Biomineralization at fluid interfaces

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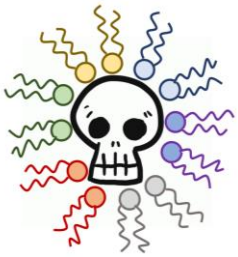
ABSTRACT

Biomineralization is of paramount importance for life on Earth. The delicate balance of physicochemical interactions at the interface between organic and inorganic matter during all stages of biomineralization resembles an extremely high complexity. The coordination of this sophisticated biological machinery and physicochemical scenarios is certainly a wonderful show of nature. Understanding of the biomineralization processes is still far from complete. The recent advances in biomineralization research from the Colloid and Interface Science perspective are reviewed herein. The synergy between this two fields of research is demonstrated. The unique opportunities offered by purposefully designed fluid interfaces, mainly Langmuir monolayers are presented. Biomedical applications of biomineral-based nanostructures are discussed, showing their improved biocompatibility and on-demand delivery features. A brief guide to the array of state-of-the-art experimental techniques for unraveling the mechanisms of biomineralization using fluid interfaces is included. In summary, the fruitful and exciting crossroad between Colloid and Interface Science with Biomineralization is exhibited.

Keywords: Langmuir Monolayer, Delivery, Biomineralization, Carbonate, Water.

GRAPHICAL ABSTRACT



* The relevance of fluid interfaces in biomineralization processes is reviewed. * The niche of the Langmuir technique for studying biomineralization is discussed. * A basic guide on the experimental techniques and procedures for studying the formation of biogenic minerals at interfaces is provided. * Biomedical applications of nanominerals are examined.

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INTRODUCTION

The IUPAC defines Biomineralization as “Mineralization caused by cell phenomena” in Schué et al.[1] Crichton defined biomineralization as “the study of processes that lead to the formation of hierarchically structured organic-inorganic materials generated by living organisms, such as shells, bone, and teeth”.[2] Both definitions are complementary and illustrate the relevance and complexity of the Biomineralization field. Biomineralization is an astonishing and wonderful phenomenon based on a highly efficient directed synthesis of inorganic and inorganic/organic materials with well-defined structure at the macro-, micro- and nano-scale. Molluscan shells are widely used models for researching biomineralization as well as a forefront example for popular science on the matter. Colourful shells with exciting shapes are one of the first examples coming to mind when mentioning inorganic materials formed by living organisms.[1] On the microscopic scale, micro-flora and fauna offer exotic and beautiful structures which catch the eye when observed by electron microscopy, as the case of Coccolithophore. The Coccolithophores are unicellular sea organisms accounting for the biomineralization of up to 40% of calcium carbonate at sea.[2] Not only in the sea can be found representative biomineralization examples but also in vertebrates´ body. For instance, bone design for mechanics and architecture enabling matching of different bones to perform global functions involving movement demonstrates the unmatched ability of nature in building functional structures, e. g., almost 30 bones in the human foot.[3] The mentioned examples share a hierarchical structure from the nanoscale to the macroscopic scale that confers unique physicochemical properties.[4] See Figure 1 for a summary of representative examples in biomineralization.

