nonlinear processes arising from chaotic mixing, vortex formation, diffusion and chemical gradients (Liesegang's rings, Turing patterns), and instabilities in hydrodynamic flow, give rise to spatial and temporal patterns in mineral deposition at the fluid–solid interface. For example, Harting[31] reported in 1872 that the growth of calcium carbonate in egg white (albumin) produced rounded inorganic concretions with banded internal structures; such structures are not dissimilar to those of fish otoliths. Many banded inorganic structures (Liesegang's rings) have also been observed in silica gels, and fractal structures have been produced in a collagen-based matrix.[32] In many cases, the periodicity and patterning of precipitation arises from diffusion-limited and mass transport processes in the fluid, and can be mathematically modelled.[33]

More complex forms can be induced by increasing the chemical reactivity of quiescent media.[34,35] For example, if silica gel is made more soluble and reactive by raising the pH above 8, then a wide range of unusual curved morphologies, such as helical ribbons and spiral foils of calcium carbonate, are obtained.[36] These shapes are the result of the interplay between localized growth and inhibition at the fluid–solid interface which arises from the indeterminate formation and rupture of a semi-impermeable calcium silicate membrane around the developing crystals, a phenomenon commonly observed in 'crystal gardens'. We have described similar effects for the growth of distorted spirals of calcium carbonate (vaterite) in aqueous solutions of polyaspartate.[37] The initial stage of crystallization consisted of globular aggregates of vaterite crystals that were coated in a semi-permeable layer of the surface-bound polymer and amorphous $CaCO_3$. Intermittently, outgrowths would erupt from these coated particles to form long spiral-shaped crystalline appendages [Fig. 4(*a*)]. Although the details are not known, circumstantial evidence supports a hydrodynamic mechanism for the spiral architecture, rather than a structure-based process involving a centralized screw dislocation in the primary particles.

The ability of soluble polymers to induce complex shapes in inorganic materials could be a general phenomenon that deserves further investigation. Recent experiments suggest that it may be possible to directly synthesize hierarchical barium sulfate structures in the presence of negatively charged polyacrylate molecules.[38,39] Under a limited set of chemical conditions, elaborate structures comprising an assembly of cone-shaped units of $BaSO_4$ are preferentially formed [Fig. 4(*b*)]. The surface of the inorganic cones consists of a coaligned array of crystalline barite filaments that are contiguous at the base of each splayed structure. How these remarkable morphologies arise from crystallization in aqueous solution is not known.

Template-directed assembly

Supramolecular assemblies of organic molecules can be exploited as chemically- and spatially-specific interfaces for the site-directed nucleation, vectorial growth and morphological patterning of inorganic materials.[40] Typically, surfactants and lipids in the form of cylindrical micelles,[41,42] rod-shaped[43] and helical[44,45] microstructures, or liquid crystals[46,47] have been used to prepare organized inorganic materials. Two strategies are currently being explored. In one approach, formation of the supramolecular architecture occurs simultaneously with mineral deposition, such that the organic and inorganic phases are coassembled. This has been exploited extensively in the synthesis of ordered silica and transition-metal oxide mesophases[41,48] in the presence of close-packed rod-like surfactant micelles. In such cases, the patterning process is predominantly internalized around a packed organic array to produce an organized inorganic 'endoskeleton'. Recently, we discovered that chiral phospholipid molecules (diacetylenic phosphatidylcholine) and silicate anions could be assembled synergistically to produce a silica–lipid multilamellar biphase with helical form[49] [Fig. 4(*c*)]. This was achieved by coupling the self-assembly of the phospholipid with the hydrolysis and condensation of tetraethoxysilane molecules bound at the choline headgroups such that organic crystallization and silica polymerization occurred simultaneously.

Another approach involves the use of preformed organic microstructures as patterning templates for inorganic deposition. For example, hollow silica tubes have been produced by incubating cylindrical phospholipid microstructures with a silica colloid.[44] Similarly, metal-ion-binding to the sugar headgroups of multilamellar tubules of a cerebroside lipid produced highly-elongated fibrous inorganic oxide–biolipid microstructures[43] by mineral decoration of the external surface of the organic template. Self-assembled templates have also been used to generate elaborate surface patterns by site-specific reactivity; for instance, helical arrays of gold crystals have been synthesized on the redox-active edges of phospholipid open-ribbon microstructures.[45]

In recent work,[50] we used an organized bacterial superstructure as a macroscale organic template for patterned inorganic deposition. The template was in the form of a macroscopic fibre which had an internally ordered microstructure of coaligned multicellular filaments of *Bacillus subtilis*. The bacterial superstructure, which is formed by slowly drawing a macroscopic thread from a web culture[51] was infiltrated with a silica nanocolloid by a reversible swelling procedure. This resulted in mineralization of the interfilament spaces to produce a 'bacterial skeleton' consisting of a continuous inorganic wall structure, 50 to 200 nm in thickness [Fig. 4(*d*)]. The organized bioinorganic material was subsequently heated to 600 °C to produce an ordered macroporous replica which consisted of an array of 0.5 μm wide channels aligned along the morphological axis of the intact fibre.

