

Report synthesizing the calcium carbonate biomineralization avenues investigated and/or already translated into technological/industrial applications and future development potential



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**Synthesizing the calcium carbonate biomineralization avenues investigated
and/or already translated into technological/industrial applications and
future development potential.**

Contributions from the CACHE (Calcium in a Changing Environment)

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I. General Introduction

At the dawn of civilization, the only materials available to man were either biological or mineral, and prior to the transformation of these materials. Afterward people have begun to use and mimic biological materials. For instance, as civilization advanced and the written word evolved, stone tablets were replaced as a writing surface by papyrus, bark, animal skins, and eventually paper, bioderived materials that provided significant advantages over stone¹. Nature offers an enormous number of materials with fascinating mechanical, optical, electrical, magnetic, chemical and biological properties. Mimicking these biological materials designs and processes could create advanced engineering materials useful for various applications ranging from portable electronics to airplanes. However, natural designs of biological materials are difficult to mimic synthetically mainly because of a lack of knowledge of materials structure–property relationship and process methods². As a result, there is a growing requirement for the academic, research, and industrial communities to understand biomimetic materials design principles and look for innovative ways of addressing these issues.

In the 1980s, a revolution of bio-inspired materials began³. Several aspects of natural biological materials came to the forefront for various reasons. Bone with its unique structure and properties, is a composite of organics and minerals⁴. A more natural artificial bone with similar composition and structure to the biological tissue would be considered as a promising avenue to medical applications. Nacre, known as mother of pearl, which is also known for its excellent mechanical properties, has been a source of inspiration to produce tough and stiff material. Its brick-and-mortar arrangement is particularly useful in the inhibition of crack formation⁵. Scientists attempt to mimic this microstructure in order to achieve similar control over mechanical properties. The spider silk with incredible strength has led researchers to investigate the possibility of reproducing artificial fibre with similar properties⁶. By biomimicry approach, inspired from the byssus of mussel⁷ and the feet of geckos⁸, bioinspired solutions have been successfully demonstrated for forming chemically or physically adhesive bonds with almost any surface. While research focuses on the development of better materials for technological applications, Nature provided a vast reservoir of solutions to engineering problems. Biological organisms are improved and refined by natural selection, only the most successful structural forms have survived, unveiling the secrets of their survival can reveal outstanding design characteristics. By imitating nature-designed structures, biological processes and methods, scientists and engineers create materials with properties and performance beyond the known materials.

In the Marie-Curie CACHE (Calcium in a Changing Environment) Initial Training Network, we are particularly interested in molluscan shells. Especially in more acidic oceans, understanding the climate change consequence over the production of four most important commercial marine shell fish species in Europe is the main challenge of the project. Understanding how these animals make shells and regulate shell production in the natural

environment is critical to the understanding of how they will fare under climate change. Also, knowledge of the natural processes is used as reference to biotechnology exploitation.

Biomimetic materials science and engineering advocate for a full appreciation and understanding which can be used to solve problems related to materials design and processing. It is helpful to refine our understanding of the structural form, processing and function of natural materials. The nature and the biomineralization process of molluscan shells have been studied extensively from macroscopic to molecular level. Biomimetic materials science inspired from molluscan shells has developed mainly from structure mimicking and processing mimicking aspects.

Composed of calcium carbonate and organic components, the molluscan shell is superior to most other composite ceramics in stiffness, strength and toughness⁹. Biomimetic materials science and engineering inspired from molluscan shells is achieved in two ways, structure and process mimicking¹⁰. The most common type of biomimetic materials is the emulation of natural material structures. Molluscan shells present a layered structure that can be categorized into seven kinds of generally accepted structures: columnar and sheet nacre, prismatic, crossed-lamellar, foliated, homogeneous and complex crossed-lamellar microstructure¹¹. The different calcified microstructures are composites of calcium carbonate together with biomacromolecules, which are spatially organized in an exquisitely controlled manner. Each microstructural organization confers distinctive mechanical properties to the shell. Aiming at mimicking these inspiring biological structures and their properties, efforts in the fields of chemistry, physics, and materials science over the years have led to a large variety of inorganic and hybrid materials, targeting several applications¹². Some analytical and numerical models have been put forward, drawing from the inspiration of extraordinary natural structures, to illustrate the basic mechanical design principles of biocomposites¹³. Numerous innovative technologies have been developed to mimic the biological hierarchical structure¹⁴. Artificial material with nacre-mimicking microstructure is an outstanding example of how the mechanical properties of a bulk material can be improved by introducing a microstructure¹⁵. Nevertheless, to date, the studies about the role of hierarchical structure on the mechanical behaviour of biological materials remain at an infant stage, and the processing of organic-inorganic hierarchical composite materials with controlled structure and morphology is still a major challenge.

Emulating nature in the process is another aspect of the biomimetic design of engineering materials. By their biologically controlled mineralization, molluscs have the ability to produce calcium carbonate with nanoscale dimensions, and also to assemble these nanoparticles into bulk materials with desired properties. Instead of relying on conventional fabrication techniques, it is possible to utilize biomolecules to fabricate artificial materials. Different from the high temperature and high pressure traditional processes, bioinspired fabrication systems could occur under eco-friendly environment. This development has an enormously potential for engineering materials. The chemical and physical properties of biominerals, such as

polymorphs, morphology, crystallographic orientation and crystal size, are strongly influenced by organic macromolecules¹⁶. With technological advancements in organic and polymer sciences, complex macromolecular templates with specific structure and functions could be achieved, which could be used for complex hybrid material formation¹⁷. We are able to control the formation of individual nanoparticles¹⁸, assemblage of nanocomposites into a highly ordered structure remains a major challenge for fabricating bulk biomimetic materials. A better understanding of biomineralization in molluscan shell will provide new insights for improving biomimetic material processing.

To date, the molluscan shell is considered in most cases as a model for the design of tough ceramic engineering materials, other functions from mollusc biological system, including optical (e.g., structural colour)¹⁹⁻²⁰, stimuli-responsive (e.g., electromechanical materials), self-healing (wet and under water)^{21 - 22}, catalytic (ion transport, biologically controlled mineralization), etc can also be taken as starting point for material engineering design. Disposed of an extensive range of materials, new attempts should be made to synthesize new materials that can overcome the limitations of biological materials and exhibit versatile characteristics such as semi-conductivity²³, special optical²⁴ and magnetic properties²⁵. This might be accomplished through collaboration among a wide variety of scientific fields, including organic chemistry, polymer chemistry, inorganic chemistry, physics, biology and engineering²⁶.

Although tremendous progress has been made in the field of advanced biomimetic materials, there still remain technological challenges, including the adaptation of laboratory processes to industrial production, as well as the environmental impact and economic burden of materials production.

II. Nature of molluscan shells

Molluscs are soft-bodied animals, many of them show complex adaptations by which they maintain their soft tissues, protect themselves against predation and avoid desiccation. These adaptations rely on the elaboration of an external calcified rigid structure, the shell²⁷. Molluscan shells are composed of calcium carbonate in an organic matrix²⁸ that are hierarchically arranged from nano- to meter-scale structures²⁹. They show remarkable mechanical toughness and provide support for the body, protection against predators and rocks moved by the current. In the last years, the field of mollusc biomineralization has known a tremendous development: complex hierarchical organization of the shell biominerals has been deciphered in a few models³⁰; identification of a huge variety of shell proteins has showed their different functions over the mineral phase formation³¹. While new designs and

applications inspired from biominerals are proposed continuously, scientists begin to realize that one must determine the relationships between structure, processes and properties. The understanding of biomineralization can further advance the field of materials science techniques.

The shell is often considered as a biomineralization product typical of molluscs: its morphological characters and ornamentation are used indeed for species identification, at different taxonomic levels, from classes to species³². Living molluscs represent about 120 000 species, grouped in eight classes of unequal species richness (Fig. 1)³³⁻³⁴. Two classes of somewhat atypical worm-like molluscs (the Solenogastrea and Caudofoveata), do not secrete a shell, but tiny spicules or sclerites on the surface of their teguments. A third class, are the polyplacophorans, better known as ‘chitons’. Chitons are grazing marine molluscs that do not produce a shell, but series of eight calcified plates that can slide with each other, when the animal cowers. The group of the true shell-forming molluscs is known as the conchiferans. It comprises five classes, i.e. the monoplacophorans, the bivalves, the scaphopods, the gastropods and the cephalopods²⁷. The bivalves are the second most speciose group in the phylum Mollusca³⁵ and are major components of marine food webs including wild harvested and farmed food source.

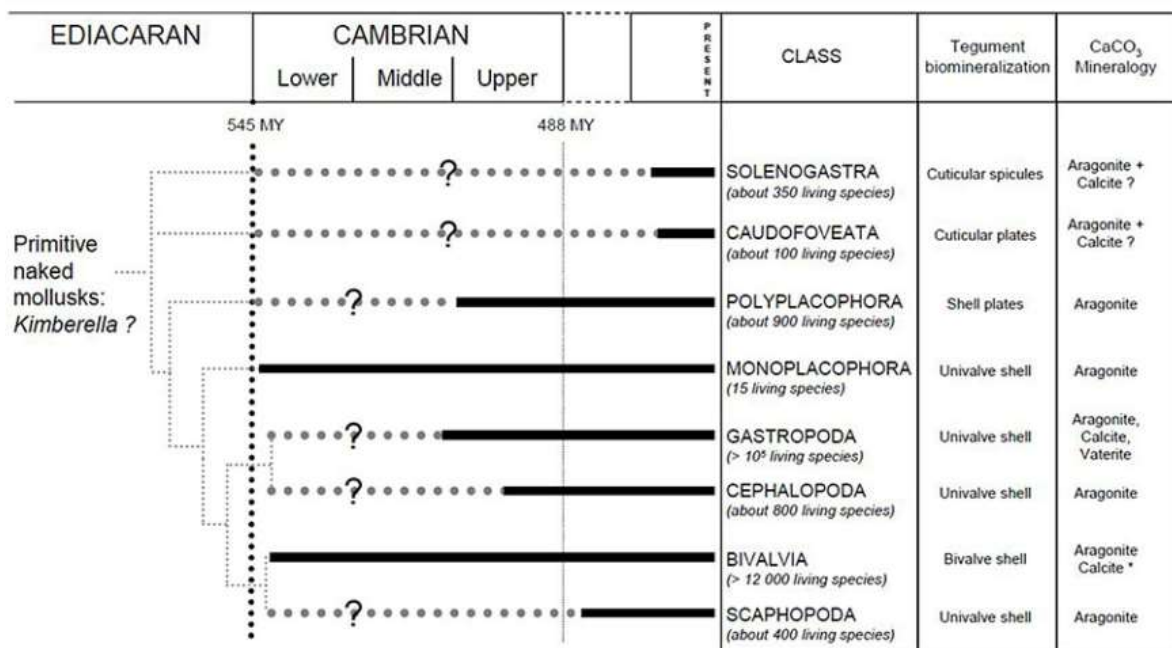


Figure 1: Phylogeny of phylum Mollusca, according to Lecointre and Le Guyader³³. (* indicates particular cases where carbonated apatites (francolite, dahllite) are also present in the periostracal layer: such examples include the rock-burrowing ‘date mussel’ (*Lithophaga*), in which these minerals increase the resistance of the shell against abrasion.)

The CACHE network focuses on four European major shellfish species: the blue mussel (*Mytilus edulis*), the Pacific oyster (*Crassostrea gigas*), the king scallop (*Pecten maximus*) and two soft-shelled clams (*Mya arenaria* and *Mya truncata*). These were chosen, not only for their commercial interest, but also for the fact that they encompass a variety of shell mineralogies and microstructures (Tab. 1). Shell mineralogy and microstructures can have a significant influence on both the energetics and ease of deposition of shells, and also on the stability of shell structure under changing conditions. Consequently, these species provide valuable case studies within which to conduct detailed mechanistic studies, while as a group, they encompass much of the variation in shell form and structure that is encountered in molluscs.

Taxa	Outer shell layer	Middle Shell layer	Inner Shell layer	Periostracal characteristics
<i>Crassostrea gigas</i>	Thin layer of calcite prisms	Foliated calcite Also may include patches of 'chalk' microstructure	Foliated calcite	Very thin <1 μm so frequently lost from shell exterior
<i>Pecten maximus</i>	Foliated calcite	Aragonite crossed-lamellar	Foliated calcite	Very thin <1 μm so frequently lost from shell exterior
<i>Mytilus edulis</i>	Fibrillar calcite prisms	Aragonite nacre	Aragonite nacre	Very thick (up to several tens of μm), tend to be persistent throughout life
<i>Mya arenaria</i>	Granular aragonite prisms	Crossed-lamellar aragonite	Complex crossed lamellar aragonite	Thin (about 3 μm)

Table 1: Four species studied in CACHE network

1. Formation of molluscan shell

The molluscan shell is composed of calcium carbonate and an organic matrix. The mineralization and shell matrix secretion begin early in larval life. The shell valves continue to enlarge during larval transformation. After metamorphosis the shell formation continues at the mantle edge. Beside the normal calcification process, molluscs have also the ability to rapidly repair shell damages, which is an undeniable advantage for overcoming external impacts.

Typically, the outer organic membrane, i.e. the periostracum, covers the external surface of the shell. It consists of conchiolin and resists to both alkaline and acid conditions, thus protecting the shell from dissolution in water. Mineralized layers, secreted by the most

peripheral cells of the mantle are deposited below the periostracum (Fig. 2). The mantle is a complex organ, comprising connective tissue, neural tissue and muscles in addition to glandular and epithelial components involved in shell secretion³⁶. When a mollusc builds its shell, the calcifying epithelium of its mantle secretes a complex mixture of proteins, glycoproteins, and polysaccharides, collectively referred to as the skeletal matrix. The matrix regulates the nucleation, orientation and growth of calcium carbonate crystals³⁷, this process is classified as the extracellular calcification process³⁸. The calcification process takes place in the extrapallial space, between the periostracum and the mantle, without direct contact with cells of the mantle epithelium.

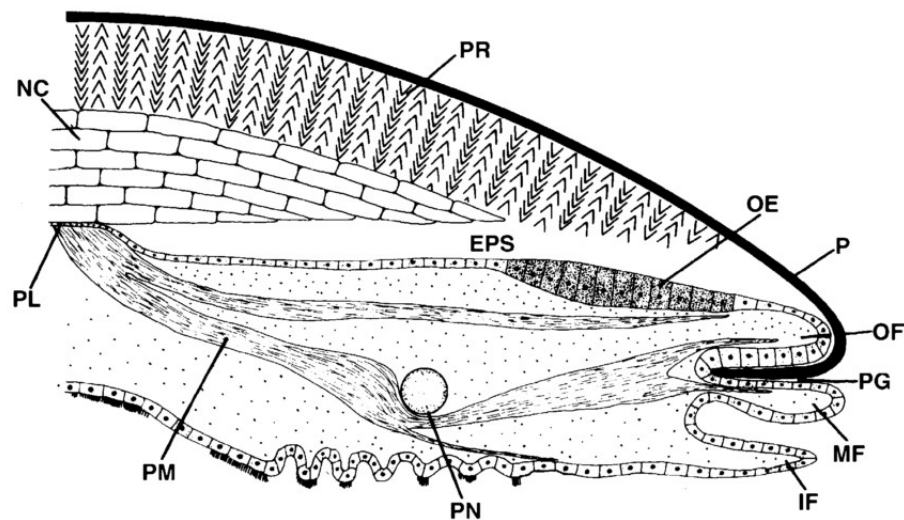


Figure 2 : Diagram of a section through the edge of the shell and attached mantle of a bivalve mollusc. The calcification occurs at the edge of the shell, at the interface between the mantle, the periostracum (P) and the shell itself, this interface corresponds to an extrapallial space (EPS). (MF), middle fold of mantle; (NC), nacre; (OE), outer epithelium of the mantle; (OF), outer fold of the mantle; (PG), periostracal groove; (PL), pallial line; (PM), pallial muscle; (PN), pallial nerve; (PR), prismatic shell layer³⁹

From a material science point of view, mineralization appears to occur within a preformed three-dimensional organic framework which acts as the template that provides the control over the morphology, crystal nucleation, and growth of mineral, along with the polymorph type and crystallographic texture^{40,41,42,43}. The structure of the shell at micro-scale varies widely within and among mollusc groups and is determined by the organic matrix⁴⁴. It is, accordingly, vital to understand the structure of the organic framework and its macromolecular layout so that critical features such as the crystal nucleation sites and the structural morphology that control mechanical behaviour. Such understanding provides possible materials design inspiration, for example, nano-scale ceramics, polymer electronic devices and synthetic bone implants. Identification of biomineralization specific genes through mantle transcriptomes contributes also to the understanding of biomineralization processes. Mantle transcriptomes, for the purpose of identifying the role of putative shell

formation genes during biomineralization, have been assembled for clams⁴⁵, pearl oysters^{46, 47, 48}, limpets⁴⁹, mussels⁵⁰ and scallops^{51, 52}.

The present report deals with the molecular aspects of shell biomineralization, we focus on the composition, the function and the organization of each component in the natural material and the relationships between them.

2. Biological components of the shell

The molluscan shell is an organo-mineral composite, the shell matrix is a mix of proteins, glycoproteins, chitin and acidic polysaccharides⁵³. Although the organic matrix is quantitatively a minor constituent in the molluscan shell (0.1 – 5.0 % w/w), it controls different aspects of the shell formation processes: the synthesis of transient amorphous minerals and their evolution to crystalline phases, the ‘choice’ of the type of calcium carbonate, and the organization of crystallites in complex shell textures (microstructures)⁵⁴. At molecular level, the matrix plays a key role in the mineralization process. At macroscopic level, the adjunction of organic components to a mineralized structure enhances the mechanical properties to the whole organo-mineral assembly.

In the last few years, an increasing number of shell matrix components have been identified and various hypotheses on their putative functions have been proposed^{31,55}. Several genes encoding the matrix components have also been characterized^{56,57,58}. Classically, the matrix is retrieved by dissolving the shell mineral phases with a weak acid or with a calcium-chelating agent like EDTA⁵⁹⁻⁶⁰. By doing so, the matrix can be separated into an insoluble fraction (referred to as conchiolin⁶³) and a soluble one⁵⁵. The ratio between the two fractions can considerably vary: while the soluble fraction represents between 0.03 and 0.50 wt %, the insoluble fraction varies in greater proportions: from 0.01 % (in some crossed-lamellar neogastropods for instance) to 4-5 wt % of the shell of the abalone or *Nautilus*⁵⁴. The major components of the insoluble fraction are often cross-linked and hence relatively insoluble in mild acids or at neutral pH⁶¹. Water soluble proteins from molluscan shells are generally acidic due to relatively high mole fraction ratios of aspartic acid. The complete amino acid content is known also for several purified soluble proteins⁶². Since Crenshaw has isolated Ca-binding glycoprotein from the shell of *Mercenaria mercenaria*⁶³, the Ca-binding property has been assigned to the water soluble proteins of many kind of molluscan species⁶⁴.