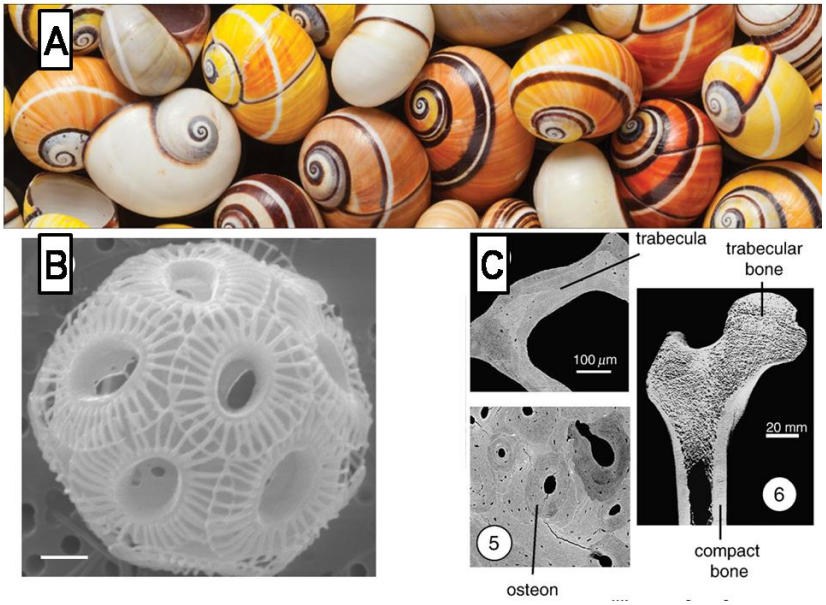

Figure 1. Representative examples of biomineralization. A) Striking colours from molluscan shells. Shells of the land snail *Polymita picta* from Cuba. Copyright from Cambridge Philosophical Society, 2016.[1] B) A single coccolithophore cell mastering the art of biomineralization. Specimens of *Emiliania huxleyi*, type O (lamella). The scale bar indicates 1 μm. Creative Commons Attribution 4.0 License.[2] C) Bones as highly hierarchical biomaterials. From trabecula and osteons to a cross section through a proximal femur. Copyright from Elsevier, 2011.[4]

Calcium carbonate in itself is a lively field of research.[5–8] The complex and hierarchical structure is a core aspect of biogenic materials. Such complexity makes the building of similar materials using lab tools a rather difficult task. The mimicking of natural nacre is a major step in this direction.[9] On the other hand, the already formed biogenic structures provide intriguing opportunities for obtaining modified materials, as reported for nanostructuring polypyrrole on biomineral templates. Using infiltration of the pyrrole monomer on sea urchin spine, electroactive and highly structured conducting polymers can be achieved.[10]

The organic/mineral interface is regarded as the core scenario in biomineralization.[11] Extraction of proteins and other biological macromolecules from biominerals provides a better perspective for designing polymers and peptides for guiding biomineralization in artificial conditions, as explored for an ascidian skeleton by Adadi et al. This study clearly proven the mismatch between calcite crystals obtained by epitaxial overgrowth and those coming from biogenic surfaces. Evidence of fundamental role of biological macromolecules for precipitating

different sorts of biogenic calcium carbonate was clearly exposed. Moreover, the results pointed to specialized macromolecules that might be classified onto two sets with function either in precipitating amorphous calcium carbonate or templating its morphology.[12] Organic/mineral interface also plays a central role in phenomena related to Astrochemistry, appearance of life and chemical gardens.[13]

The works by Mann et al. opened an avenue of research that is still expanding, with Langmuir monolayers of classic surfactants mimicking the organic/inorganic interface for controlled crystallization of different phases of calcium carbonate.[14–16] Mann discovered the relevance of the organic/inorganic interface on crystal growth over other classical bulk chemical considerations. These experiments were seminal and greatly illustrate the potential of the Langmuir technique for studying the biomineralization at the organic/mineral interface. We therefore envision that detailed insights on the biomineralization mechanisms would be attained with the availability of state-of-the-art experimental techniques and a non-classical approach of the Langmuir technique.

Liquid/solid interfaces are also relevant models for understanding biomineralization through self-assembled monolayers. The study by Lee, De Yoreo et al. is a representative example, in which the influence of the crystallization of calcium carbonate on the organic surface was examined in detail.[17] The molecular orientation of the organic groups emerged as a highly relevant parameter, tuned by a subtle chemical difference in the substituting group of a thiolated phenol. The structural coevolution of both the mineral and the organic monolayer were monitored, pointing to a reciprocal templating. he concept of directing the formation of mineral crystals at a ligand coated inorganic surface can be used also in curved surfaces, e.g., nanoparticles.[18]

Excellent reviews on biomineralization are available, as the exhaustive review by Cölfen et al. on the matter with a general perspective in the non-classical crystallization.[19] Crystallization by particle attachment has also received excellent attention.[20] Herein, we focus on the relevance of the organic/mineral interface and highlight the unique opportunities provided by fluid interfaces, mainly the Langmuir technique.

BULK VS. INTERFACIAL BIOMINERALIZATION

Biomineralization always takes place at an interface when rigorously considered: surface/solution, surface/crystal and later crystal/solution interfaces in the simplest approach.[21] Emulsions and multi-solvent systems are valuable examples for obtaining nanostructured biominerals.[22] These colloidal systems present a confined space, with confinement as the scenario in biogenic mineralization in all cases.[23] Bulk biomineralization will be referred as those processes developed with no purposeful introduction and specific consideration of interfaces, e. g., formation and growth of carbonate particles in solution. Interfacial biomineralization will be referred as the biomineralization occurring at whichever interface, both in the formation of inorganic nuclei and subsequent growth. A remarkable study by Bertinetti´s group on the crystallization mechanisms of amorphous calcium carbonate (ACC) in presence of different surface adsorbates greatly illustrates this effect as opposed to bulk crystallization.[24] Note that directed crystallization of amorphous biogenic minerals is a core strategy for living organisms, as shown for crystallization into calcite in sea urchin.[25] In this direction, biogenic minerals from bacteria have been proposed as a source for bioconcrete.[26]