Materials replication of organized reaction fields

Organized reaction media, such as vesicles, microemulsions and biliquid foams, are compartmentalized liquids in which chemical reactions can be spatially confined. In principle, the restriction of mineralization reactions in the shaped environments of these self-organized systems should constrain and pattern the deposition of inorganic materials by spatial delineation of the reaction field. The simplest case would constitute the direct replication of a static or transitory environment. For example, mineralization of the interstitial spaces and boundary edges of

an oil-droplet foam produced by microphase separation in supersaturated bicontinuous microemulsions gives rise to the imprinting of cellular frameworks of aragonite[52] or transition-metal oxides[53] [Fig. 5(*a*)]. Typically, the cellular films have continuous, branched mineral walls 20 to 100 nm in width, and enclosed cells of average size 45 to 300 nm, depending on the reaction conditions. Because the foam is a transitory structure, mineralization and oil droplet self-assembly must occur almost simultaneous if the interstitial spaces are to be filled with a continuous inorganic framework of calcium carbonate or metal oxide. This is achieved, respectively, by outgassing of CO_2 from or O_2 diffusion into the microemulsion. Both these processes are accelerated as the air–water interfacial area increases during foam formation, and give rise to rapid increases in supersaturation by shifting the carbonate–bicarbonate and redox/hydrolysis equilibria, respectively. The resulting micro-skeletal structures resemble the porous frameworks of diatoms and radiolarians, particularly when the synthetic forms are fabricated as hollow micro-spheres by using polystyrene beads as external substrates[52] [Fig. 5(*b*)].

A number of other studies have shown that the imprinting of inorganic morphologies in organized organic media results in materials that are not commensurate in size or shape with the associated reaction fields. It turns out that interactions between the incipient mineral and reaction compartment result in local reconstruction of the biphasic medium such that new patterns and morphologies evolve (metamorphose) by synergistic mechanisms. For example, mesolamellar aluminophosphate spheroids and hollow shells with complex surface patterns, similar to those of silicified diatoms, have been directly synthesized by a process involving adhesion, fusion and reshaping of primary vesicles of the hybrid inorganic–organic phase.[54,55] Recently, we showed that the precipitation of barium sulfate in supersaturated water-in-oil microemulsions produces highly elongated crystalline nanofilaments of barite many of which are twisted and occasionally coiled[56] [Fig. 5(*c*) and 5(*d*)]. The unusual filamentous form appears to evolve from the unidirectional fusion of 4 nm sized surfactant-stabilized water droplets containing either Ba^{2+} or SO_4^{2-} ions. Initially, aggregation and exchange of the droplets results in mixing of the reactants in a collision pair, and the concomitant increase in supersaturation gives rise to rapid nucleation of $BaSO_4$. Fusion is irreversible because some of the sulfonated surfactant molecules are immobilized by being strongly adsorbed onto the incipient inorganic phase. However, the presence of bulk water (the phenomenon is not observed in 'dry' reverse micelles) enables unbound surfactant molecules, positioned at sites away from the $BaSO_4$ cluster, to undergo further exchange with other microemulsion droplets. Each collision pair therefore leads to the unidirectional propagation of a nanofilament of $BaSO_4$, perhaps one microemulsion droplet wide, through the organized reaction medium.

In other work, we have used bicontinuous microemulsions, assembled from mixtures of tetra- and hexa-decane, water and the cationic surfactant didodecyldimethylammonium bromide (DDAB), as nanodimensional channel-like reaction fields for inorganic materials synthesis. These microemulsions are structured as compartmentalized liquids in which the oil and water components are separated into highly branched and interconnected conduits, approximately 2 nm wide. By using a supersaturated aqueous phase in place of water, and freezing the oil channels at temperatures above 0 °C, our aim was to transcribe the complex morphology of the assembled reaction field into a nano-textured inorganic replica. However, studies of calcium phosphate precipitation in bicontinuous microemulsions[57,58] produced materials with remarkable interconnected micro-architectures on length scales two orders of magnitude greater than that of the compartmentalized reaction environment [Fig. 5(*e*)]. Although transmission electron microscopy (TEM) studies suggest that nucleation and initial growth of the inorganic phase replicate the arrangement of the nanoscale water conduits, later stages of growth were accompanied by reconstruction of localized regions of the microemulsion structure. This seems to develop from interactive coupling of the crystallization process and surrounding reaction environment, such that morphological patterns at the micrometre scale develop over a period of several days from the nanoscopic materials replica of the compartmentalized liquid.

Micro-skeletons consisting of silica have been synthesized in bicontinuous microemulsions by sol–gel reactions involving hydrolysis and condensation of tetraethoxysilane (TEOS) present initially in the oil phase.[59] Reaction of TEOS at the oil–surfactant–water interface results in phase partitioning as the hydrolysis products are water soluble. This, together with freezing the oil prior to reaction, gives rise to the controlled precipitation of silica in the nanoscopic water channels. If left for several days however, extended three-dimensional microscale frameworks of amorphous silica are produced [Fig. 5(*f*)]. The results indicate that the composition, viscosity and structure of the microemulsions have a profound influence on the general morphological features of the associated silica phase. Like the calcium phosphate materials, although there is morphological correspondence, the length scales of the reaction media and silica framework are very different. In the latter case, one significant factor influencing the local reconstruction of the reaction field is the production of ethanol as a by-product from the silicification process.

Conclusion

This article has focused on a new area of bioinorganic chemistry research concerning the synthesis of complex inorganic form in biomineralization and biomimetic materials chemistry. Recent progress indicates that biological concepts such as self-organization, morphogenesis and pattern replication have much in common with new chemical strategies concerned with the synthesis of complex inorganic form. Thus, the traditional view of inorganic solids as 'condensed-matter' is currently being reshaped to accommodate the notion of *organized-matter chemistry.* This transformation represents a shift in emphasis away from solid phases as thermodynamic states of consolidated matter, towards solids as organizational states determined by local (rather than global) energy minima and constructional mechanisms. Research at the biology–chemistry–materials interface will continue to play a pivotal role in the development of this new paradigm.

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