The shell matrix is not only present between the mineral particles, but also within mineral particles in the form of an organic intracrystalline matrix⁶⁵(Fig. 3). Nacre tiles are separated by thin interlamellar layers of biopolymer framework with a thickness in the shell of approximately 30 nm⁶⁶. There is also evidence of a continuous intracrystalline matrix surrounding coherent nanograins in individual nacre tablets⁶⁷. The intracrystalline matrix

would impart increased toughness to the structure via the energy absorptive capacity of a visco-elastic matrix.

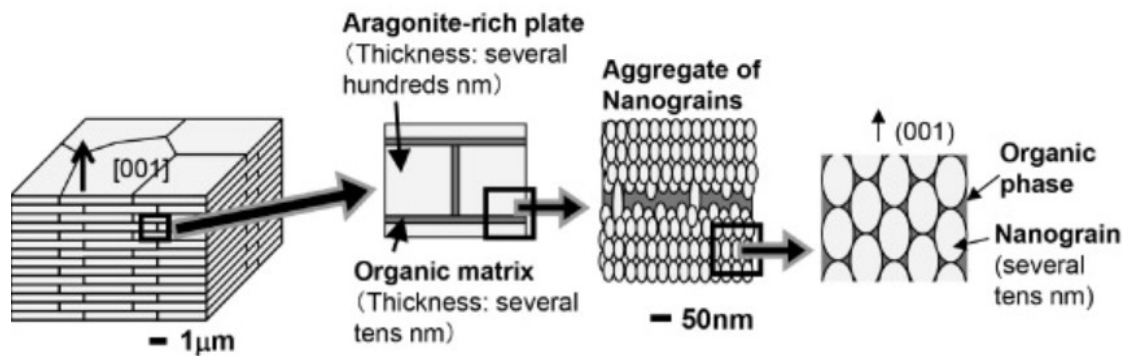


Figure 3: Three-dimensional model for nacre structure. It shows the “brick-and-mortar” structure, consisting of horizontal layers of polygonal aragonite plates within shell matrix. Single plates are uniformly aligned vertically in the c-axis [001] direction. The platelet consists of 'nanograins', aragonite particles of the order of several tens of nanometres and the organic phase surrounding the nanograins⁶⁵.

Until recently, the biological mineralization was considered to occur by antagonist mechanisms: crystal nucleation and growth inhibition⁵⁴. Emerging models try to translate a more complex reality, illustrated by the huge variety of shell proteins, characterized so far. Nowadays, the shell matrix appears as a whole system, which regulates protein-mineral and protein-protein interactions, as well as feedback interactions between the biominerals and the calcifying epithelium that synthesizes them⁶⁸.

Here below, we briefly describe several typical shell macromolecules and their function in the biomineralization process. The macromolecules in the shell matrix have already been reviewed extensively in the literature^{31,54,69,70}.

2.1 Insoluble macromolecules

The major components of macromolecules are hydrophobic, often cross-linked and is hence relatively insoluble in mild acids or at neutral pH. These macromolecules are referred to as ‘frame-work macromolecules’, because they provide (1) a three-dimensional matrix in which the mineral phase is formed and (2) a substrate from which some of the control proteins interact with the mineral phase⁷¹. Their nature can vary considerably from tissue to tissue and in many cases, they are indeed tissue specific. They can often be extracted or degraded chemically in the presence of mineral, implying that they are less intimately associated with the mineral phase. Common examples of framework macromolecules are Gly- and Ala-rich proteins (structurally similar to silk-fibroin) in molluscan shells⁷². These repetitive acidic motifs would induce CaCO_3 nucleation and promote epitaxial growth of the crystals or, in contrast, terminate crystal growth when covering growth surfaces⁷³.

A. Chitin

Chitin is well-known to be a key component in molluscan shell and nacre formation^{74,75, 76, 77,78}. It is one of the first biopolymers identified in the organic part of molluscan shells^{79,80} and plays a major structural role in the current concepts for understanding mollusc biomineralization^{81,82,83,84}. Biomineral orientation^{75, 85} and the activity of certain aragonite-inducing proteins⁸⁶ are closely related to the chitin orientation. Moreover, the fact that an inhibition of chitin synthesis disturbs the larval molluscan shell development *in vivo* indicates that chitin and its supramolecular architecture rigorously influences the biomineralization of molluscan shells on various levels of hierarchy⁸⁷.

Chitin ((C₈H₁₃O₅N)_n) (Fig. 4) is a biopolymer resulting from the copolymerisation of at least 50% of N-acetyl-β-glucosamine⁸⁸ in its β-conformation^{89,90,91} and D-glucosamine. Structurally, chitin is composed of a linear chain of (1→4) linked 2-acetamide-2-deoxy-β-D-glucopyranose units⁹² also designated as N-acetyl-β-glucosamine units. In its extracted crude form, chitin has a highly ordered crystalline structure with strong inter- and intramolecular hydrogen bonds, forming two allomorphs: α and β⁹³. α-chitin is by far the most abundant, being present in arthropods, and is characterised by an antiparallel arrangement of the chains, which leads to stronger intra- and intermolecular hydrogen bonds. The rare β-chitin, found in association with proteins in squid pens, is characterised by a parallel chain arrangement and weaker intermolecular hydrogen bonds rendering materials with higher reactivity and higher affinity towards solvents⁹⁴. The α and β forms can be differentiated by infrared and solid-state nuclear magnetic resonance spectroscopy together with X-ray diffraction⁹⁴.

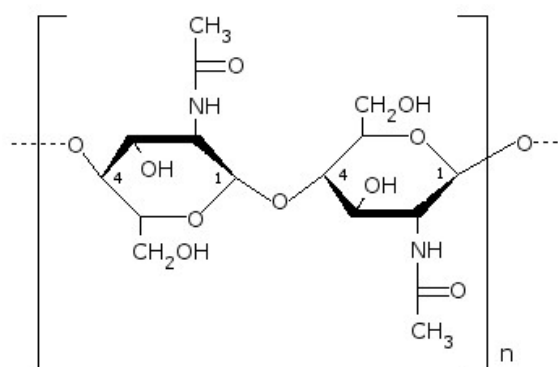


Figure 4 : Structure of chitin molecule, two of N-acetylglucosamine units repeat to form chitin long chains.

Chitin, the second most abundant natural polymer, just after cellulose^{92,94}, is part of the organic matrix of exoskeletons of crustaceans (e.g. crabs, lobsters and shrimps) and shells of molluscs.^{93,94} To obtain chitin from molluscan shells, an acid treatment is needed to dissolve the minerals followed by purification with an alkaline treatment to remove the proteins retained from the surrounding tissue. The alkaline treatment can also remove some acetyl groups bound to the amine. When enough acetyl groups are removed (50 % deacetylation or more) the polymer is called **chitosan**. In acidic solutions, the primary amine is protonated and chitosan becomes a soluble, positively charged polyelectrolyte. As a **polycation**, chitosan has

extensively been used in a layer-by-layer technique, which allows the build-up of multi-layered thin films made of polymers of opposite charge.

Chitin polymorphism (α - versus β -chitin) is another important issue. β -chitin in molluscan shells was identified only from cephalopods by using X-ray diffraction⁹⁵ and solid NMR⁹⁶. In fact, most chitin research on molluscs was on non-calcified squid chitinous shells (pens); the chitin polymorphism in other calcified conchiferan shells remains unknown, although many biomineralization researchers believe that all molluscan shells contain β -chitin^{97,98}. Yet, α -chitin is found in the radula sheath of polyplacophorans⁹⁹ and the non-mineralized portion of the *Nautilus* siphuncle¹⁰⁰. The presence of chitin-protein complexes was reported in various molluscan species^{78, 101, 102}. However, little is known about the complex formation in molluscan shells, due to the complexity of organic matrix deposition during shell formation *in situ* and the difficulty to recreate interactions between supramolecules using *in vitro* systems.

B. Silk fibroin gel protein

Silk fibroin, the main protein of spiders and worm threads, was originally studied in arthropods. Two types of silk were distinguished: *Bombyx mori* (cocoon) silk and spider silk¹⁰³. Molluscan shell silk fibroin proteins have been defined as Gly- and Ala- rich insoluble proteins with a β -sheet domain; such proteins are similar to silk proteins, e.g., spider dragline silk¹⁰⁴ and cocoon silk¹⁰⁵. Pereira-Mouries et al.¹⁰⁶, for example, reported a silk fibroin-like protein in *Pinctada* nacre due to the high proportion of Gly and Ala. In contrast, the proportions of Gly and Ala were not always high, and they are not always the same in the insoluble shell organic matrices of other molluscs¹⁰⁷. Furthermore, there is no evidence of internal ensemble repeats in molluscan shell proteins, nor is there evidence of a clear crystalline region and other motifs (e.g., helical structure and β -spirals) that are important characteristics of silk fibroin. Indeed, Weiner and Traub⁹⁵ reported only β -sheets in insoluble shell organic matrices (e.g., *Nautilus*) but no helical or β -spirals as seen in silk fibroin proteins.

The silk is initially present in a hydrated gel-like state filling the space between two layers of chitin before mineralization^{86, 106, 108, 109}. As minerals fill this space, the silk-like proteins are pushed aside by the crystal and in the mature shell overlie the chitin sheets^{Error! Bookmark not defined.}. The silk is a mild inhibitor of crystal nucleation¹¹⁰. Its function during mineral formation is thus possibly to provide a hydrophobic environment, such that nucleation of the aragonite crystals will occur only at the nucleation site, when induced by the acidic proteins adsorbed on the chitin sheets. It has been shown that the silk fibroin may be essential in determining the conformation of the soluble macromolecules on adsorption¹¹¹. The silk fibroin may also influence ion diffusion or the accessibility to the chitin surface or both⁸⁶.

C. Other insoluble shell proteins

The definition of insoluble proteins is not always clear due to different solubilities which are related to the extraction methods¹¹². For example, Lustrin A (*Haliotis rufescens* nacre) has been extracted with urea and SDS¹¹³, N14 (*Pinctada fucata* nacre) with NH₄OH alkaline extraction¹¹⁴, and Schematrin (*Pinctada fucata* non-intra crystal protein from prismatic layer) with urea. The characteristics of insoluble shell proteins differed from one another. Thus, N14 is rather acidic, LustrinA is a large and multi- functional protein, Schematrin families possess Gly repeats and a lower proportion of Ala. The protein MSI31, that was sequenced from the prismatic layer of *Pinctada fucata*, is also Gly rich, but not homologous to silk¹¹⁵. Only the Schematrin2 Glycine-rich part was similar to MSI31⁵⁴.

2.2 Acidic soluble macromolecules

Investigations on molluscan shell proteins have made significant advances and tens of proteins have been characterized, including for most of them the identification of the corresponding transcript³¹. The soluble proteins are rich in Asp and Glu, bind Ca²⁺, and play a vital role in mineralization. It is assumed that acidic macromolecules with a negative charge are important for the calcium carbonate crystallization process and could control crystal nucleation and growth by lowering the interfacial energy between the crystal and the macromolecular substrate. The main acidic macromolecules are thought to be aspartic acid-rich proteins¹¹⁶ and sulphated GAG¹²⁹.

The biomineralization proteins have a non-globular shape and multiple charges and post translational modifications, which further hampers the protein separation¹¹⁷. It is even more difficult to reveal their function as they are not only present in a polymer mixture with the associated possibility of polymer interactions but are furthermore often only active for a certain time.

A protein which was recently isolated from the nacreous layer of the pearl oyster *Pinctada fucata*¹¹⁸, called Pif, has an essential role in nacre formation, but how it functions has not yet been clarified. Pif contains a separate chitin-binding domain¹¹⁹ and an acidic part that facilitates selective binding to aragonite¹¹⁸. Because of its ability to bind to both of the main elements of nacre, it is possible that a protein such as Pif, in addition to its demonstrated role in the formation of the nacreous structures, might have a function in the mechanical strength of the composite materials by 'gluing' components together.

Since the first full sequence of nacrein was published¹²⁰, the number of identified proteins has grown exponentially. Recent structural information on various molluscan shell proteins indicates that the regulation of crystal growth may be more subtle and complex; proteins such as nacrein¹²⁰, MSI60⁵⁷, lustrin A¹²¹, N16¹²², N14, and N66¹²³, all from molluscan nacre, are clearly multifunctional. However, the putative functions of these proteins remain unclear. Yet,

the functions of these proteins are related not only to their structures, but probably also to their neighbouring macromolecules and the microenvironments in which they are located. Testing these functions requires an *in vitro* assay that bears some relation to the *in vivo* environment. Thus, relating structure to function for these unusual proteins is extremely difficult.

Polysaccharides are of importance as glycosaminoglycans (GAGs), which are polysaccharides bound to a protein core. Since Abolins-Krogis' work¹²⁴ a slow but increasing interest has been developed to explore the role of polysaccharides in biomineralization, despite the fact that their involvement in biomineralization seems to appear very early in evolution. There is no single type of polysaccharide associated with biominerals, but such polysaccharides are mainly hydroxylated, carboxylated, or sulfated or contain a mixture of these functional moieties. Glycoproteins are also not well studied as biomineralization polymers despite a great number of biomineral associated glycoproteins^{125 126,127}.

With regard to sulphated GAGs and glycoproteins as a nucleation factor, Wada¹²⁸ emphasized the potential importance of sulphated sugars for molluscan shell formation. Dauphin¹²⁹ conducted XANES analyses on *Pinna* and *Pinctada* prism and found a specific distribution pattern of sulphate (SO_4^-) and protein S: sulphate, for example, was localized at both the outer wall and central part of prisms in both species, although its function is still speculative.

The most important point established about the growing region of the shell is that the structural matrix is indeed formed first and has mechanical integrity; the second point is that the matrix within the compartment contains other macromolecules that may be involved with crystal nucleation¹³⁰. The structural macromolecular framework may not have nucleation capability.

Studies of the shell matrix provided information on the early evolution of the molluscan shell and biomineralization mechanisms. Jackson *et al.* compared the nacre-secreting mantle transcriptomes of a bivalve (*Pinctada maxima*) and a gastropod (*Haliothis asinina*), finding that the majority of the secreted proteins had no similarity to sequences in public databases, and less than 15 % of the secreted proteins were shared between the two species¹³¹. These results indicate that the two taxa use different gene sets to construct their shells. This is in line with observations that both the crystallographic orientations of nacre tablets and their growth modes differ between these taxa, and strongly suggests that bivalve and gastropod mother-of-pearl nacre evolved convergently.

3. Mineral phases in molluscan shell

The mineral phase in shell, calcium carbonate, accounts for 95 to 99 % of the weight. Shells exhibit higher toughness than calcium carbonate obtained from a chemical

precipitation¹³². The mineral phase in biological ceramics is controlled in terms of the size, shape, orientation, chemical composition, crystal type and degree of bonding within the matrix. With control like this, it is perhaps not surprising that biological ceramics can perform at least as well as the best man can make, and usually much better. Their only weakness is the inability to stand high temperature because de protein denatures at 60 °C or so¹³³.

The calcium carbonate minerals are the most abundant biogenic minerals, both in terms of the quantities produced and their widespread distribution among many different taxa¹³⁴. Of the eight known polymorphs of calcium carbonate, seven are crystalline and one is amorphous. Three of the polymorphs, calcite, aragonite and vaterite, are pure calcium carbonate, while two, monohydrocalcite and the stable forms of amorphous calcium carbonate, contain one water molecule per calcium carbonate¹³⁵. Surprisingly, the transient forms of amorphous calcium carbonate do not contain water¹³⁵. The atomic structure with the lowest lattice energy, and hence the most stable, is calcite. Somewhat less stable, and with a slightly different lattice structure, is aragonite. Even less stable is the polymorph dubbed vaterite, which is unstable in its inorganic form, though it is present in spicules of some molluscan species¹³⁶. One of the major challenges in the field of biomineralization is to understand the mechanism by which biological systems determine which polymorph will precipitate.

Aragonite and calcite have very similar crystal structures and thermodynamic stabilities¹³⁷. The former is slightly less stable than the latter at ambient temperatures and pressures but is very common in biomineralization^{134, 138}. The calcium ions are located almost in the same lattice positions in (001) layers, alternating with layers of carbonate ions (Fig. 5)¹³⁷. The major differences between the two polymorphs occur in the organization and orientation of the carbonate molecules. The presence of other doubly charged ions in CaCO₃ solutions, in particular Mg²⁺ as well as a variety of small organic molecules, favours the formation of aragonite¹³⁹. In aragonite, 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 different¹⁴⁰. This shift is the basis for the very different properties of these two phases.

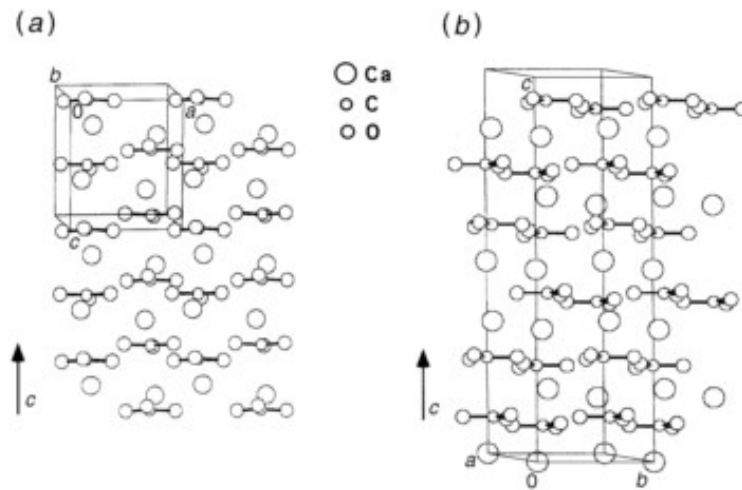


Figure 5: Crystal structures of (a) aragonite and (b) calcite.