Relevant work employing the block copolymer poly(ethylene glycol)-*block*-poly(methacrylic acid) (PEG-*b*-PMAA) for reaching different nanostructures of calcium carbonate has been performed in bulk solution.[27] Cölfen and Qi designed a smart strategy using the different hydrophilic ratio of the block copolymer to adjust directing the formation of calcium carbonate with the solvation of the precipitated calcium carbonate. Not only could the shape of the nanostructures of calcium carbonate be adjusted, but also the kinetics of formation, being the pH of the solution the most relevant parameter. This concept appears as easily exported to fluid interfaces, e. g., building monolayers and vesicles of PEG-b-PMAA. Indeed, the authors discussed the formation of a given polymorph in terms of the interface energy that varies due to the adsorption of the polymers which might contribute to stabilize a certain phase. The purposefully design of block copolymers for directing and inhibiting precipitation of calcium carbonate has been explored by Cölfen more in depth.[28] The interplay of PAA in bulk solution and arachidic acid on the water surface on the growth of amorphous calcium

carbonate has been described using synchrotron X-ray experiments.[29] The use of self-assembling polymers for controlling biomineralization is undoubtedly a relevant strategy that will continue being explored with additional possibilities unfolding. Polymers can tune the precipitation rate of a given biomineral, and also can be used to the selective solubilization of relevant components. Block copolymers containing poly(ethylene glycol), peptide chains and cholesterol moiety, have been proposed for treatment of atherosclerosis plaques, which are composed mainly of calcium phosphate and cholesterol.[28,30] The block copolymers included anionic groups for Ca^{2+} binding and hydrophobic regions for a great performance as solubilizer of cholesterol. Therefore, this multifunctional approach for designing solubilizing polymers appears therefore quite promising.

There are a number of key experimental parameters that will likely differ when comparing bulk and interfacial biomineralization. For example, moluscan shells are a relevant model in biomineralization. Zlotnikov et al. reported on a thorough analysis of the structure and crystalline properties of the *Unio Pictorum* shell.[31] The directional solidification from a soft interface was demonstrated to induce an ordered and layered structure from an initial scenario of disordered packing in the biomaterial. We herein discuss the most relevant parameters that are taken into account when establishing an experimental design.

Temperature is a fundamental factor in biomineralization processes.[32] Bulk experiments can be performed under any value of temperature, provided suitable care on monitoring all physicochemical events. A fine control of the temperature of the subphase using a coupled thermostat to the Langmuir trough is easily incorporated by the Langmuir technique when considering the air/liquid interface. However, studies at the air/liquid interface under high values of temperature involve high evaporation rate values of the subphase, leading to non-reproducible composition of the subphase and interference of water vapor with the measuring instruments. There is no experimental difficulty in using low values of temperature, e. g., low temperature experiments that help stabilize amorphous calcium carbonate.[33]

Hydration is a key parameter that can be used to engineer biomimetic crystallization, as recently shown by Bertinetti et al. ACC might be regarded as storage of disordered mineral

matter, ready for crystallizing on demand by varying the hydration.[34] The effect of hydration in the structure of amorphous calcium carbonate and the role of hydrogen binding was studied by the mentioned authors, using an interesting combination of theoretical studies and experimental techniques using X-ray and neutron from synchrotron sources. Hydration of a phospholipid surface at the air/liquid interface can be quantitatively monitored by sum-frequency generation spectroscopy (SFG).[35] The hydration state of the phospholipid surface directing the biomineralization might influence the interaction with the inorganic matter and the phase of the formed crystal. Additionally, phase change can be induced by local changes in the hydration. Note that the highly relevant influence of hydration is present not only for phospholipids but for any given organic surface during biomineralization.