In biology the two polymorphs are used widely as building materials and the choice of polymorph used is almost always under strict genetic control¹⁴⁰. 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 crystals 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.

4. Shell microstructures

Molluscan shells, whatever their taxonomic origin, are always made of the superimposition of few calcified layers, generally two to five, and one organic layer. From outside to inside the shell, one finds a thin organic leathery layer called the periostracum, followed by several mineralized layers with composition and microstructure distinct. For instance, the shell of the Pacific oyster (*Crassostrea gigas*), which is composed of more than 99 % CaCO_3 and of around 0.5 % of occluded organic matrix, exhibits a shell with a multilayered organo-mineral structure (Fig. 6)¹⁴¹. The outer calcified layer is composed of calcitic prisms of 20–200 μm in height and 5–40 μm in width (Fig. 6b)¹⁴²⁻¹⁴³. Chalky structures (Fig. 6c) are constituted of platy calcite petals of various sizes interlocking with each other, leaving a large void space between them. They form lenticular bodies that occur randomly, interrupting the foliated microstructure. Folia (Fig. 6d) are composed of arrow point ending tabular laths 2–5 μm wide and 200–250 μm thick that join laterally to form a series of thin sheets of folia, which are stacked vertically to form the dense calcitic foliated microstructures.

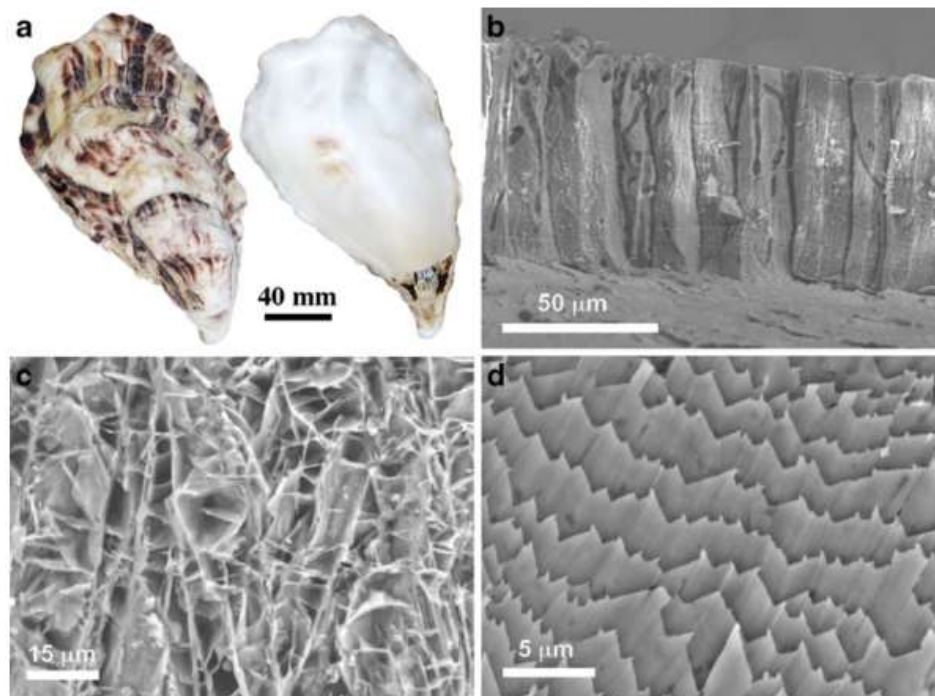


Figure 6: Shell of the Pacific Oyster *Crassostrea gigas*.¹⁴¹ **a** General Macroscopic view of the shell. **b** SEM micrograph of texture detail of the calcite prismatic layer. **c** Scanning electron micrograph of texture detail of the calcitic chalky layer. **d** Scanning electron micrograph of texture detail of the calcitic foliated layer showing individual laths that appear to be coated with organic material.

The mineralized layers are composites of calcium carbonate together with biomacromolecules, which are spatially organized in an exquisitely controlled manner. This organisation of mineral crystals into very complex microstructural architectures is one strategy that molluscs have developed during their evolution to reinforce the protection of the shell. Numerous publications on the classification of shell microstructure are available^{144, 145, 146}. The very wide diversity of shell microstructures includes “prismatic”, “nacreous”, “foliated”, “crossed-lamellar”, “granular”, “composite-prismatic” and “homogeneous”¹⁴⁷. Figure 7 gives a set of examples of microstructures found in diverse molluscan shells.

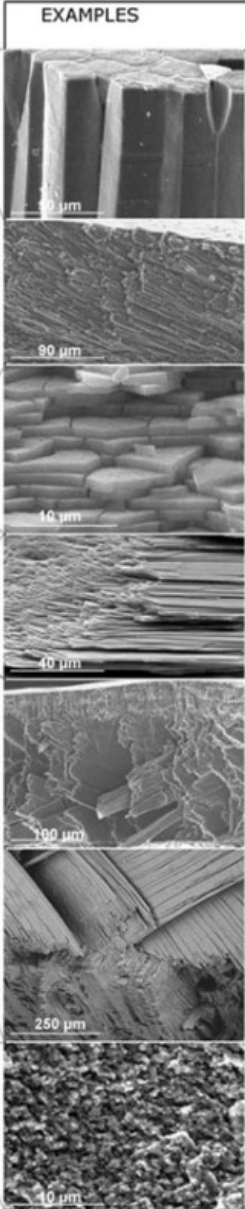
CATEGORY	TYPES	SUB-TYPES	EXAMPLES	Genus species
PRISMATIC	Simple prismatic	Regular simple prismatic		<i>Atrina rigida</i>
		Radially elongate simple prismatic		
		Asymmetric prismatic		
		Irregular simple prismatic		
		Blocky prismatic		
	Fibrous prismatic	Lath-type fibrous prismatic		
		Rod-type fibrous prismatic		
		Anvil-type fibrous prismatic		
		Simple lamellar fibrous prismatic		
	Spherulitic prismatic	Irregular fibrous prismatic		
		Regular spherulitic prismatic		
	Composite prismatic	Irregular spherulitic prismatic		
Denticular composite prismatic				
Non-denticular composite prismatic				
Compound composite prismatic				
Crossed-composite prismatic				
SPHERULITIC				
LAMINAR	Nacreous	Sheet nacreous		
		Row stack nacreous		
		Columnar nacreous		
	Semi-nacreous			
	Lamello-fibrillar			
	Crossed-bladed			
CROSSED	Crossed-lamellar	Regularly foliated		
		Semi-foliated		
		Simple crossed lamellar		
		Rod-type crossed lamellar		
		Linear crossed lamellar		
		Branching crossed lamellar		
		Crisscross crossed lamellar		
		Irregular crossed lamellar		
	Complex crossed lamellar	Compressed crossed lamellar		
		Triangular crossed lamellar		
		Diffuse crossed lamellar		
		Fine complex crossed lamellar		
HELICAL	HOMOGENEOUS	Intersected crossed acicular		
		Intersected crossed platy		
ISOLATED SPICULES	ISOLATED CRYSTAL MORPHOTYPES	Dissected crossed prismatic		
		Irregular complex crossed lamellar		
		Cone complex crossed-lamellar		
		Fine grained homogeneous		
		Coarse grained homogeneous (= granular)		
				<i>Pleiodon speckii</i>
				<i>Anomia ephippium</i>
				<i>Helix aspersa</i>
				<i>Strombus gigas</i>
				<i>Venerupis philippinarum</i>

Figure 7: Classification of the shell microstructures according to Carter and Clark¹⁴⁵ (From Marin et al. 2012¹⁴⁸).

The strategy of superimposing calcified layers of different microstructures was invented soon after the invention of the shell, since well-preserved conchiferan molluscs of the Lower Cambrian of China already exhibited layered shells¹⁴⁹. Fossils show that the inner structure of the early molluscan shells reflects the intensity of predation, providing information about the earliest arms race between molluscs and their predators¹⁵⁰. Each microstructural organization confers distinctive mechanical properties to the shell (Tab. 2). Shell microstructures such as nacre and crossed lamellar layer, which have a nanoscale laminate architecture, are the toughest. The prismatic layer with a relatively high organic content, provides a high degree of flexibility. Combination of several microstructures improves the preformation of shell.

Type	Tension (MPa)	Compression (MPa)	Bending (MPa)	<i>E</i> (Young's modulus) (GPa)	Hardness
Columnar nacre	78–116	320–401	193–267	47–64	122–211
Sheet nacre	35–86	304–419	117–211	31–58	106–221
Cross lamellar (Gastropoda)	31–60	198–336	58–165	30–58	162–270
Cross lamellar (Bivalvia)	9–43	163–336	35–106	50–82	242–298
Foliate	40–42	73–133	44–110	29–34	11–110
Cross foliate (Patella)	16–33	196–208	39–171	18–60	173
Prism (Bivalvia) calcitic	60–62	210–295	139	21–39	162
Cnidaria (Aragonitic coral skeleton)	X	22–47	25	10–62	X
Bryzoa semi nacre	X	X	24–50	42–65	X
Echinodermata (calcitic test)	X	X	95–190	31–69	X

Table 2: Mechanical properties of calcified tissues¹⁵¹.

4.1 Three-dimensional organisation of nacreous layer

Nacre is the strongest modern shell microstructure. Its resistance to fracture was > 1000x higher than that of its chemically precipitated counterpart, geological aragonite¹³². It is also slow-growing and energetically expensive¹⁵². Nacre is widespread in molluscs, e.g., in the shells of bivalves, gastropods and cephalopods¹⁴⁷. Its intrinsic beauty, its incredible mechanical toughness¹³², and its occurrence in the three major mollusc classes¹⁴⁷ have indeed contributed to make it the unique model for molluscan mineralization¹⁵³. The interest in nacre has also been driven by its economic importance in the pearl industry¹⁵⁴ and recently by its promising use for promoting bone repair¹⁵⁵. Nacre is also an interesting natural composite that serves as a model for the development of synthetic biomimetic materials, because of its apparent geometrical simplicity in the inhibition of crack formation.

The characteristic structure of nacre at microscale is a composite made of “brick-and-mortar”, which is composed of 95 % mineral calcium carbonate in the aragonite form. The mineral platelets are typically hexagonal, their thickness varies between half a μm to one μm , for a lateral extension of few μm . Depending on the stacking mode of the tablets, nacres are defined in ‘sheet nacre’, ‘columnar nacre’ or ‘row stack nacre’¹⁵⁶. In sheet nacre (Fig. 8A, B), deposition takes place over most of the inner surface of the shell, and the tablets are stacked in a ‘brick wall’ pattern, spanning the interface between underlying tablets. It is the most frequent and almost exclusively observed among bivalves. Columnar nacre (Fig. 8C, D) has tablets of rather uniform size with coinciding centres that determine the nucleation site of the overlying tablet¹⁵⁷, viewed from the top, the polygonal tablets from neighbouring layers overlap in such a manner that the inter-tablet boundaries form tessellated bands perpendicular to the lamellae boundaries. This nacre microstructure is common in gastropods. ‘Row-stack nacre’¹⁴⁵ (Fig. 8E, F), is described as a nacreous microstructure in which mutually parallel elongate tablets show vertical stacking in vertical sections perpendicular to their length axes, and brick wall and/or stair step stacking in vertical sections parallel to their length axes. This particular type is found in the bivalve *Pinna nobilis*, for example.

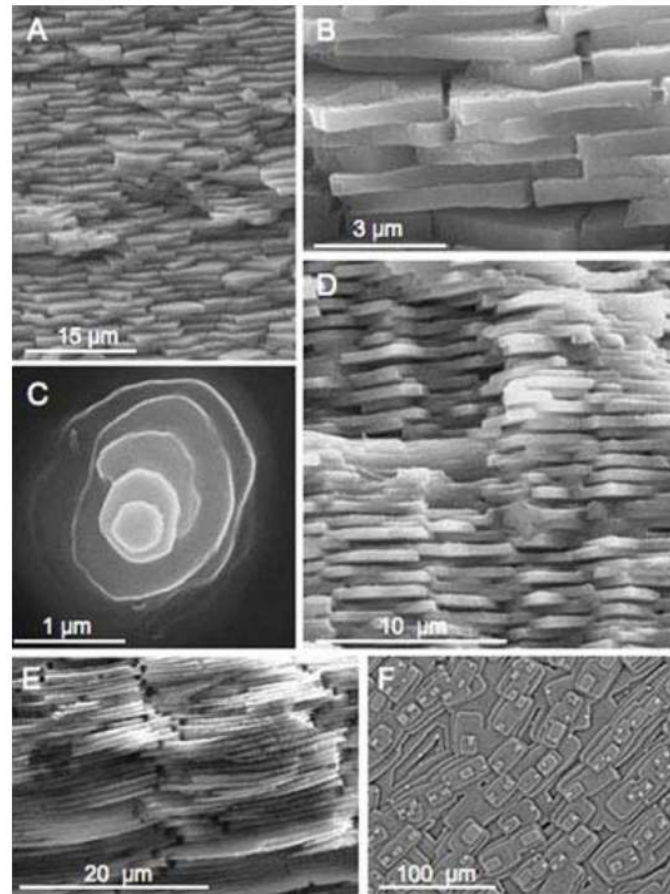


Figure 8: Different nacre microstructures found among molluscs. Sheet nacre: A, B. Cross-section of the sheet nacre of *Pleiodon spekii*. Columnar nacre: C, D. Columnar nacre of the green ormer gastropod *Haliotis tuberculata*. Row stack nacre: E, F. Row stack nacre of the noble fan mussel *Pinna nobilis*.²⁷

As in most structural natural materials, nacre exhibits a hierarchical structure (Fig. 9)¹⁴. The longitudinal cross section of nacre-containing shells, such as the abalone shell (Fig. 9a), exhibits two layers with distinct microstructures: a prismatic calcite layer (P) and an inner nacreous aragonite layer (N) (Fig. 9b). Such arrangement permits to optimize the protective function of the shell¹⁵⁸: the outer layer prevents penetration of the shell, while the nacreous layer is capable of dissipating mechanical energy through inelastic dissipation. In the next hierarchical level, one can detect the so-called ‘growth bands’ (Fig. 9c). Such periodic growth arrests create mesolayers that can also play an important role in the mechanical performance of nacre, acting as powerful crack deflectors¹⁵⁹. The thin organic layers separating the mesolayers are thought to be formed upon shell growth in periods of less calcification¹⁴. In a higher magnification level, one can detect that, in some cases such as in the abalone shell, the aragonite platelets are organized in columns, in which the intertablet boundaries form tessellated bands perpendicular to the lamellae boundaries (Fig. 9d). In a 1 mm level, we can observe the accumulation of polygonal tablets made of aragonite crystals (orthorhombic; CaCO_3), with a thickness of approximately 0.5 mm (Fig. 9f). The mortar fraction in nacre may reveal a few nanoscale mineral columns through the organic matrix layers (see arrow in Fig. 9f), randomly distributed in the surface of the aragonite tablets. Such mineral bridges have

diameters of approximately 46 nm and a height of 26 nm¹⁶⁰. Moreover, the surface of the aragonite platelets is not smooth, but presents nanoscale asperities that could also influence the mechanical properties of nacre (Fig. 9e)¹⁶¹. In a lower hierarchical level, the tablets are then composed by the assembly of nanograins with an average size of 32 nm delimited by a fine three-dimensional network of organic material (Fig. 9g)¹⁶².

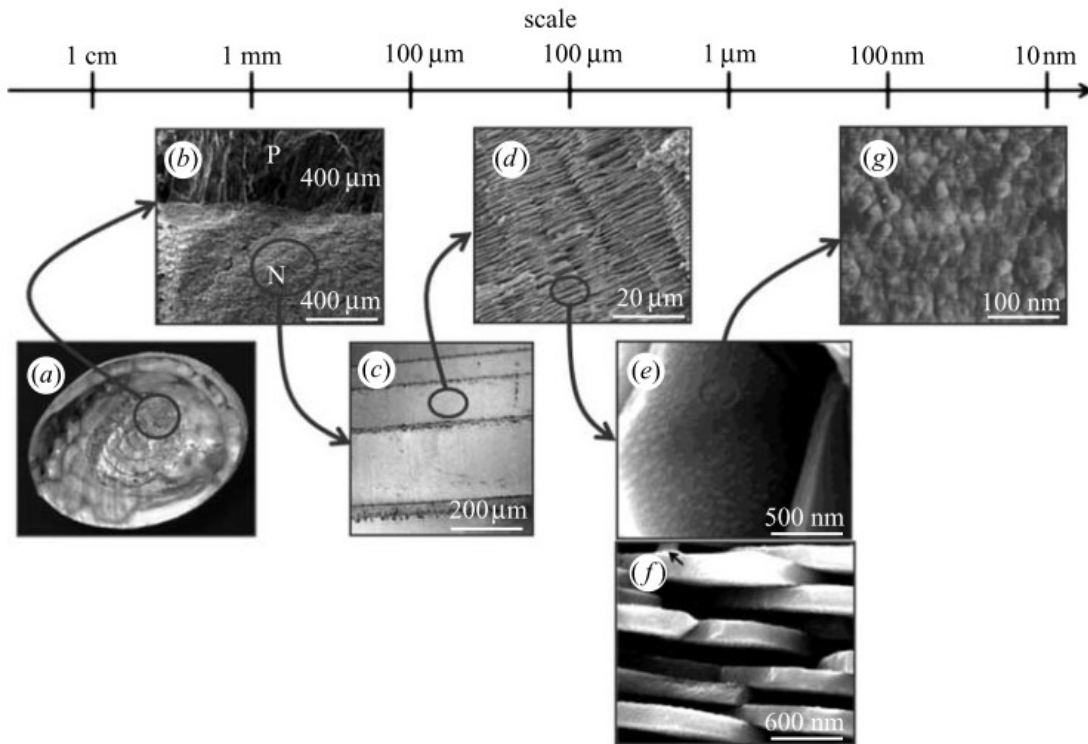


Figure 9: Hierarchical organization in nacre showing at least six structural levels¹⁴.

Extensive work on nacre, which consists of stacked nanoparticles-assembled aragonite platelets sandwiched with organic biopolymer, has shown that the toughening strategy is not simply derived from the zigzag breakage along the biopolymer interlayer between staggered arrangements of aragonite platelets¹⁶³. It is essential to understand that the superb mechanical properties of nacre are a direct result of optimal geometry, existence of nanoscale elements such as pillars¹⁶⁴ and asperities¹⁶⁵, and an organic matrix with outstanding mechanical properties.

4.2 Crossed lamellar structure

The crossed lamellar structure is the most widespread structure in molluscs. In crossed lamellar structure, the mineral lamellae are organized in more hierarchical levels than in nacre¹⁶⁶. Although their resistance to fracture is less than that of nacre (Tab. 2)¹⁶⁷, crossed lamellar structures represent a remarkable strategy that molluscs have set up to combine a 'cheap' cost of calcification (due to the secretion of low amounts of shell organic matrix¹⁶⁸) and interesting mechanical properties, among which an aptitude to stop cracks¹⁶⁹. This structure may have consequences in the superior mechanical properties found in seashells

owing to the enhanced ability for crack arrest at interlamellar boundaries across the different hierarchical levels^{169,170}.

The crossed lamellar microstructure is always aragonitic. In contrast to non-biogenic aragonite, which typically grows as needles elongated along the fast-growing *c* axis, and to the large aragonite plates of nacre that develop in the *ab* plane, the crystals in the crossed lamellar microstructure have a variable orientation in different species but are almost always elongated in a direction approximately perpendicular to the crystallographic *c*-axis^{171,172,173,174}.

From a geometrical viewpoint, crossed lamellar structures are the most complex ones and can be described as microstructures with ‘two or more non-horizontal dip directions of their elongate structural units relative to the depositional surface’. Carter & Clark¹⁴⁵, based on the mineralogical composition and the morphology of the first and second order lamellae, have proposed a classification of 15 subgroups of crossed lamellar microstructures (Fig. 10).

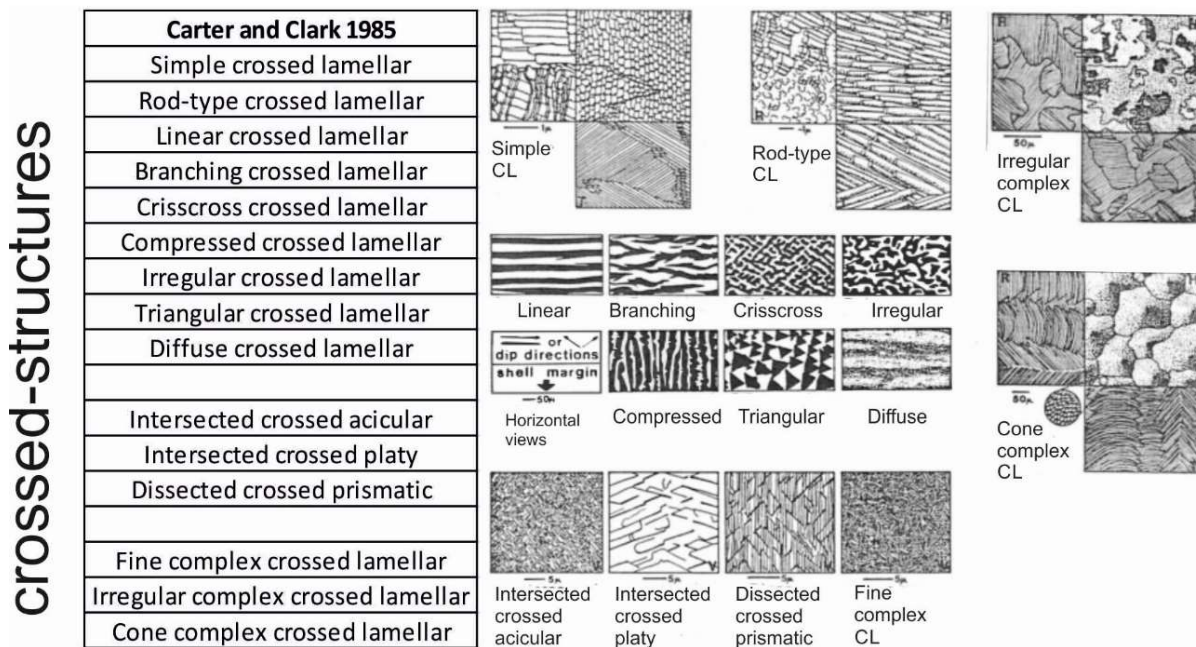


Figure 10: Classification of crossed lamellar structure (modified from Carter & Clark¹⁴⁵).

Regular crossed-lamellar organization (Fig. 11) is, among the variety of microstructural types described, one of the most commonly found, often forming one or several shell layer(s) of many bivalves or gastropods. Its fundamental architecture consists of a nicely ordered arrangement of lamellae, perpendicular to the shell growth surface and around 5 to 30 μm thick (usually called 1st order lamellae), that are themselves composed of sheet-like arrangements (2nd order lamellae) of submicrometric, elongated individual rods or fibres (3rd order lamellae). The 2nd order lamellae are dipping in opposite directions between two consecutive first order lamellae (typical angle comprised between 90° and 130°)¹⁷⁵. The third-order lamellae, reported to be the basic building blocks for the shell structure, have the

dimension of 60-150 nm by 120-330 nm in cross section and hundreds of μm in length. The individual third-order lamellae are bundled up with biopolymer to form the larger structure – the second-order lamellae with 5-30 μm in thickness and 20-50 μm in width. Likewise, the second-order lamellae are stacked together to form the first-order lamellae of 10-70 μm in thickness and several micrometres in width. The AFM and SEM images jointly uncover nanoparticles with a diameter ranging from 20 to 45 nm in individual third-order lamellae. A single third-order lamella is not just a string of nanoparticles, but composed of a few bundles of nanoparticle strings.

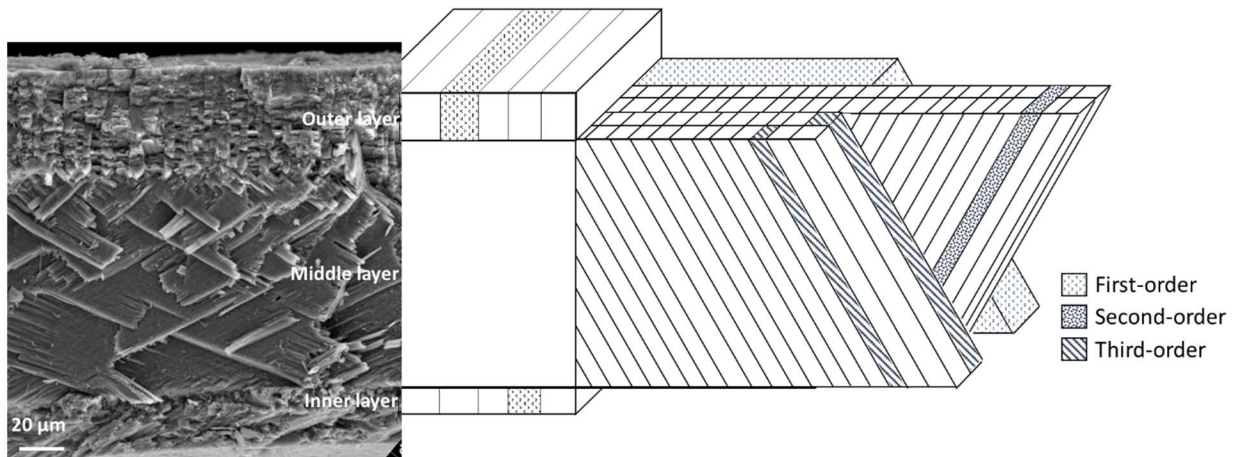


Figure 11: Crossed-lamellar shell microstructure of *Strombus gigas*.

The third order lamellae are surrounded by a rather homogeneous and monotonous organic membrane (conchiolin)¹⁷⁶. No organic membrane exists along the border surfaces of first-order lamellae. The boundaries between 1st order lamellae are faintly enriched in organics but deprived in S-polysaccharides¹⁷⁷. The unique construction of such crossed-lamellar structures provides many complicated interfaces, which can effectively localize the deformation during indentation tests by invoking microcracking mechanisms¹⁷⁸. As such, it would theoretically be advantageous for many molluscs to possess relatively hard cross lamellar structures without secreting much organic matrices.

4.3 Prismatic microstructure

The prismatic layers are an important shell texture found most frequently in molluscan outer shell layer, in particular, in gastropods, cephalopods, and bivalves. With individual prisms arranged nearly perpendicular to the shell surface and surrounded by organic envelopes (also called organic walls), the prismatic layers have a relatively high organic content. Indeed, values reported by Taylor et al.¹⁷⁹ and Checa et al.¹⁸⁰ are higher (4 %–6 % of the shell weight) than for any other molluscan shell microstructure (except for a value of 7 % recently recorded for the homogenous layer of the anomalodesmatan bivalve *Entodesma*¹⁸¹). The distribution of organic walls provides the prismatic microstructure with a high degree of flexibility. They are often associated to nacre, it has been proposed that nacre evolved through

simple horizontal partitioning of vertical prisms¹⁴⁵. This appealing idea, based on simple geometric considerations, needs to be re-evaluated with accurate crystallographic and biochemical criteria. This may help to understand the transition from one microstructure to the other and to reconstitute primitive shell textures⁵⁴.

Prisms, which can be made of calcite or aragonite, includes very different objects, fine, medium or coarse, such as the big-sized regular simple calcitic type, oriented perpendicularly to the outer shell surface, encountered in the outer layer of the fan mussel, *Pinna nobilis*. They include also the tiny oblique and straight prisms of the edible mussel (simple prismatic, Fig 12a), *Mytilus edulis*, or the composite curved prisms of the Manila clam *Ruditapes philippinarum*, that grow almost parallel to the shell surface and diverge in a fan-like manner towards the edge of the shell (spherulitic prismatic, Fig 12b)¹⁸². Such heterogeneity implies that all prisms are not produced in a single manner and that there must be very different mechanisms of crystal growth. Several parameters, such as the initiation of prisms at the internal surface of the periostracum, geometrical constraints, competition for space, position of the calcifying epithelium facing the mineralization front, are obviously crucial for explaining how prisms emerge¹⁸³.

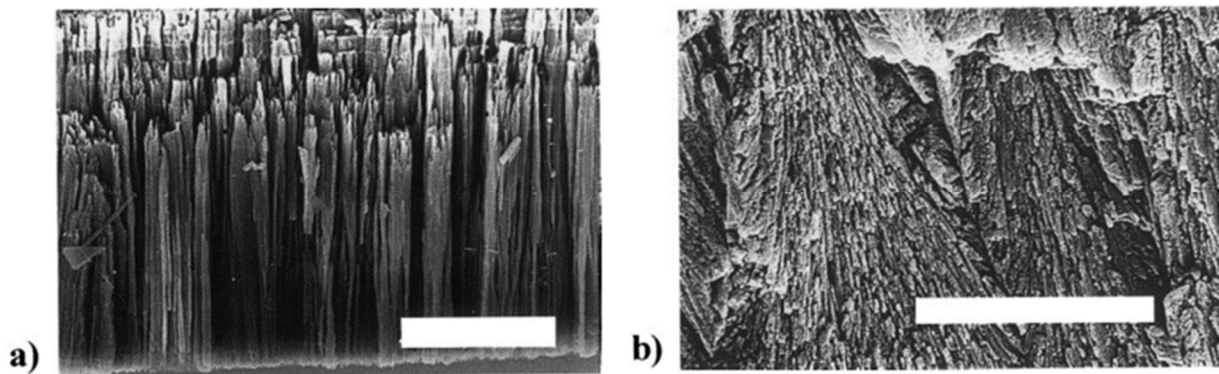


Figure 12: Scanning electron micrographs of prismatic microstructures: (a) regular simple prismatic of the innermost layer of *Turbo petholatus* (scale bar = 15 μm); (b) irregular spherulitic prismatic layer of *Tectus conus* (scale bar = 20 μm)¹⁸⁴.

The prismatic texture has been studied far less than nacreous layer, only few proteins have been genetically characterized: MSI31, prismalin, aspein, asprich, caspartin and calprismin^{57, 185,186,187,188}. Prism-like biominerals are often considered to represent an efficient and simple strategy adopted by calcifying metazoans for rapid mineralization¹⁸⁹. This layer is believed to represent an archaic type of texture, analysis of associated matrix components may clarify the molecular mechanisms of early biomineralization¹⁹⁰⁻¹⁹¹.

4.4 Foliated microstructure

Foliated microstructures are thin calcitic laths, arranged in superimposed sheets¹⁹². They are extremely developed in edible oysters and scallops, for example. This microstructure

comprises parallel and overlapping calcitic flat laths that have typical arrow-pointed endings and are inclined at a small angle (2° – 3°) to the shell surface. The arrangement of the laths can be very variable, and several subtypes of foliated microstructure have been described on the basis of this arrangement^{142, 193}. Folia are composed of arrow point ending tabular laths 2–5 μm wide and 200–250 μm thick that join laterally to form a series of thin sheets of folia, which are stacked vertically to form the dense calcitic foliated microstructures¹⁹⁴. By contrast to prismatic calcite, the foliated microstructure contains very little organic matrix, with measured values ranging from 0.4% to 1.5% weight. Although foliated layers have been shown to be low density and weak in most mechanical tests, compared to other molluscan microstructures, they represent presumably an efficient strategy developed by bivalves to rapidly mineralize and increase the thickness of their shell.

4.5 Homogeneous microstructure

Homogeneous microstructure is described as “aggregations of more or less equidimensional, irregularly shaped crystallites lacking clear first-order structural arrangement except for possible accretion banding” by Carter and Clark¹⁴⁵. In other words, when a shell structure has no other identifiable elements than minute granules, it is homogeneous. They can be fine grained, when the crystals units are minute ($< 5\mu\text{m}$) or coarse grained, for crystallites higher than 5 μm . This microstructure does not present an apparent organization of their crystallites, when observed with optical or with electronic microscope (Fig. 13). Homogeneous microstructure is extremely frequent in heterodont bivalves¹⁹⁵.

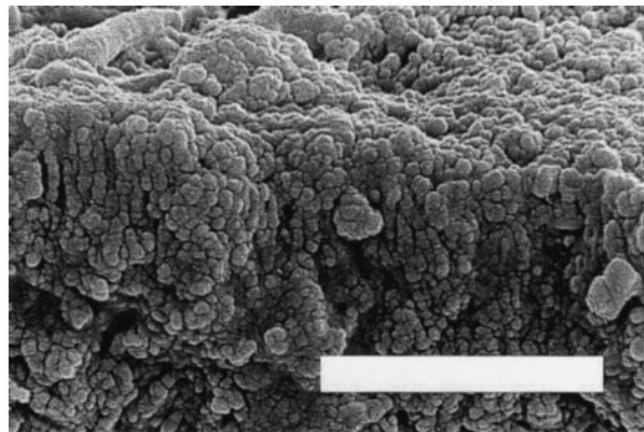


Figure 13: Scanning electron micrograph of the homogeneous outermost layer of *Lotia granulosa* (scale bar = 5 μm)¹⁹⁶.

We have shown here the complexity of molluscan shell for its chemical composition and physical structures. The hierarchical structures seem to be the general rule for hard biological materials¹⁹⁷. As a general principle, the formation of biominerals consists of a four-stage process: 1) the fabrication of a hydrophobic solid organic substrate or scaffolding; 2) onto which nucleation of the crystalline phase takes place closely associated with some

polyanionic macromolecules; 3) crystal growth is then controlled by the addition of gel-structuring polyanionic macromolecules; 4) finally mineralization arrest is accompanied by the secretion of a new inert scaffolding of the same type or the deposition of other hydrophobic inhibitory macromolecules. The essential mechanism of the formation molluscan shell though remains unclear. A better understanding of the relationships between materials architecture and mechanical response, spanning not simply microstructure but encompassing the influence of structural parameters acting at multiple length scales, from atomic to the macro levels and their interactions, will enable us to reveal secrets to achieve combinations of stiffness, strength and toughness.

III. Materials biomimetics inspired from molluscan shell

The high-performance properties of molluscan shell compared to inorganic minerals and synthetic composites attract attention of chemists, material scientists. The technology transfer with the objective of duplicating these properties in artificial man-made materials is called biomimetics¹⁹⁸. The field of biomimetics involves the understanding of biological functions, structures, design and fabrication of biological materials. And devices of commercial interest are also indispensable. Many new materials have been developed by using biomimetic concepts for a variety of applications, especially to improve some mechanical properties of structural materials^{12, 199, 200}. Some common features found in structural mineralized natural composites have provided clues for the design of new materials¹⁴.

The biomimetic engineering of materials does not result from the observations of natural structures alone, but requires (1) a thorough investigation of structure-property relationships in biological materials, and (2) the application of these relationships to engineering materials through existing technologies. Therefore, the feasibility of the biomimetic transfer of technology from nature to actual engineering materials is analysed in a four steps process²⁰¹: (1) identify a high-performance natural model; (2) infer key mechanisms, structure and design principles; (3) transfer and design biomimetic materials structures; (4) fabricate and implement biomimetic material.

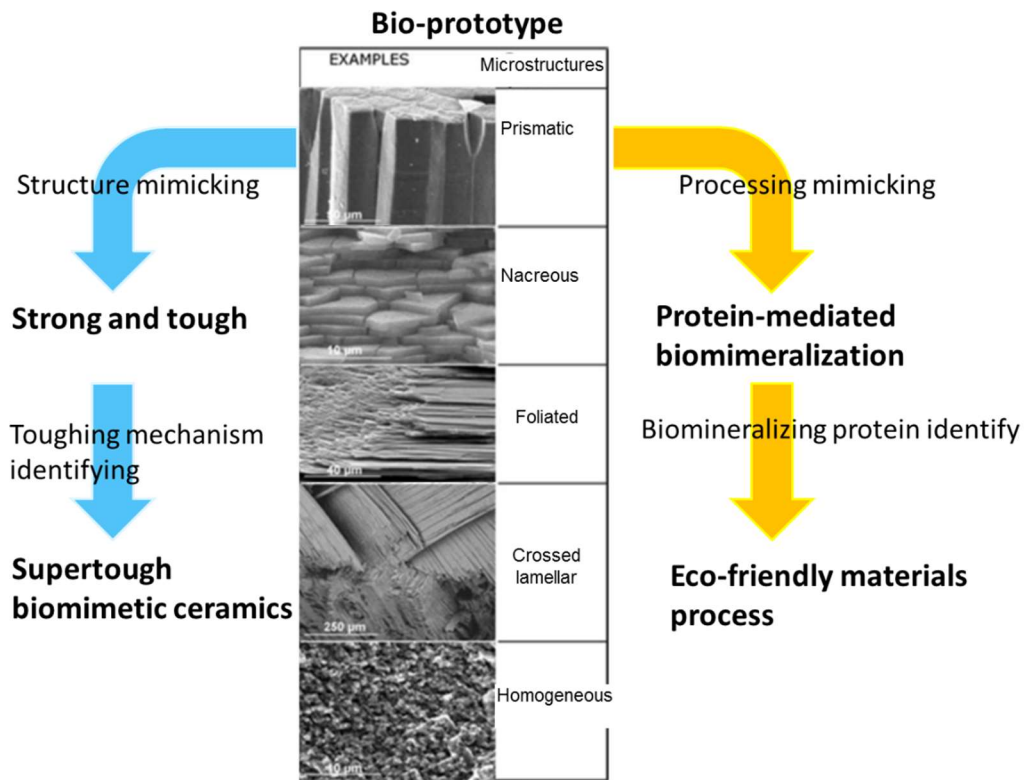


Figure 14: Two aspects of biomimetic materials science and engineering: Structure and Process mimicking²⁰².