The biological conditions resemble a significantly higher complexity in terms of chemical composition and local heterogeneity in physical properties when compared to the lab conditions. Therefore, in vivo scenario in biomineralization displays a highly complex and dynamic biochemical composition, in which the different components leading the formation, growth, and crystalline phase shifting that usually varies along the entire process of biomineralization, see Figure 2.[36,37] Proteins play a relevant role on the biomineralization, with amino acid sequence and protein conformation as key parameters to be studied. While the structure of biogenic inorganic crystals is determined by the organic interface, the mineral can simultaneously template the protein structure. The concept of simultaneous two-ways templating is highly relevant on the current understanding of biomineralization. In this direction, Cölfen et al. reported on the role of intrinsically disordered proteins by including four short peptides responsible for the disordered domains in a remarkable publication.[38] The chemical composition of model organic interfaces is easily established when building a monolayer or similar model system.[39]

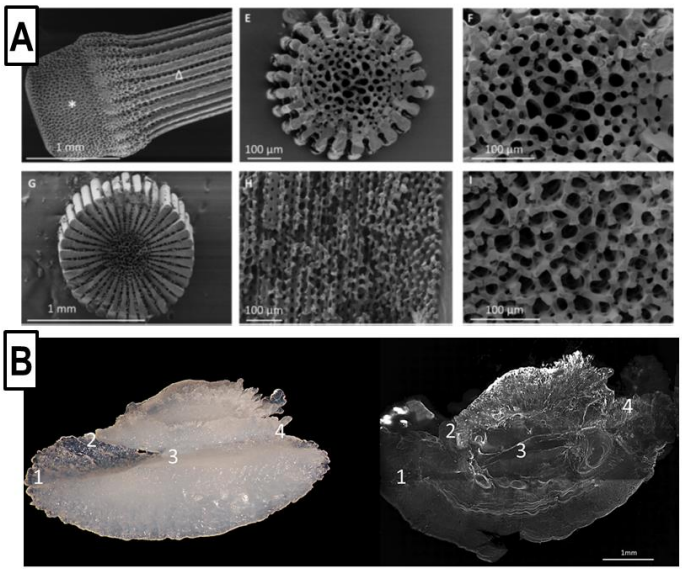

Figure 2. Complexity in biogenic minerals directed by biomolecules. A) Scanning electron microscopy picture sea urchin skeletal elements, illustrating the striking complexity of biogenic minerals attained by a directing organic surface.[36] B) Optical microscopy pictures of the protein fraction from a biogenic mineral from *Salmo salar*.[37]

The role of interfaces in biomineralization cannot be stressed enough. Wetting properties of a surface can greatly determine the kinetics of mineral crystallization in contact with the surface, as demonstrated by Koishi and Montes-Hernandez et al. for calcium carbonate.[40] In an elegant study, similar surface structures with different wetting values were tested for crystallization of calcium carbonate. In situ characterization by grazing incidence small angle X-ray scattering was included. The formation of ACC was found to be not influenced by wetting. On the other hand, wetting has a much more profound impact in the crystallization kinetics of the amorphous calcium carbonate layer. Slightly smaller particles of calcium carbonate were obtained for a hydrophilic surface when compared to a relatively hydrophobic surface. The crystallization kinetics was comparatively slower, probably due to an enhanced interaction between the surface and the amorphous layer. Indeed, the ACC layer was continuous on the surface only for the hydrophilic case. This study greatly illustrates the profound impact of interfaces in the complete process of biomineralization.

UNIQUE OPPORTUNITIES FOR BIOMINERALIZATION AT INTERFACES

Langmuir monolayers allow building a well-defined surface in terms of surface charge and chemical composition.[41] The possibility of tuning the molecular area per surfactant molecule additionally provides fine control on the surface pressure and the interacting organic surface for crystal formation and growth. Therefore, the influence of 2D pressure on the precipitation and crystallization of biogenic minerals can be studied in detail using Langmuir monolayers as model organic surfaces. Insights from the effect of bulk 3D pressure on such processes can be related to those observed at the organic/mineral interface.[42]

The biomineralization driven by proteins and short peptide fragments is being revealed as an exciting topic in grasping the actual biological mechanism for the directed formation of biogenic minerals. Small amphiphilic molecules as lipids and fatty acids as templates in surfaces for biomineralization are able to render interesting inorganic particles.[43] However, natural matrix proteins are required for obtaining biogenic materials. At least the most relevant peptide fragments should be present. In this direction, forming monolayers containing well-defined assemblies of peptides allows reaching the adequate level of structural complexity and the fine study of the peptide conformation in relation to the biomineralization at the peptide/solution interface. Understanding the relation of the peptide sequence to the mineralized phase of calcium carbonate is a relevant task, as well as extraordinary complex challenge. The mechanism and selective growth of a given phase of calcium carbonate remains poorly understood. Langmuir monolayers provide the possibility of probing the interface of sequence-chosen peptides with the inorganic ions, as demonstrated by Lu and Weidner et al.[44] The sequence of two model peptides was chosen to tune the hydrophobicity and the residence at the air/liquid interface. Langmuir monolayers of the peptides were prepared with subsequent injection of calcium and carbonate ions in the subphase. Highly detailed information in the coordination of Ca^{2+} ions to specific chemical groups of the peptide molecules was obtained by sum frequency generation spectroscopy (SFG). Conformational changes of the peptides could be also monitored by SFG. The idea of organic surface and mineral simultaneously inducing a templating effect on each other was also referred in such study, with conformation of the