Biomimetic materials science and engineering inspired from molluscan shells is achieved by structure and process mimicking²⁰² (Fig. 14). The most common type of biomimetic materials is the emulation of natural material structures. Molluscan shells present a layered structure that can be categorized into seven kinds of generally accepted microstructure: columnar and sheet naces, prismatic, crossed-lamellar, foliated, homogeneous and complex crossed-lamellar structure²⁰³. Numerous innovative technologies have been developed to mimic this biological hierarchical structure¹⁴. Emulating the processes that are at the base of the structures is another aspect of the biomimetic design of engineering materials. By the biologically controlled mineralization, molluscs have the ability to produce calcium carbonate with nanoscale dimensions, and also to assemble these nanoparticles into bulk materials with relevant properties. Relying on mineralization controlling molecules, it is possible to synthesize mineral nanoparticles with desired polymorphs, morphology, crystallographic orientation and crystal size²⁰⁴⁻²⁰⁵. Thus, complex macromolecular templates with specific structures and functions can be established, which then can be used for complex hybrid material formation²⁰⁶.

Aiming at mimicking these inspiring biological structures and their properties, efforts in the fields of chemistry, physics, and materials science over the years have led to a large variety of inorganic and hybrid materials, targeting several applications. Nevertheless, to date, designing organic-inorganic composite materials with controlled structure and morphology is still a major challenge. Scientists began to realize that one must determine the relationships between structure, processing and properties for purpose to combination of high strength

and toughness. The essence of biomaterials design is the hierarchical structures across the spectrum of length scales from nanometres to millimetres, based on which our understanding of biomineralization can further advance the field of materials science techniques. Beyond describing the state of the art development of biomimetic materials, we also underline the advantages and limitations of the different techniques applied and to suggest some critical areas for further work.

1. Process mimicking: biologically controlled mineralization

Molluscan shell is formed under “organic matrix-mediated” processes, in which the mineral type, orientation of crystallographic axes, and microarchitectures are under genetic control²⁰⁷. Only recently, researchers have begun to understand the first principles of how the shape and organization of crystals together with the included organic matrix provide the remarkable properties of biomineral²⁰⁸. Organic molecules or surfaces can influence the development of the crystalline state by the stabilization of a certain polymorph or even a specific crystal face. This process is the key to the formation of oriented crystals in biomineralization. It is evident that by mimicking the design and synthesis of organic matrix, organic-inorganic hybrid materials may open a new horizon in the field of materials science²⁰⁹. When different materials are merged at nanometre scales, the hybrid materials show unique properties compared with microscale composites^{210, 211, 212}.

Our understanding of organic molecule functions during the biomineralization is indispensable to chemical approach of biomimetic process. *In situ* biomineralization study is generally an experimental technique in which the crystallization of minerals or mineralization takes place *in vitro* from supersaturated solutions in the presence of organic macromolecules that influence the size, type and morphology of the crystals and act as templates for their nucleation¹⁵. The macromolecules can accelerate or inhibit the growth of the crystals depending on their concentration, molar mass and functional groups. This technique is inspired by nature, where well-defined and ordered hierarchical structures form with a chemical approach, in which an organic phase is the template for the nucleation and growth of inorganic crystals from a solution. In order to apply this technique, it is fundamental to choose the most appropriate organic template to form a desired inorganic layer and to understand how the organic layer influences the nucleation, the orientation and the shape of the growing inorganic layer^{14, 213}. This process has been widely studied to understand the underlying fundamental processes and then extensively applied to develop new materials²¹⁴.

The application of both soluble and insoluble directing agents inspired from biomineralization organic molecules has been extensively explored for the formation of a wide range of materials²¹⁵. The growth of calcium carbonate crystals on a range of organic

molecules and templates has been studied experimentally²¹⁶. As such, it appears that an exact match between the structure of the organic molecules template and the developing inorganic phase is not required for an organic surface to control the formation of the mineral²¹⁵. The possibility of the template to rearrange is shown to strongly influence its ability to promote the nucleation of oriented calcite.

1.1 *Molecular template formation*

In biomineralization, the organic matrix plays an important role in templating the growth of mineral phase, but also in improving adhesion between mineral interfaces. For instance, a recently isolated protein from the nacreous layer of the pearl oyster *Pinctada fucata*¹¹⁸, called Pif, has multi-binding domains, containing a separate chitin-binding domain¹¹⁹ and an acidic part that facilitates selective binding to aragonite. Therefore, this protein, in addition to its role in the formation of the nacreous structures, might have a function in the mechanical strength of the composite materials by 'gluing' components together.

Genetic engineering allows the design and development of novel proteins with tailored structures and functionalities²¹⁷. Hence, different protein domains that are found in nature can be genetically tailored together to add more complexity into a single recombinant protein with multi-domain structure. The enormous versatility of combinations of functional units that can be achieved using recombinant proteins has resulted in numerous studies. One recombinant protein can have separate domains, such as a hydroxyapatite nucleating dentin protein combined with spider silk protein²¹⁸, or each with a specific affinity for different materials²¹⁹. Such a recombinant protein that is made by combining different parts of unrelated proteins is called a fusion protein or a chimeric protein. Wang and co-workers have used elastin-like polypeptides (ELPs) as a matrix for binding HAP crystals together to form a composite. ELPs were produced by recombinant DNA techniques and are long repetitive protein sequences derived from natural elastin sequences²²⁰. The compressive and flexural strength of the composites containing the fusion proteins was enhanced by more than 65 % compared to the reference materials. Another study showed that very long filamentous viruses can be used as scaffolds for mineralization²²¹, i.e. by modifying the surface protein of the M13 virus with dentin protein fragments that nucleate HAP crystallization. The formation of HAP on the long fibril-like viruses was observed, confirming that a virus–HAP composite had been formed.

The possibility to engineer the properties of recombinant proteins could yield composite materials which may surpass the performance of synthetic materials. Although our understanding of biomineralization mechanisms and the role of the organic matrix remain limited, it is a major progress to understand the underlying principles and then to apply this knowledge to construct simpler systems that reproduce the essential material features.

Mimicking composites and engineering the interactions responsible for their properties might enable us to make better hypotheses about the biomineralization process.

1.2 Two-dimensional templating surfaces

The growth of calcium carbonate crystals on two-dimensional templates has been studied toward Langmuir monolayers^{222,223} and self-assembled monolayers^{224,225}. These synthetic model matrices have been successfully used to mimic biological control by an organic matrix over crystal orientation. A Langmuir monolayer can be formed by spread amphiphilic molecules at the air-water interface, and this method provides some control over the spacing between functional groups through applied surface pressure²²⁶. Mann et al. demonstrated that under Langmuir monolayers of fatty acids, calcite predominantly nucleates from its (1 -1 0) face²²⁷. Self-assembled monolayers, which can be patterned on substrates using microcontact printing, provide templates of well-defined chemistry and spacing of functional groups. Aizenberg et al. elegantly demonstrated the application of this technique for examining crystallization processes, by showing that patterned self-assembled monolayers could serve as templates for locational control of the nucleation event, as well as modulate crystal orientation²²⁸. The monolayers could be used to oriented nucleation and stabilize specific crystal face (Fig. 15). Several research groups have explored the use of reversed micelles or surfactant stabilized emulsion systems to create similar interface to monolayers systems²²⁹.

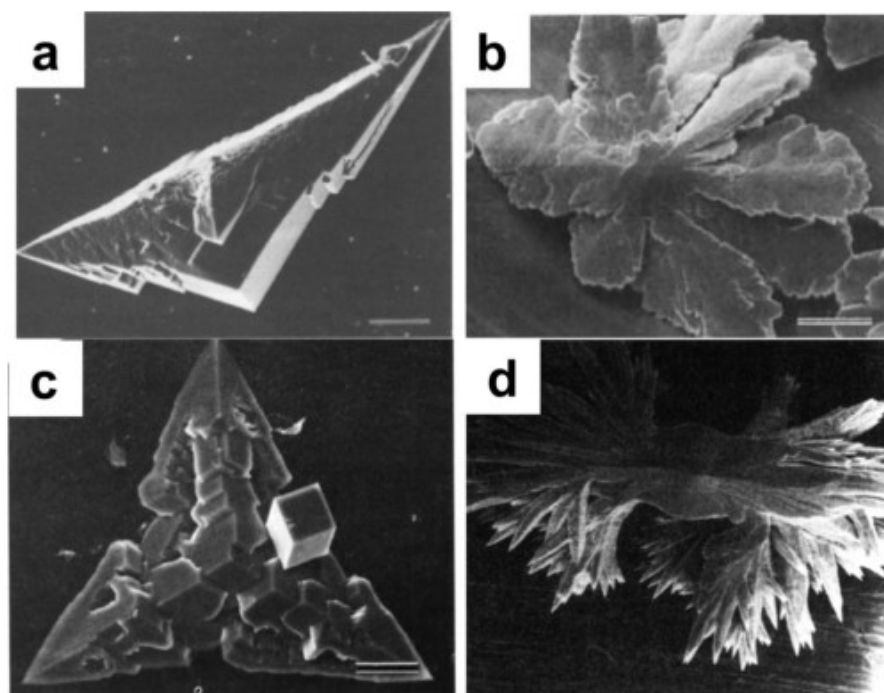


Figure 15: Scanning electron micrographs of CaCO_3 crystals formed under a Langmuir monolayer of (a, b) stearic acid and (c, d) eicosyl sulfate: a, (11-0) oriented calcite; b, (00-1) vaterite; c, (00-1) calcite; d, (001) aragonite formed in presence of Mg^{2+} ions²¹⁵.

These synthetic procedures show exceptional performances for biomineral shape optimization or crystal orientation. Two-dimensional templating surface could also yield complex and functional materials. Lee et al. have reported calcium carbonate microlens arrays with optical properties at the micro- and macroscopic scale by organic molecule controlled bioinspired monolayer precipitation²⁴. These techniques suffer a number of limitations, as they are inflexible in terms of preparation technique and they offer patterning on a limited length scale only.

1.3 Nucleation and growth control in solution

The control of mineralization can be archived in solution by using additive. In the past two decades, many experiments have been performed which give a rather interesting overview of the factors involved in controlling the morphology and shape of a crystal²¹⁵. Systematic studies into the effect of different additives on the formation of calcium carbonate investigated a series of inorganic and organic additives for their ability to modify the growth of calcite²³⁰. The use of additives can inhibit the development of the energetically most stable product to obtain one of the other polymorphs²³¹. The amorphous phase can be effectively stabilized by polymeric additives such as polyaspartate²³², polyglutamates²³³, polyacrylate²³⁴ and DNA²³⁵. The presence of additives is an important factor in controlling the morphology and growth direction of mineral crystals. It has been demonstrated that calcite can form into divers shapes by interacting with different additives (Fig. 16).

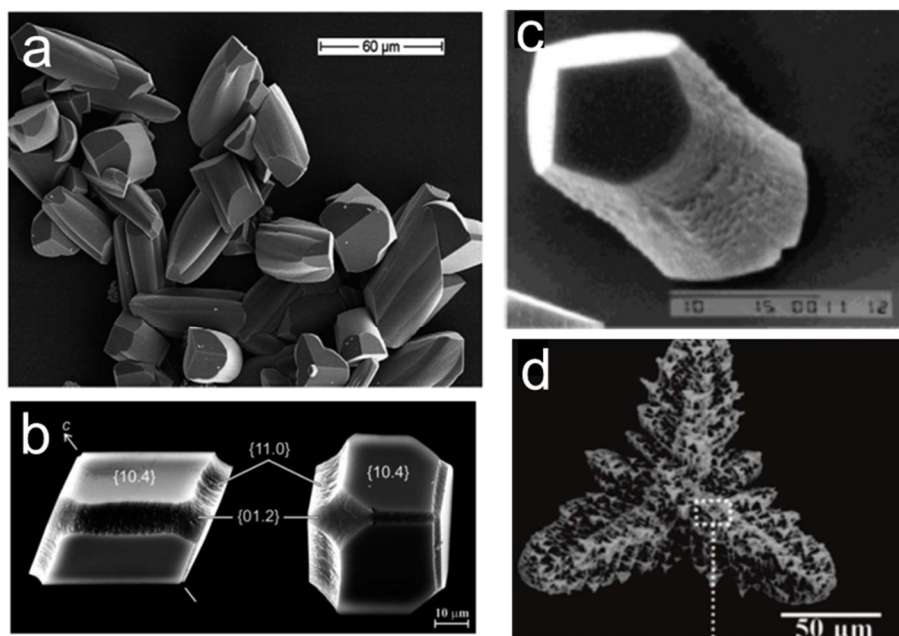


Figure 16: Scanning electron micrographs of calcite grown in the presence of (a) N-2-hydroxyethyl-iminodiacetic acid²³⁶; (b) (Phe-Asp)₄²³⁷; (c) Poly(isocyanopeptides)²³⁸; (d) silica ions²³⁹.

These synthetic procedures remain restricted to small sample sizes, are still very complex and also require further steps for preparing or removing biological or artificial templates^{232, 240, 241}.

1.4 Mineralization in confined environments

Biomineralization occurs generally in confined space (vesicles or organic matrix) and doing so in synthetic systems allows to control the size and the assembly of crystals. Container systems, such as micellar systems²⁴², protein capsules²⁴³, vesicles²⁴⁴, synthetic membranes²⁴⁵ and microemulsions²⁴⁶ have been developed for confined growth of minerals and complex calcium carbonate architectures have been formed according to the method used.

Droplets in microemulsions can act as a closed compartment in which mineralization can be performed and can be confined to the shape of the droplet²⁴⁷. Nanostructured films (using nanoimprint lithography) of generic polymers have been employed to direct the growth of crystals, and the organic layer was incorporated within the crystal²⁴⁸, while biomimetic hydrogel matrices have been used to control the oriented nucleation of the crystals²⁴⁹.

The chemical and physical properties of biominerals, such as polymorphs, morphology, crystallographic orientation and crystal size, are strongly influenced by the additives²⁵⁰. With the technological development in organic and polymer sciences, a variety of complex hybrid materials have been developed by using designed macromolecular templates. However, the construction of hybrid materials by this biomimetic method remains challenge. Novel technological advance will be essential to overcome the limitations of additive formation and extend the material design from molecular to macroscopic level.

2 Structure mimicking: Strategies to produce shell microstructure inspired biomaterials

The variety of shapes, composition and microstructure in molluscan shells is immense. Owing to the apparent simple morphology and peculiar properties, nacre has attracted considerable attention for novel material design. One of the special features of nacre is its mechanical strength: it is about 3000 times more fracture resistant than pure aragonite, its main component. The remarkable performance of nacre is largely related to its “brick-and-mortar” structure, where the bricks are densely packed layers of microscopic aragonite polygonal tablets (about 5–8 μm in diameter for a thickness of about 0.5 μm) glued together by 20–30 nm thick layers of organic materials.

Several biomimetic strategies have been proposed to produce “brick-and-mortar” structure by a variety of routes¹⁴. The approaches, such as layer-by-layer²⁵¹, freeze casting²⁵²,

hot-press assisted slip casting²⁵³ or processes at interfaces²⁵⁴ are excellent methods to produce materials with a nacre-like structure. Almost all methods related to the production of nacre microstructure are based on laminates processing¹⁴. The practical applications of a material are strongly dependent on it being a film or a bulk material. The techniques explored by numerous research groups can be divided into two groups: one producing bulk and free-standing materials; one producing coatings with a nacre-like structure, as schematically summarized in figure 15¹⁵. Several reviews and books focus on the production of nacre-like structures,^{255,256,257,258,259}. Despite the simplicity and elegance of the reported laboratory fabrication processes, challenges immediately arise with respect to scalability, dimensionality, material flexibility, and cost for industrial application. Addressing these issues, we give here particular attention to the processes that have been implemented on an industrial-scale or that can be transformed into high-throughput industrial technology.

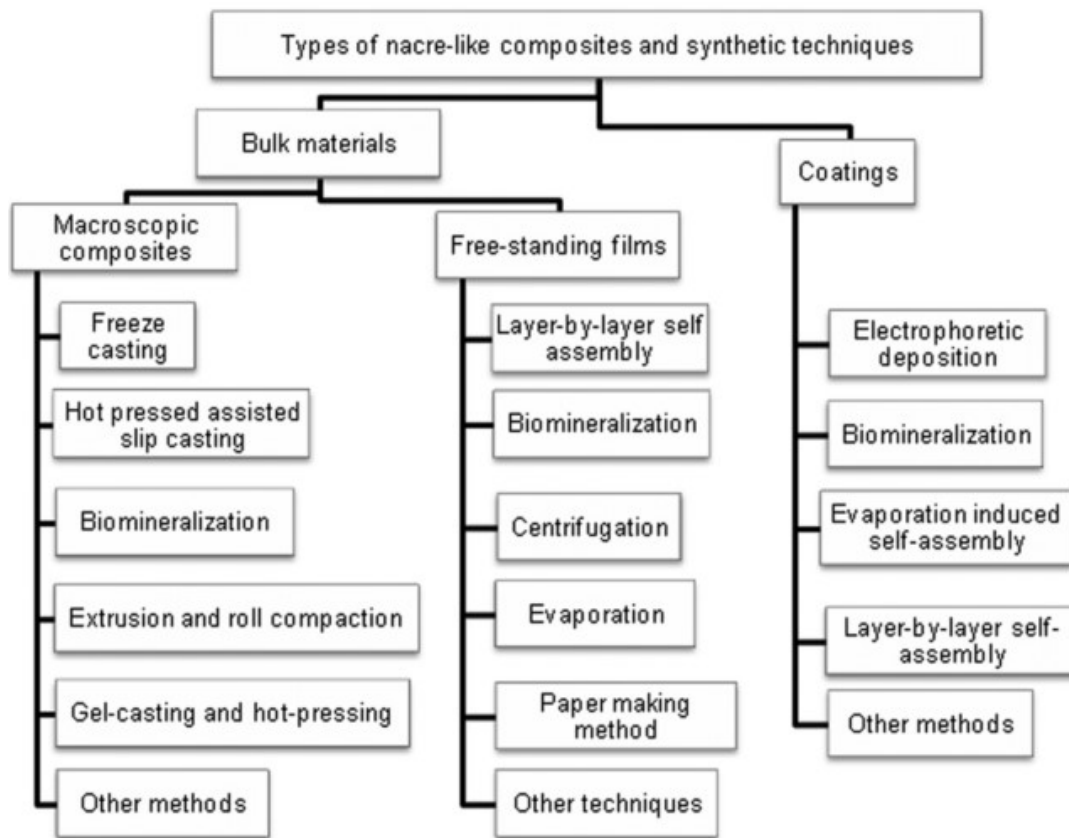


Figure 17: Schema of the main techniques explored to produce nacre-like materials as macroscopic composites, free-standing films and coatings^{Error! Bookmark not defined.}.

2.1 Layer-By-Layer (LBL) assembly

LBL assembly is a simple and versatile technique for fabricating functional thin films with a layered structure by alternately immersing a clean substrate into two solutions, which can interact and assembly accumulatively upon varying the driving force (Fig. 18). The LBL

assembly allows precise thickness control at nanoscale, and it can be used to combine a wide variety of inorganic compounds with polymers, thus merging the properties of each type of material. In particular, inorganic colloidal particles with a plate-like shape, such as platelets of clays, ceramics, layered double hydroxides, and graphene, can be readily assembled with various polymers to form such layered composite structures mimicking the natural nacre, where the inorganic building blocks act as the bricks and the polymers act as the mortar.

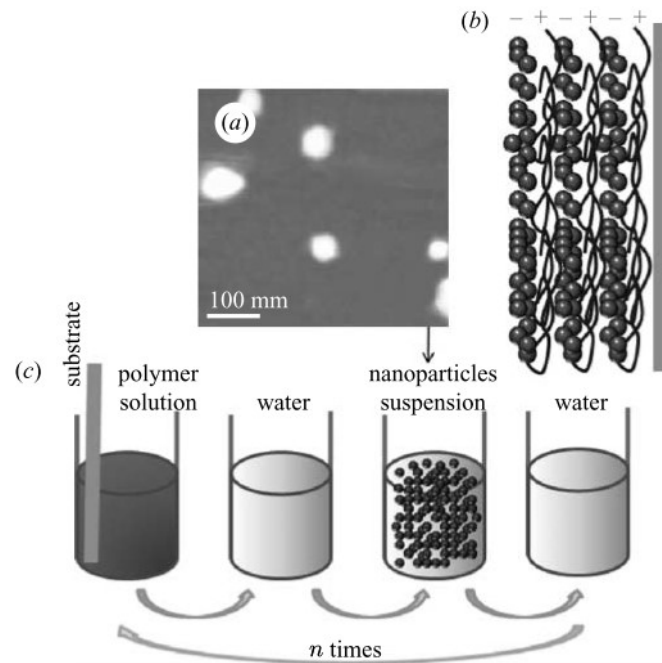


Figure 18: Layer-by-layer nanostructured hybrid coatings obtained by the sequential deposition of a substrate in a suspension of (a) nanoparticles (exhibiting a negatively charged surface) and a solution of a positively charged polymer (chitosan)²⁶⁰.

The LBL assembly technique can easily produce thick nanocomposites with pre-defined complex geometries. The mechanical properties are remarkably high and can sometimes exceed those of nacre²⁶¹. The structure of nacre was mimicked by using a LBL approach in which organic polymer layers constructed by the LBL self-assembly method were alternated with layers of calcium carbonate prepared by the ammonium carbonate diffusion method²⁶². Many LBL films with various compositions and properties have been developed by changing the building blocks or exploiting different driving forces for the film build-up. Layered nanocomposite structures were prepared by LBL assembly of cationic poly(diallyldimethylammonium chloride) (PDDA) and anionic montmorillonite nanoplatelets (layered silicates)²⁶³. The tensile strength and stiffness of the resultant PDDA–MTM composite film reached 106 MPa and 11 GPa, respectively, very closed to those of natural nacre.

The only drawback is the requirement of repeating the deposition steps numerous times in order to achieve a film with a practical thickness; therefore, this technique could be time-consuming. For instance, 300 bilayers are typically needed in LBL to reach an

approximate thickness of $1.5 \mu\text{m}^{261}$. Other methods proved to be faster than the usual dip-coating method, such as spin-coating methodology²⁶⁴. The process takes from days to a week despite progress in automation or identification of exponential LBL growth mechanisms²⁶⁵.

2.2 Field-assisted assembly

To overcome limitations of LBL process, field-assisted assembly techniques, such as electric field-assisted electrophoretic deposition and force field-assisted macroscopic self-assembly have been developed for the fabrication of nacre-like materials.

2.2.1 Electrophoretic deposition

Electrophoretic deposition is colloidal process wherein colloidal particles are shaped directly from a stable colloid suspension by a direct current electric field. The depositing electrode is the shape of the ware required, and it is designed such that deposit release is facilitated. A dc field causes the charged particles to move toward, and deposit on, the oppositely charged electrode (Fig. 19)²⁶⁶. All colloidal particles form stable suspensions carrying a charge can be used in electrophoretic deposition, a large variety of materials included, such as polymers, pigments, dyes, ceramics and metal.

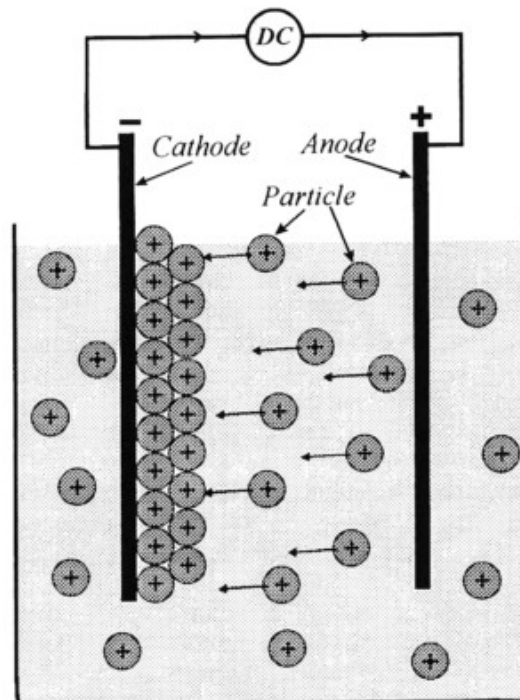


Figure 19: Schema of an electrophoretic deposition cell.

Electrodeposition has advantages such as short processing time, the possibility of room temperature processing, and no requirement for cross-link agents. It has been applied to

develop bioactive glass/polymer composite coatings with or without HA nanoparticle inclusions for biomedical purposes²⁶⁷ and to prepare clay-modified electrodes with a uniform and continuous polymer/clay composite film of brick-and-mortar nanolaminated structure, mimicking nacre²⁶⁸.

2.2.2 Thermal Spray process

Thermal spray processing was used to incorporate nacre-inspired design to materials engineering²⁶⁹. In this processing, thick coatings or free-standing structures are fabricated by feeding material powder, rod, or wire feed-stock into a high-temperature, high-velocity gas jet, forming micrometre-sized droplets that are driven toward a solid surface to be coated (Fig. 20a)²⁷⁰. Droplet impact ($100\text{--}600\text{ m s}^{-1}$) on the surface causes spreading into disc-shaped splats ($0.5\text{--}2\text{ }\mu\text{m}$ thick) that rapidly cool (10^6 K s^{-1}) and solidify resulting in fine columnar microstructures and “mud-cracking” if the material is sufficiently brittle (Fig. 20b). Successive impingement of thousands of such droplets leads to a layered or lamellar structure (Fig. 20d,f). A comparison of typical thermal spray processed cross-sectional microstructures with those of nacre (Fig. 20e,g) immediately reveals a striking architectural similarity, with splats as “bricks” but with the absence of organic “mortar.” There are also some clear differences, in that splats are generally relatively thick compared to nacreous lamellae, and the arrangement is not as ordered.

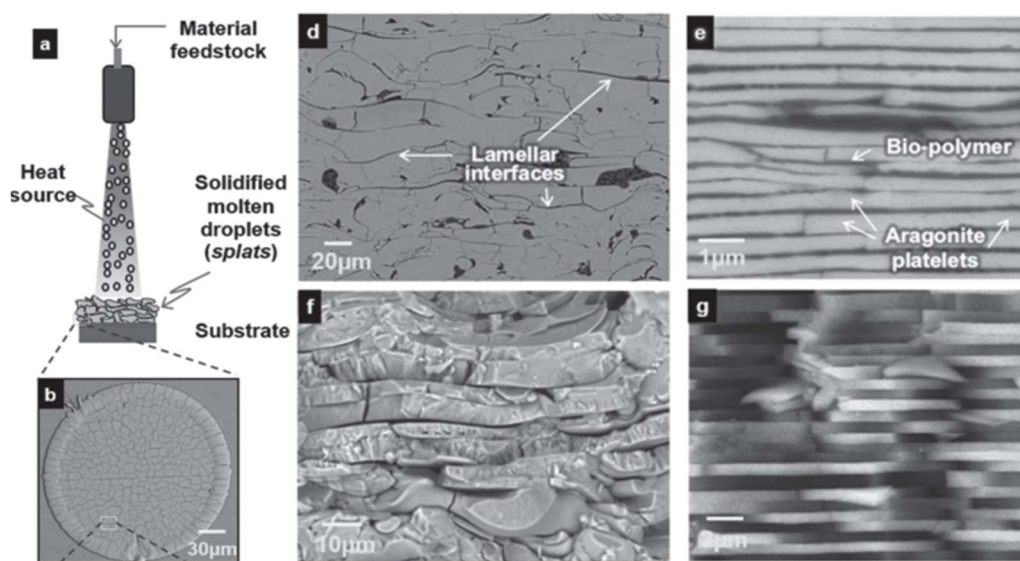


Figure 20: Illustration of the thermal spray process and associated microstructural and mechanical behaviour similarities between thermal spray formed ceramics and nacre. a) Diagram of thermal spray highlighting basic components. b) Scanning electron micrograph of a typical single solidified ceramic droplet (splat) with the quench-crack network (“mud-cracks”) associated with solidification shrinkage. d) Cross-section of sprayed material showing the lamellar structure resultant from droplet-based assembly of thermal spray. e) Comparative scanning electron micrograph of nacre (obtained from a commercially purchased abalone shell). f) Fracture surface images of sprayed material and g) nacre corresponding to (e)²⁶⁹.

This method allows rapid exploration of different experimental configuration, in terms of template architectures, materials and dimensions, it can be potentially implemented in an

industrial-scale, rapid fabrication method that has been part of the manufacturing pipeline in a host of technologies for several decades.

2.3 Paper-making method

The paper-making method is essentially a filtration, heating and pressing process. Walther and co-workers produced nacre-like sub-millimetre films using modified montmorillonite clay platelets individually coated with a Poly(diallyldimethylammonium chloride) polymer and then forced to self-assemble by paper-making process²⁷¹. This film showed outstanding mechanical properties. The authors then proposed an improved method by combining paper-making technique with doctor-blading and painting processes (Fig. 21). They all combine robustness, time-efficiency, and potential for scale-up. Wan et al. have used graphene oxide (GO) and molybdenum disulphide (MoS₂) nanosheets in a vacuum-assisted filtration self-assembly process to produce a robust ternary artificial nacre²⁷². The artificial nacre showed also high electrical conductivity.

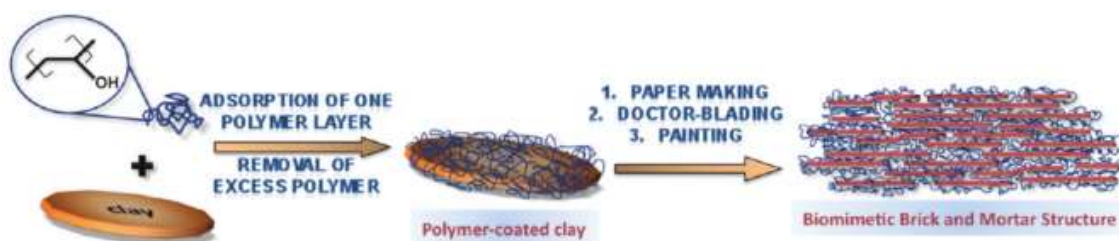


Figure 21: Strategy toward the preparation of biomimetic self-assembled brick-and-mortar structures: First, nanoclay particles are coated with an individual layer of polymer; then, the polymer-coated particles are ordered into nacre-mimicking layered composites via paper-making, doctor-blading and painting techniques²⁷³.

This process is a simple and rapid methodology for nacre-mimetic films with superior material properties; it is also ready for scale-up and to be applied in specific applications. Another major benefit is the “green” strategy, involving a benign aqueous solvent, a minimum of up-scaled apparatuses, as well as readily available and biodegradable components.

2.4 Freeze casting assembly

A more recent attempt at mimicking nacre in bulk material involved the use of freeze casting (also known as ice templating) to provide a new class of bioinspired ceramic materials with exceedingly high toughness^{274 - 275}. This technique (Fig. 22a,b) consists of freezing directionally a liquid suspension, the metastable growth of the ice crystals repels and concentrates the particles present in the suspension. The concentration of the particles occurs at a length scale where self-assembly of platelets can occur. Then, followed by sublimation of

the solidified phase from the solid to the gas state under reduced pressure, a layered homogeneous ceramic scaffold is obtained that, architecturally, is a negative replica of the ice. The scaffold can then be filled with a second soft phase so as to create a hard–soft layered composite. It is also possible to create brick-and-mortar structures by compressing the layered ceramic scaffolds before the introduction of the soft phase. This technique is a relatively inexpensive procedure to mimic natural structural designs over several length scales. Also, by controlling the freezing kinetics and the composition of the suspension, the architecture of the material can be adjusted at several length scales so as to replicate some of the microstructural features responsible. Furthermore, the thickness of the lamellae can be manipulated by controlling the speed of the freezing front, such that materials with lamellae as thin as 1 μm (close to that of nacre) ordered over macroscopic dimensions can be fabricated in practical sizes (Fig. 22c)²⁵⁹.

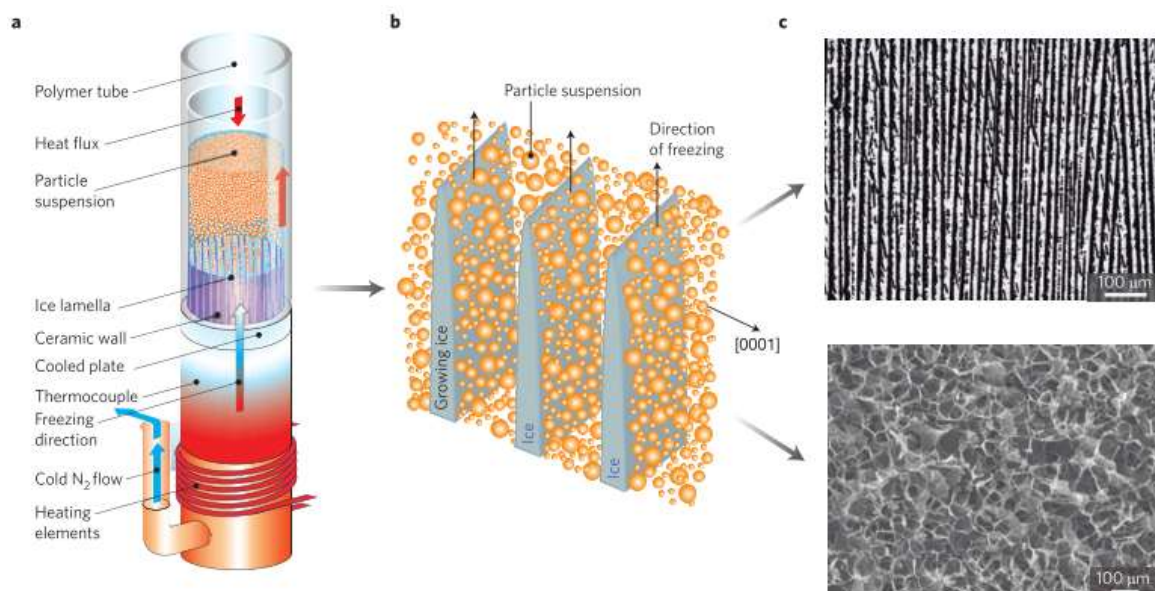


Figure 22: Processing of nacre-like structures by freeze casting. a, freeze casting uses both the directional freezing of, generally, ceramic suspensions and the microstructure of ice to template the architecture of scaffolds, and thus can be used to create porous, layered materials²⁷⁴. b,c, Controlled freezing results in the formation of lamellar ice crystals that expel the particles and/or dissolved molecules as they grow²⁷⁶. The particles accumulate in the space between the crystals, leading to the formation of a lamellar material (exemplified by the freeze-cast lamellar alumina (top) and porous chitosan (bottom) micrographs in c) after the ice has been sublimated by freeze drying and the material has been sintered. Particles trapped by the ice crystals form bridges between lamellae, and these bridges make a critical contribution to the mechanical properties of the layered material. The relevant microstructural dimensions — pore and lamellae widths and wavelengths from one to hundreds of micrometres — can be controlled by adjusting the composition of the suspension (solid content and solvent formulation) and the speed at which the ice grows²⁵⁹.

Freeze casting has been used to fabricate diverse hybrid materials such as metal/ceramic and polymer/ceramic with fine lamellar or brick-and-mortar architectures²⁷⁷. It can also be used for making efficient cellular structures. Although foaming techniques are well established, freeze casting and 3D printing both offer many advantages in creating such structures. For

example, directional solidification during freeze casting of platelet-based slurries can be used for the development of highly porous honeycomb-like scaffolds with a nacre-like cell-wall structure, which arises from the self-assembly of the ceramic platelets during processing²⁷⁸.