peptides appearing more relevant than any specific interaction with chemical groups.[45] This concept can be readily exported to other biominerals, e. g., the highly stable calcium oxalate that might offer insights in the formation of kidney stones. An alternative strategy for adjusting the hydrophobicity of short-sequence peptides to build Langmuir monolayers is the inclusion of hydrophobic groups such as the 9-Fluorenylmethyloxycarbonyl (Fmoc) group.[46]

Langmuir monolayers can be designed for directed assembly of inorganic particles mediated by organic matter.[47] A purposefully designed Langmuir monolayer formed by an amphiphilic porphyrinoid surfactant was used for building Janus-like thin films of calcium carbonate.[48] While the face in contact with the subphase showed a significant roughness, a smooth side was found in touch with the monolayer. Thus, the possibility of adjusting the roughness at the micro- and nano-scale of an inorganic surface could be provided using the directed biomineralization by a Langmuir monolayer. As opposed to synthesis of mineral nanoparticles, engineered biomineralization of continuous thin films is also an interesting approach provided by an interface. Expanding this concept on a relevant study, Groves et al. achieved large thin films of apatite using a Langmuir monolayer of stearic acid that formed a negatively charged surface, see Figure 3.[49] Modest chemical modifications on the polar headgroup of the surfactants might have a profound impact on the obtained crystals when comparing Langmuir monolayers.[50] A suitable Langmuir monolayer can provide local supersaturation of ions while bulk subphase remains below the saturation limit. In this way, biomineralization processes can be studied at the air/liquid interface in great detail.[51]

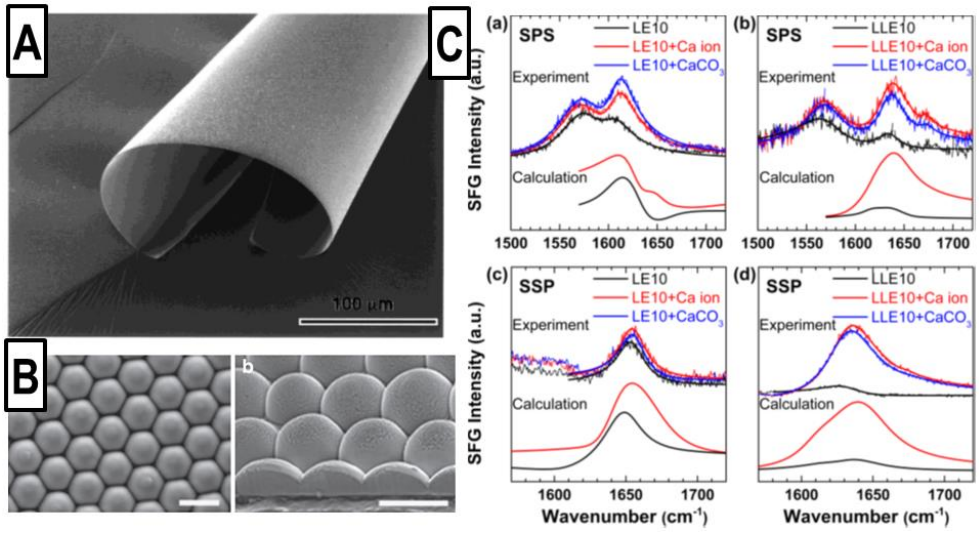

Figure 3. Fluid interfaces offer exceptional opportunities for biomineralization. A) Scanning electron microscopy picture of a calcium phosphate thin film templated by a Langmuir monolayer of stearic acid.[49] B) Microlens array of calcium carbonate particles formed at the air/liquid interface covered by a monolayer of Tween 20. [55] C) Experimental and calculated spectra sum frequency generation spectra of Langmuir monolayers of model short peptides LE10 and LLE10 before and after mineralization of calcium carbonate at the monolayer interface. [44]