Munch and co-workers reported a composite of alumina with 20 vol% poly(methyl methacrylate) infiltrate resulted in a material with an ultimate strength of about 210 MPa and a fracture toughness of 5.1 MPa m^{1/2}²⁷⁹. Using observations of rising crack-growth-resistance behaviour, it was further concluded that a stable crack propagation toughness of greater than 20 MPa m^{1/2} was achievable through crack deflection and bridging, as well as inelastic deformation of polymer. Later, Bouville et al. substituted the organic matrix with mineral bridges of ceramic nanoparticle suspensions giving strength and fracture toughness of 400 MPa and 6.2 MPa m^{1/2}, respectively, which could also be sustained at relatively high temperatures²⁸⁰. This method has been used for high-performance thermally insulating material²⁸¹. Wicklein et al. described lightweight (7.5 kgm⁻³), highly porous foams that are produced by freeze-casting colloidal suspensions of cellulose nanofibres (CNF) and graphene oxide (GO), together with sepiolite nanorods (SEP)²⁸². The anisotropic nanocomposite foams are mechanically stiff in the freezing direction and able to sustain a considerable load. The thermal conductivity of the nanocomposite foams is sufficiently low to allow for a more than 50 % reduction in the passive house standards insulation thickness compared with commercial EPS, and the nanocomposite foams also show a high fire-resistance.

Freeze casting produces a ‘bricks-and-mortar’ structure very similar to that of nacre, even if the dimensions and the polymeric content are much higher. This methodology can easily be scaled-up to fabricate samples bigger than a few cm. The limitation could be the difficulty in uniformly infiltrating the polymer into the ceramic scaffold without creating porosities if the samples are much larger in the direction parallel to the lamellae. Moreover, this approach requires extensive amounts of energy during rapid freezing. Reaching the actual size of nacreous components (µm thick bricks, nm thick organic) is at the lower extreme of this strategy²⁸³.

2.5 Hot-press-assisted slip casting assembly

Another method to produce nacre-like bulk composites is the hot-press-assisted slip casting method (Fig. 23), in which the liquid matrix is a mixture of several components in a homogeneous suspension. With the applied pressure liquid matrix is forced to flow through a porous mould decreasing the volume fraction of the matrix phase and forcing the flakes to align perpendicular to the applied pressure direction²⁸⁴. Additionally, the applied pressure contributes to the reduction of residual porosity in the matrix.

Using this method Al_2O_3 flake-reinforced epoxy matrix nacre-like bulk composites have been successfully fabricated. Bulk nano-laminar composites revealed a high degree of flake alignment and high-volume content of the inorganic phase²⁸⁴.

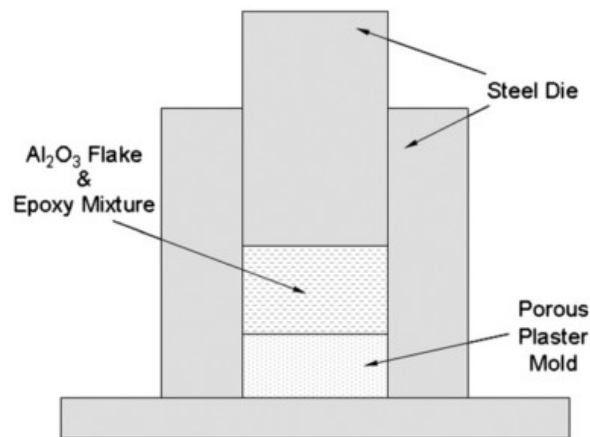


Figure 23: Schema of the Hot-press-assisted Slip Casting method²⁸⁴.

Considering that both hot-pressing and slip casting are widely employed industrial techniques, after optimizing the interfacial bonding between the two phases, this new methodology might be expected to be easily applied within an industrial context to fabricate nacre-like bulk materials. The sobering fact is that the slip/tape casting combined with hot pressing, cannot provide the degree of microstructural control down to the nanoscale that is needed to manipulate and optimize the mechanical response of the material.

2.6 Other techniques

Different techniques such as centrifugation²⁸⁵, sintering²⁸⁶, vacuum-filtration²⁸⁷, extrusion and roll compaction²⁸⁸, and combinations of these techniques have been adapted for formation of material with lamellar nacre-like structure^{12, 14, 15, 289}. Wei-Han and co-workers produced MMT nanoplatelets-poly-vinyl alcohol nanocomposites with a brick-and-mortar structure by a simple vacuum filtration²⁹⁰. This methodology is easily scalable and the nanocomposites showed tensile strength and toughness higher than those of nacre. Yao et al. fabricated chitosan-MMT nanocomposite films by vacuum-filtration, taking advantage of the strong electrostatic and hydrogen-bonding interactions between the MMT and the chitosan²⁹¹. The ultimate tensile strength of these composites with “brick-and-mortar” structure is 2–3 times higher than the property of the films made by simply mixing MMT and chitosan. Liu et al. produced TiC-metal multilayer coatings by ion beam sputtering deposition²⁹². The toughness of the multilayered films was much higher than that of pure TiC, but the hardness was much lower.

3 From laboratory scale methodology to industrial production

The natural material shows a diverse range of complex structures, using a biologically controlled self-assembly. The molecular interactions and structural studies have revealed that the molluscan shell is an excellent model for lightweight and tough structural materials.

Biologically controlled mineralization yields precise interface structure and mineral formation. Genetic engineering provides scientists with extensive possibilities to design recombinant proteins which might mimic essential functions of biological matrix on biomineralization. Simple fabrication of bulk biomimetic materials is conceivable by tuning the structural details of proteins by genetic means. The controlled growth of mineral phase and the building of structures from molecules (self-assembly) are powerful techniques, which once harnessed will allow a greater control over shapes and patterns over multiple length-scales²⁹³. Moreover, compared with traditional fabrication techniques self-assembly uses very little energy and, therefore, offers a sustainable approach to fabricating materials. However, role of the organic matrix remains unclear, and for large-scale production it can generate a bottleneck especially for industrial applications. Even though only a few percent of the final mass of a composite would be protein, availability and cost are currently limiting progress. However, industry is moving towards these types of products: BASF is working to produce hydrophobic proteins (trade name H STAR PROTEIN) for materials applications on a large scale²⁹⁴.

Nacre microstructure is the most studied biomimetic model, it is critical to identify its exact microstructural features and its hierarchical structure spanning over various length scales (from nanometres to micrometres to centimetres and larger). Numerous nacre processing techniques are proposed in the literature to achieve the brick-and-mortar biological structure, ceramic platelets (such as MMT, gibbsite, talc and Al_2O_3) have been used to create composites, able to transfer the load to the ceramic 'bricks' improving the overall mechanical properties of the composite. As discussed above, some process, such as freeze-casting, hot-press-assisted slip casting, and paper-making methods are susceptible to be adapted to large scale production. Especially, freeze-casting is probably the best-suited technique to produce bulk material. To our knowledge, this technique is employed to produce high quality construction materials with divers functions. Despite numerous efforts to elucidate the state of development of biomimicry in direct industrial application and its specific application, little information has been released under confidential justification.

Molluscan shells are also prominent material that can be mimicked to fabricate human body armour²⁹⁵. LAST® armour tiles, built by Foster– Miller (<https://www.qinetiq-na.com/>), consist of SiC or B₄C hexagonal tiles covered in a Kevlar® thermoset laminate which are held together with a Velcro®-type adhesive. This nacre-like structure provides energy absorption and toughening through many of the same mechanisms as its natural counterpart. The armour

has been implemented onto various ground and air vehicles including over 1000 Humvees for the US Marines. However, the method of preparation of this material is not communicated.

Despite such success to mimic nacre, the duplication of key features in artificial materials remains a challenge. No technique can currently generate small-scale features and integrate them into larger structure with a sufficient degree of control. Reproduction of the complex architecture typical of nacre has not been realised yet. However, considering the significant improvement achieved in resistant materials, it would be interesting to carry out further research to determine if additional enhancements could be obtained by inserting in the structure some other structural features typical of nacre. Furthermore, it would be extremely interesting to add macromolecules involved in biomineralization, which will recreate the interface structure in order to improve mechanical properties at molecular level. These studies would give new insights into the composite materials and provide information on the further directions that need to be taken to further improve the mechanical properties of nacre-like synthetic materials.

Molluscan shells give us lots of inspiration and design principles for the construction of artificial materials. The biomimetic approaches offer a sustainable solution to fabricating materials. However, production time, manufacturing facility and cost are currently limiting progress in fabrication on a large scale. The sophisticated bio-inspired methodology, with low environmental impact and low carbon footprint at the end of product cycle, is suitable for high-end product with outstanding properties.

IV. Potential avenues for biomimetic studies inspired from molluscan shell

The nacre microstructure in shells with its excellent fracture toughness has provided design inspiration to produce material with remarkably good mechanical properties. Its brick-and-mortar organization which inhibits crack formation in nacre, is mimicked to achieve similar control over mechanical properties in artificial materials. Much research on mollusc biomineralization has so far focused on structure-property characterization. Technological applications inspired from molluscan shell biomineralization have led mainly to microstructural design inspired from nacre for material with remarkably good mechanical properties. Yet, novel biomimetic functional material design can inspire from molluscan shell for a wider range of microstructures than nacre and for more properties than mechanical property.

While the molluscan shell is considered in most cases as a model for the design of tough ceramic engineering materials, other functions from molluscan biological systems, including optical (e.g., structural colour), stimuli-responsive (e.g., electromechanical materials), self-

healing (wet and under water), catalytic (ion transport, biologically controlled mineralization), etc. can also be taken as starting point for material engineering design. Here we have focused on certain particular native properties and functions of molluscan shells, which are of interest for both social and industrial applications.

1. Structure mimicking

1.1 *Inspiration from other shell microstructures*

As mentioned above, molluscan shells present a layered structure that can be categorized into several kinds of microstructure¹¹. Nacre is the most studied biomimetic model^{15,296,297}, very limited efforts have been devoted towards the mimicking of the other microstructures which present also very interesting mechanical properties.

The crossed lamellar microstructure, which is associated with higher fracture toughness than nacre, contains less than 1 wt % organic molecules^{298, 299}. Its superior mechanical strength and low-energy-cost secretion make the crossed lamellar microstructure much more used by molluscs for shell constitution than nacre³⁰⁰. It offers an excellent biomimetic solution with its complex shell architectural elements³⁰¹.

Due to the high complexity of the crossed lamellar microstructure, only few attempts have been made to reproduce its architecture in material science. Suzuki and co-workers tried to produce a particular bunched structure consisting of c-axis-elongated aragonite nanorods about 100 nm in diameter³⁰². The obtained highly ordered bunched structure and the morphology of the aragonite nanorods were similar to those of the third-order lamellae of crossed lamellar microstructure. Kaul and Faber developed a process to produce a crossed-lamellar-type microstructure in mullite by combining tape casting, oriented lamination and templated grain growth³⁰³. Chen et al. fabricated a second-order lamella by using micro-electro-mechanical systems³⁰⁴. The brittle and strong aragonite and the tough and relatively weak organic matrix were taken on the roles by silicon and photoresist, and mechanical tests indicated an estimated increase in energy dissipation of 36 times than that of simple silicon bulk. Karambelas et al. utilized a process called SHELL (sequential hierarchical engineered layer lamination) to mimic the crossed-lamellar structure based on Si_3N_4 and h-BN serving as the mineral and organic matrix³⁰⁵. The work of fracture of these biomimetic ceramics can reach 3.62 kJ/m^2 , which is much higher than that (2.86 kJ/m^2) of the fibrous monolithic $\text{Si}_3\text{N}_4/\text{h-BN}$.

The complex and effective crossed lamellar microstructures give us lots of inspiration for engineering and functional materials design. Therefore, more sophisticated innovative processing techniques need to be developed to reproduce these biological structures. Further

investigations are also expected for the other microstructure to reveal their formation mechanism and structural feature for biomimetic materials design.

1.2 Structural colour from molluscan shell

Structural colour is a phenomenon of wave properties of light. Biological systems are able to produce structural colour using highly precise and sophisticated nanometre scale architectures³⁰⁶. Structural colour in biological tissue is due to the interaction of the light with biological photonic systems, which mainly consist of highly ordered organic materials, including chitin (bugs), guanine (fish and spiders), pterin (butterflies), keratin (birds), melanin (birds) or carotenoids (birds)^{307,308,309,310,311}.

Li et al. described a localized, structurally complex and entirely mineralized photonic system observed in the blue-rayed limpet *Patella pellucida*³¹² (Fig. 24). This photonic structure consists of a nanoscale-periodic layered arrangement of crystallographically co-oriented calcite lamellae with regular thickness and spacing, which selectively reflects in the blue and green spectral range. The mineralized photonic architecture likely has evolved to satisfy an optical purpose without overly compromising the shell's mechanical performance. The underlying design principles could inspire and inform the technological generation of transparent, mechanically robust, multifunctional optical surfaces with incorporated, controllable display capacity.

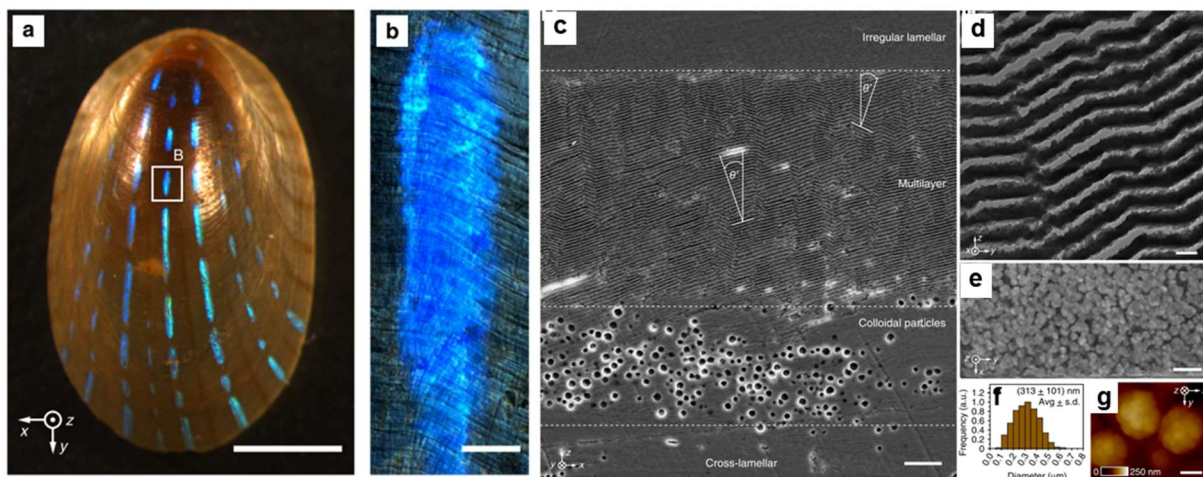


Figure 24: The blue-rayed limpet *Patella pellucida*. (a) Optical image of a limpet shell showing the reflection of light from the shell exterior. Scale bar, 2 mm. (b) Reflection optical micrograph of a single stripe. Scale bar, 100 μm. (c) Scanning electron micrograph of photonic architecture, two different structural components: a regular multilayer on top of a disordered array of colloidal particles (marked with the white dashed lines), are embedded within the normal densely packed lamellar microstructure, which lacks the detectable gap spacing present in the multilayer region. The characteristic angle θ' between the local multilayer surface normal and the shell surface normal is marked in two positions. Scale bar, 2 μm. (d) Transmission electron micrograph showing the regularity in width and spacing of the mineralized lamellae in the multilayer region. Scale bar, 200 nm. (e) Plan-view z-stack overlay showing the distribution of colloidal particles underneath the multilayer, based on X-ray nanotomography reconstructions. Scale bar, 2 μm. (f) Size distribution of the colloidal particles. (g) Atomic force

microscopy height image of the intact colloidal particles, demonstrating their non-faceted fused-granular surface morphology. Scale bar, 100 nm³¹².

The investigation of the morphogenesis of the limpet's photonic architecture could inspire the design of transparent, mechanically robust, multifunctional optical surfaces with incorporated, controllable display capacity and also provide new ideas for the technological realization of inorganic photonic materials for a wide range of applications.

2. Process mimicking: molluscan shell protein applications

Several studies have demonstrated that proteins play an important role in biomineralization. They take part in each step of shell formation such as the nucleation of crystals (polymorphism), crystal growth (morphology) and termination of the crystal growth, and they are also essential in assembling nanocomposites into highly ordered structures³¹³⁻³¹⁴. Yet, the organic matrix in molluscan shells might also exhibit novel functions other than biomineralization. For instance, the soluble matrix proteins from the shell of *Pinctada maxima* have osteogenic potency on human mesenchymal stem cells³¹⁵. Arivalagan and co-workers in the CACHE project identified specific domains such as immunomodulatory, lipocalin, myosin tail and LCD rich in shell matrix proteins³¹⁶. Thus, the molluscan shell organic matrix might provide emerging novel proteins for diverse applications.

2.1 Anti-lime scale device

Lime scale deposition may concentrate in industrial heat transfer equipment and decreases the heat exchanger performance and wastes heating energy, resulting in a tremendous economic loss. Anti-lime scale inhibitors, especially the organic inhibitors present a significant advantage from the environmental point of view³¹⁷. Green corrosion inhibitors, in particular of the organic diphosphonic acid³¹⁸ and citric acid³¹⁹, have long been known in the art of detergent to reduce lime scale build up to washing machine parts.

During molluscan shell formation, organic matrix could participate in the inhibition of the crystal nucleation and growth. It is hypothesized that Ca-binding COO⁻ groups of polypeptide chains may initiate or inhibit crystal growth, depending upon their spacing relative to the spacing of Ca of the CaCO₃ crystal lattice³²⁰. Moreover, ubiquitylated proteins may inhibit the spontaneous crystallization of aragonite and slow down the rate of calcite precipitation³²¹. The inhibition of crystal formation is also reported in the sponge *Clathrina*³²², the ascidian *Pyura pachydermatina*³²³ and the crayfish *Cherax quadricarinatus*³²⁴.

The molluscan biomineralization offers important potential insights and designs for anti-lime scale system. A better understanding of the biologically controlled biomineralization and the role of proteins, is desirable for adaptation of biological system to industrial application.

2.2 *The other potential applications*

Molluscan shell contains very small amount of organic matrix that is essential for controlling the shell biomineral deposition. Among a large number of identified shell proteins, some of these molecules have structural similarities with proteins found in vertebrates, especially in humans. This similarity justified the usefulness of testing the biological effects of molluscan shells on many mammalian tissues.