The Langmuir technique offers a unique opportunity to mimic the dynamic environment at the organic/mineral interface during in vivo biomineralization. As opposed to bulk experiments, the Langmuir trough allows a detailed change of the surface during the experiments by introducing and removing molecules, as well as performing chemical reactions at the air/liquid interface.[52] Note that even the chemical composition of the surface is not modified, the reversible application of the surface pressure provides a fine control over surface charge and surface concentration of interacting chemical groups that defines the crystallizing surface including surfactant, lipids, peptides and other relevant biomolecules.[53] The physicochemical situation at the interface with the phospholipid layer prior to precipitation is also relevant, given the local high concentration of ions.[54]

Biomineralization at the air/liquid interface from an initial scenario of surfactant and salt in bulk solution is also an interesting strategy as shown by Fratzl et al.[55] The strategy included the surfactant PS20 (Tween 20) in a calcium hydroxide solution. A short period of time of minutes was required for adsorption of the surfactant at the air/liquid interface to a surface pressure of 15 to 30 mN/m. Despite the formation of the calcium carbonate film took place on the water surface, the process could be followed in bulk by monitoring the pH value. Formation of carbonic acid and dissociation onto carbonate ions led to acidification of the bulk solution. A 2D array of ordered microlenses of calcium carbonate with a clear hexagonal patterning was achieved. No X-ray diffraction signal indicated the composition of the film by amorphous calcium carbonate. Confocal Raman spectroscopy was smartly applied: carbonate ions, water and organic components were semi-quantitatively analyzed from the interface plane using Z-analysis.

The Langmuir monolayers for biomineralization might include non-standard surfactants, as demonstrated by Volkmer et al. in a calixarene monolayer for growing calcite crystals.[56] The composition of the subphase can be adjusted to almost any chemical composition using aqueous media for Langmuir experiments. Moreover, the composition of the subphase can be tuned within the experiment by using injection and movable Langmuir trough. Inspired by the work of Simon and Rosseeva et al. in which a fusion of already formed inorganic particles by mineral bridges was demonstrated, we propose that planned adjustment of the subphase composition after creating mineral particles at a Langmuir monolayer might lead to directed bridging between the particles.[57] In this direction, oriented attachment is regarded as main phenomenon accounting for fusing and growth of nanoparticles.[58] Oriented attachment might also be studied in detail at the monolayer interface. The distance between the primary particles might be adjusted by varying the surface pressure of the Langmuir monolayer, leading to different values of surface charge and interparticle distance. Moreover, introducing different monolayers might help to elucidate which interactions are more relevant during oriented attachment. Detailed study of the organic/mineral interface can provide insights in the biomedical field, as shown by He and Fischbach et al. for skeletal metastases of breast cancer.[59] The interaction of tumor cells with hydroxyapatite surface was demonstrated to depend on the size and nanostructure of the crystals. Model membrane models for the tumor cells in combination with purposefully designed hydroxyapatite crystals might point at the relevant molecular interactions that ultimately lead to adhesion of tumor cells and metastases of breast cancer. In summary, the Langmuir technique provides a unique platform for studying biomineralization at an organic interface in high detail.

INTERFACIAL BIOMINERALIZATION FOR BIOMEDICAL APPLICATIONS

Despite the chemical composition of synthesized minerals can be coincident to those of biological origin, significant differences might exist concerning the microscopic structure and roughness between natural and lab-made minerals. Evidently, the synthesis conditions have a great influence on the resulting features of the biomineral. Purely chemical routes for

biomineralization might involve temperature and concentration conditions that are certainly far from the physiological scenario. Moreover, minerals from biological origin often display intricate shapes that are highly structured. This level of complexity is difficulty achieved in lab conditions even for the simplest cases. We hypothesize that directed biomineralization using purposefully designed fluid interfaces will make a significant contribution to fill this gap. Engineered formation of thin films of biomimetic minerals on the air/liquid interface opens the possibility for coating and functionalizing hard surfaces regardless the macroscopic shape, as discussed below.

Hybrids materials containing biogenic minerals and organic molecules with biological activity are highly interesting not only in basic research but also aimed at biomedical purposes. Colaco and Landoulsi et al. assessed the crystallization of calcium phosphate mediated by an enzyme, i. e., alkaline phosphatase.[60] The intermediates and dynamics of phase transformation were characterized by electron microscopy and X-ray diffraction, with light scattering for monitoring the kinetics of crystal growth. Crystals of calcium phosphate from purely chemical origin were compared with crystals grown mediated by the enzyme. The relevance of the surface tension for the kinetics effects on crystallization were thoroughly described.

The fate of biogenic minerals under in vivo environment is highly relevant not only in basic understanding but also from the biomedical perspective. Calcium phosphate in amorphous phase might crystallize to apatite and brushite in physiological conditions, leading to formation of kidney stones on calcium oxalate nuclei. A study on the inhibition of the transformation of brushite onto hydroxyapatite by the osteopontin proteins revealed a multistage process in which crystallization of amorphous calcium phosphate was inhibited by the proteins.[61] The mechanism of dissolution and phase transformation was discussed in terms of chemical potential and thermodynamics. A complete characterization by Atomic Force Microscopy and Raman spectroscopy pointed to aspartic acid residues forming complex with Ca^{2+} ions, which might be used as a future strategy in preventing and treating kidney stones and related diseases.

On an inverse strategy, the on-demand formation of calcium carbonate and related biominerals might lead to new avenues for bone regeneration. Nano- and micro-structuring would be required for adequate vascularization and connection with tissue. Water-in-oil microemulsions formed by micelles containing either Ca^{2+} or CO_3^{2-} ions were studied for micelle disruption upon a chemical stimulus, i.e., addition of ethanol.[62] The rupture of the micelles was required for obtaining precipitate of calcium carbonate, whereas fussion of the micelles led to globular objects including supersaturated solution with no solid calcium carbonate. Using a more biological approach, Wei and Zhang et al. used extracellular vesicles from bone-marrow derived mensechymal stem cells to obtain a biomimetic extracellular matrix with outstanding results in bone regeneration.[63]

Nanostructured calcium carbonate nanoparticles are highly suitable vehicles for imaging and nanotherapy due to the excellent biocompatibility and chemical flexibility providing hollow or porous particles.[64] Vaterite as a thermodynamically unstable and relatively water-soluble phase of calcium carbonate appears as a good candidate for drug delivery vehicles.[65–67] Hybrids materials are also an interesting possibility, as proposed by Sahai et al.[68] Hydroxyapatite nanoparticles were doped with silver nanoparticles. The combination with other functional nanostructures opens interesting possibilities for prosthetics, given the relevance of hydroxyapatite as orthopedic material. Protein corona, biocompatibility and antimicrobial features were extensively studied for such material. The acidic environment of the tumor cells combined with the fast degradation of calcium carbonate offers an interesting opportunity for the targeting of such cells. Dong and Liu et al. reported on hollow calcium carbonate-polydopamine nanoparticles containing the photosensitizer chlorine e6.[69] Such composite hollow nanoparticles were successfully applied against a model animal tumor. The key ideas that greatly illustrate the potential of directed synthesis of mineral nanoparticles were the fundamental role of dopamine in coordinating Ca^{2+} ions and the controlled release. Dopamine coordinated to the Ca^{2+} ions enabled the growth of the nanoparticles. The acidic pH led to decomposition of the polydopamine and dissolution of calcium carbonate for on-demand release at tumor cells, see Figure 4. The proposed nanoparticles could be coated by any ligand using

non-covalent interactions, as phospholipids.[70] Calcium carbonate can be alternatively used as a coating rather than as constituent material for the particles, as reported by Wan and Tang et al.[71] The coating of calcium carbonate offers a programmed release due to its dissolution in the slightly acidic environment in tumor cells, preventing leakage of the cargo. In this case, the loaded particle was based on metal-organic framework architecture composed of iron cation and porphyrin, with the anticancer drug dihydroartemisinin as cargo.[72] Taking advantage of the selective dissolution of biogenic minerals under acidic pH opens a strategy that might be combined with different nanostructures. This sort of approach will most likely be a relevant alternative to metallic nanoparticles that still cast doubts on biocompatibility.[73] Basing on these concepts, Chang and Ma et al. engineered extracellular yeast cells producing biogenic nanosilica within the living cells. By including doxorubicin in coordination with the nanosilica, the extracellular yeast cells were used as containers for drug delivery.[74]