In 1992 Lopez and co-workers discovered that natural nacre from the pearl oyster *Pinctada maxima* is simultaneously biocompatible and osteoinductive³²⁵. Nacre shows osteogenic activity after implantation in human bone environment³²⁶. In previous studies as reviewed in Westbroek and Marin, it has been shown *in vivo* and *in vitro* experiments that nacre can attract and activate osteoblasts³²⁷. Furthermore, nacre powder has been used as an implantable material for reconstruction and regeneration of maxillary alveolar ridge bone in humans³²⁸.

Recent work by Latire et al. has demonstrated the biological activities of *Pecten maximus* shell matrix on human skin fibroblasts in primary cultures³²⁹. Water soluble matrix components have beneficial effects on human skin, including enhanced wound healing³³⁰. Another *in vivo* study using a rat skin incisional injury model, showed that implanting powdered nacre between the epidermis and dermis at the incisional site increased collagen synthesis by dermal fibroblasts³³¹.

Extensive investigations have indicated that molluscan shells exhibit desirable properties for bone/skin tissue engineering applications. Hence, the molluscan shell is becoming widely used for diverse biomedical applications and the cosmetics industry³³². A number of studies has shown that the release of some biotically active factors from the shell influences tissue regeneration. However, the identification of these bioactive molecules present in the molluscan shell remains a challenge.

During the formation of molluscan shells, CaCO_3 is taken up from the aqueous environment. Since the calcitic deposition reaction is exergonic³³³, an acceleration of the reaction velocity can be reached by lowering the activation energy either allowing the process of CaCO_3 deposition to proceed on a functionalized organic surface³³⁴ or by coupling of the membrane-bound bicarbonate transporter with the soluble enzyme, the carbonic anhydrase³³⁵. The carbonic anhydrase enzyme (CAe) can form reversibly bicarbonate by

hydration of carbon dioxide. The CAe is among the fastest catalysing enzymes³³⁶ and represents a promising component for CO₂ capture and storage in order to achieve deep emissions reductions³³⁷.

The solvent-based post combustion CO₂ capture process (PCCC Fig. 25) is one of the most mature and promising technologies for capturing the CO₂ emitted from large point sources like fossil fuel-based power plants^{338,339}. The challenge facing this capture process is the energy loss during desorption. Solvents such as aqueous monoethanolamine tend to bind CO₂ tightly such that the parasitic energy loss in desorbing the CO₂ would result in a near doubling of the cost of electricity³⁴⁰.

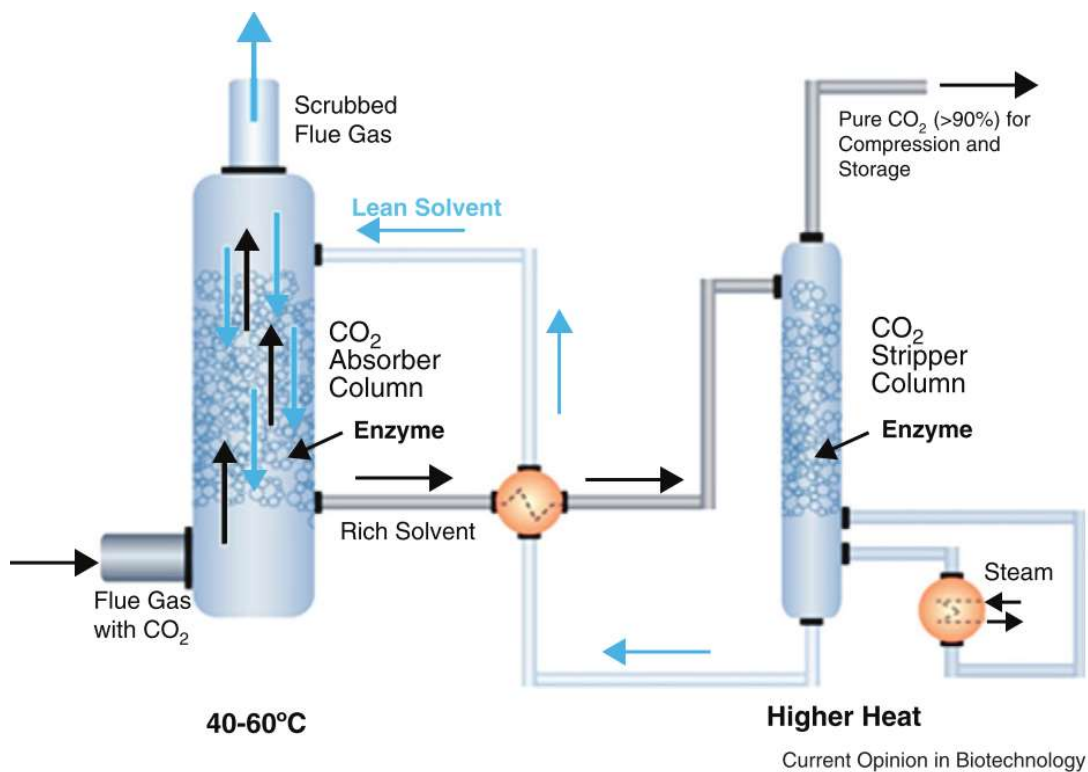


Figure 25: Solvent-based PCCC schema: CO₂ is removed from the flue gas stream in the absorber column and then desorbed in a heated stripper column to give relatively pure CO₂ for compression and storage³⁴¹.

CAe facilitates the use of aqueous solvents with a far lower heat of desorption (e.g. hindered and tertiary amines), thus enabling a far lower energy penalty on CO₂ capture and sequestration³⁴². Several studies have described process formats for capture of CO₂ from combustion sources using CAes entrapped in a gas permeable membrane^{343, 344, 345}. CO₂ Solution (Quebec, Canada) uses an CAe approach for CO₂ capture from cement-factory flue stacks³⁴⁶. They describe the use of protein to accelerate capture in solvents such as MEA, MDEA, and piperazine³⁴⁷.

The potential of CAe for CO₂ capture has been demonstrated, a number of strategies have been proposed to overcome stability limitation of protein engineering³⁴⁸. These approaches should help biocatalysis to spread further into industrial application.

3. Antifouling effect

Fouling processes (corrosion, crystallization, suspended particles, oil and ice) are responsible for numerous industrial losses. Another engineering challenge plaguing a variety of industries, is biofouling, i.e. the accumulation of unwanted biological matter on surfaces. The antifouling system is one of best protection methods to avoid degradation of metal surface. Since traditional inhibitors which had great acceptance in industries, showed negative environmental impacts, more attention has been paid to the study of new green scale inhibitors^{349,350,351}.

Marine organisms face intense fouling pressures. Defence mechanisms of the body surface consist of physical, chemical and ecological mechanisms that are common among marine organisms³⁵². These defences can provide biomimetic and bio-inspired solutions for fouling problems on man-made structures³⁵³ and also for biological fouling problem. Potential chemical defence mechanisms of molluscs have been rarely studied and appear to function as general protection mechanisms against predators.

The periostracum, the outermost proteinaceous layer on the shell surface, prevents the initiation of boring by fouling organisms^{354,355} and is a fouling deterrent^{356,357}. Studies on the periostracum of blue mussels, *Mytilus edulis* and *Mytilus galloprovincialis*, showed a multi-level antifouling defence consisting of cumulative filtration³⁵⁸, a distinct microtopography³⁵⁹ and chemical repellents³⁶⁰. Studies of antifouling mechanisms in Mytilidae have highlighted the distinct microstructure of the periostracum, which inhibits the settlement of larvae of various barnacle species^{361,362} and other fouling organisms³⁶³. This microtopography appears to inhibit settlement of macrofoulers rather than microfoulers, when tested without the chemical components of the periostracum. By producing topographies with the lowest number of attachment points for the settlement and growth of propagules and larvae of fouling organisms, bio-inspired anti-settlement surface have been produced^{364,365}. These structural parameters provide a template for the industry to move towards in the development of coatings with increased resistance to fouling and improved fouling-release properties. These goals can be achieved by the development of new materials or the incorporation of surface features to current anti-fouling surfaces³⁶⁶.

Benthic micro- and macro-organisms produce bioactive molecules that can kill, impair or inhibit the settlement of bacteria, spores or larvae by chemical defence mechanisms³⁶⁷. The chemically active substances investigated are generally found within the body tissue, whereas antifouling defences need to be located at or released from the surface of the organisms. Bers et al. have investigated the chemical defence strategy of the periostracum of blue mussel³⁶⁸. They have shown that a biofilm on resin replicas of periostracum micro-ripples reduces the antifouling efficiency within a few weeks³⁶². However, the identification and quantification of bioactive components in anti-fouling process remain challenging at present. A lack of

knowledge on the bioactive molecules production, storage and release, makes it difficult to adapt this technique to biomimetic application.

A combination of physical, chemical and mechanical defence mechanisms should provide an opportunity to maximise anti-fouling effects, while minimising environmental effects. The design and manufacturing of a surface that can prevent or control chemical or organism fouling is an enormous challenge. However, molluscs have successfully managed this challenge and the molluscan shell can provide inspiration for developing sustainable technologies.

4. From shell formation to building construction

Concrete, the most man-made material, plays an important part in our daily lives. Cement, as the essential component in concrete, reacts with water to bind aggregates and sand. The world's yearly cement production has reached 2.8 billion tonnes and is expected to increase to some 4 billion tonnes per year³⁶⁹. This production accounts for about 7 % of the global loading of carbon dioxide into the atmosphere, making it a major cause of global warming. Cement industry is singled out for its environment impact.

The cement production includes quarrying and calcination of raw material, grinding of clinker and transport. The main energy consuming processes during cement production are calcination and grinding of clinker. They represent huge inputs of energy, producing a ton of Portland cement requires about 4 GJ energy and release a ton of CO₂³⁷⁰. The main reaction occurring in kiln was the formation of calcium silicates from calcite and silicate at 1400 °C. Release of CO₂ from carbonate during calcination of raw material represents 60 %, power production about 40 %³⁷¹. Several approaches of reducing the environmental impact of concrete are investigated: alternative fuels, replacement of clinker, energy efficiency and carbon capture and storage³⁷². Despite the tremendous efforts made to recover CO₂ released during cement production³⁷³ and to recycle alternative materials³⁷⁴, annual anthropogenic global CO₂ production contributed by cement production continues to increase due the growing demand of this material for construction.

Biomimetic materials science which contributes to economy and acts in favour of global warming limitation can provide solutions for cement industry. Traditional cement companies and a growing number of start-ups are exploring biomimetic production methods to reduce or even eliminate their net output of CO₂. Several companies have proposed biomimetic solution coupled with environmental and economic sustainability. Calera³⁷⁵ proposes a process that essentially mimics marine cement, which is produced by taking the calcium and magnesium from seawater and using bicarbonate from hydration of CO₂ (Fig. 26).

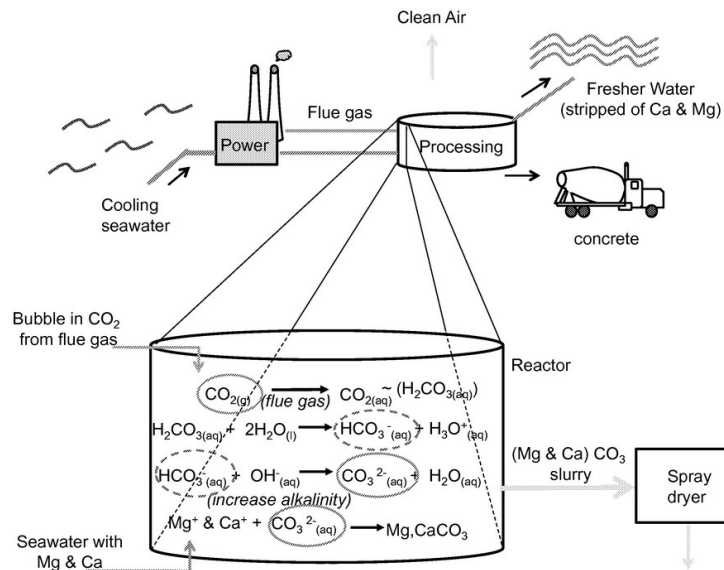


Figure 26: Process proposed to produce concrete by drawing CO₂ from the flue gas stream⁴³¹.

Calera claims its process achieves an effective reduction of CO₂ emissions³⁷⁶. However, the formulas used by Calera are unclear and unconvincing³⁷⁷. Hence, the efficacy of this carbon sequestration process remains controversial.

CarbonCure proposes to increase the CO₂ uptake capacities of concrete by injecting recycled CO₂ into wet concrete during the mix. CO₂ would form carbonate and then react with CaO to form calcium carbonate. CarbonCure claims that its carbonation curing technology could result in a net sequestration efficiency of over 80% and can offer a promising tool for greenhouse gas mitigation. Calcium carbonate reaction is exergonic³⁷⁸, but one can notice that CaO based carbonation process assumes a 50% conversion of CaO over repeated cycles in solution condition³⁷⁹. The high CO₂ conversion efficiency claimed by CarbonCure reminds uncertain.

All in all, currently, there is little, if any, convincing biomimetic approach for cement production. Still, the biomineralization mechanism of molluscan shells could inspire alternative cement production methods. Molluscs extrude inorganic precursors of calcium carbonate by membrane pumps and channels: calcium ions are released in the site of mineralization by transmembrane pumps, for instance, Ca-ATPases³⁸⁰; hydration of CO₂ to bicarbonate is catalysed by carbonic anhydrases³⁸¹. The biomineralization occurs then in the extrapallial space, regulated by the shell matrix composed of proteins, glycoproteins and polysaccharides.

Acidic, negatively charged macromolecules that play a vital role in the calcium carbonate crystallization process, could control crystal nucleation and growth by lowering the interfacial energy between the crystal and the macromolecular substrate³⁸². The macromolecules with separate functional domains facilitates selective binding to different surfaces. Pif, a protein

isolated from the nacreous layer of the pearl oyster *Pinctada fucata*, contains a chitin binding domain and an acidic part that binds to aragonite. This protein has a function in the mechanical strength of the composite materials by 'gluing' components together. Introduction of these functional organic molecules in cement would accelerate the hydration/carbonation reactions and enhance mechanical performance. Investigation of the shell matrix molecules inspired additives offers important potential insights for more sustainable concrete production, although the cement formation mechanism and industrial production process remain unchanged. The environmental challenge for the concrete industry remains similar.

Cement is not the most energy-intensive materials of construction; its large carbon footprint is mainly due to the huge quantities used all over the world. All the environmental efforts made by the cement industry consist of reducing CO₂ emission of CaCO₃ decarbonation process. Resolution of this fundamental issue might be replacement of limestone decarbonation/carbonation by using other alternative process.

While scientists propose various solutions for enhancing the man-made cement stability, reducing energy consumption and increasing the CO₂ capture and storage efficiency, marine organisms have been using environmentally friendly systems to attach hard substrates. Many different means and strategies of attachment have been developed³⁸³. We give particular attention to biological cement, a biologically mediated precipitation of inorganic cement. The process of biological cementation has been most intensively studied in oysters where a crystalline calcium carbonate cement is developed between the periostracum and the substratum³⁸⁴. The spherulitic nature of this cement resembles those secreted under dominantly physico-chemically controlled conditions rather than direct biotic control³⁸⁵. This extraperiostracal calcification requires very low additional energy input³⁸⁶.

Due to the huge functional similarity between biological cement and man-made cement, one can imagine a bio-inspired cement composed of organic molecules which would induce inorganic cement formation from supersaturated solutions. This environmentally friendly process would allow overcoming all intense energy consuming steps during traditional cement production.

Although, replacing clinker with alternative bio-inspired cements is an approach to tackling concrete CO₂ emissions, the potential of cement replacement is limited. The challenge is to translate laboratory findings into industrial applications. The biomimetic process is attracting little interest, compared to the well-functioning cement manufacturing. Cement production is a straightforward process: from the mining/quarrying large quantities of raw materials, grinding of clinker, transport operations.

Further studies on bio-inspired cement is indispensable to clarify the principle of biologically induced mineralization. To highlight the feasibility of this material at industrial

level, there is a need to assesses the product durability, the cost, the product value and profitability of the business enterprise.

V. Conclusion

The human needs are constantly growing due to population growth and demands for higher quality of life. As such, our modern-day society uses natural resources ~1.5 times faster than the Earth's systems can renew them³⁸⁷. Humankind is now significantly changing the quality of the atmosphere, mobilizing various minerals at many times their natural rates, vastly altering the land surface, and polluting the entire planet with toxic substances. Overwhelming evidence shows that greenhouse gas emission from human activities is a major contributor to global climate change with potentially devastating effects. Hence change is needed. One of the major challenges of humanity is to provide sustainable technologies, which means a development that satisfies the needs of the present without impacting the needs of future generations. Biomimetic design potentially offers approaches to creating new materials whose production imposes lower constraints on the Earth's rare raw materials and energy resources.

Molluscs, which operate within restricted living constraints without creating waste or irreversible damage to the ecosystem, provide various inspirations to support sustainable and/or ecological design. The nacre microstructure has been a source of inspiration to produce tough and resilient material. Numerous innovative technologies have been developed to achieve this biological structure. Our understanding of the influence of shell matrix on mineral growth provides a possible control over chemical and physical properties of mineral at nanometre range.

As noted, technological applications inspired from shell biomineralization have led mainly to microstructural design for material with remarkably good mechanical properties. Nevertheless, the introduction of hierarchical structures from nano- to meter-scale similar to molluscan shells is well beyond the reach of present technology. Thus, our understanding of biomineralization remains incomplete and the self-assembly of nanocomposites into highly ordered structures remains a major challenge for bulk biomimetic materials. The ultimate objective is to develop smart materials that can both detect an event and structurally change in response to it. The need for novel processing routes imposes tremendous technological advances.

Much research on mollusc biomineralization has so far focused on structure-property characterization. We would like to emphasize that mimicking molluscan shell designs and processes could create advanced engineering materials for various other applications, such as structural colour, antimicrobial medication, antifouling process, construction material

production, etc. The key to mimicking biominerals lies in understanding the involved cellular processes. However, the role of the shell matrix in the mineral formation remains unresolved. Further progress in disentangling the mechanisms that control these biomineralization processes should yield clues for the design of novel biomimetic materials.

Nature gives us materials with outstanding performance that we try to mimic by focusing on their essential features. Forming a material based on biological design principles and observing the biomimetic model behaviour might enable us to make better hypotheses about the design secret of biological materials.

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