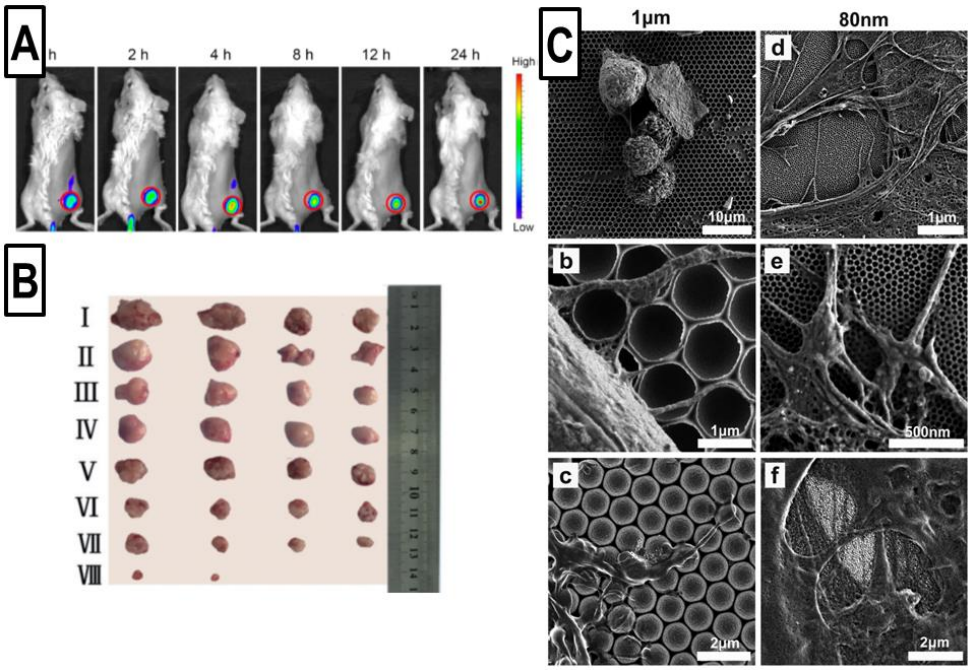

Figure 4. Biomineral-based nanostructures for diverse applications in Biomedicine. A) In vivo fluorescence imaging of 4T1 tumor-bearing mice intravenously injected with nanocomposites based on calcium carbonate for delivery of the Ce6 photosensitizer. The fluorescence signal is produced by Ce6.[69] B) Optical pictures of dissected tumors demonstrating the superior performance of the biomineral-based nanocomposites.[71] C) Scanning electron microscopy pictures showing the morphology of ST2 cells on patterned substrates. Concave and convex surfaces can determine cell adhesion and migration.[75]

As commented above, biomineralization at interfaces can provide an adjustable roughness of the inorganic surface when applying the adequate experimental parameters. This possibility is highly relevant when considering the effect of the roughness of a surface for promoting cell growth and arrangement. The air/liquid interface provides an advanced platform for the fabrication of on-demand coatings for prosthetics and other applications of medical materials with any required shape. Using polystyrene particles and silica surface based on a sol-gel route, Vogel et al. achieved concave/convex architecture of silica surfaces, with the particle monolayer as sacrificial agent for sputtering.[75] When cells were seeded, large periodical topography led to reduced number of anchoring points and elongation of the cells. Therefore the interplay of cell-cell and cell-substrate interactions could be tuned by adjusting the topography.

EXPERIMENTAL TECHNIQUES FOR RESEARCH IN BIOMINERALIZATION AT INTERFACES

Unraveling the fine structure and hierarchical levels of biogenic minerals require a combination of experimental techniques probing the different scales of the samples.[76] Devoted experimental techniques can offer a high level of detail for model systems on researching the organic/inorganic interface at fluid interfaces. While the attained information might be comparatively simplified with the biological scenario, this model systems of study are able to provide highly valuable insights in the formation and evolution of biominerals.

Crystals in the micron scale at the organic interface on a Langmuir monolayer might be already observed by Brewster Angle Microscopy, providing information on the early stages of biomineralization.[77] Infrared spectroscopy has proven a valuable tool in direct and relatively easy identification of the different crystalline phases of biogenic minerals.[78] The Infrared spectroscopy can be applied in situ at the air/liquid interface using the InfraRed Reflection Absorption Spectroscopy mode.[79,80] This spectroscopy admits the polarization modulation, then named as PM-IRRAS.[81] Infrared spectroscopy can obviously be applied ex situ to bulk samples.[82]

Ex situ techniques provide complementary and often necessary information to that obtained at the air/liquid interface. The major concern is the modification of the sample, i. e., mechanical stress and chemical oxidation due to the transfer procedure to a solid support.[83] Indirect experimental proof of a safe transfer is usually provided by comparing in-situ and ex-situ spectroscopy data.[47,84]

Fine structure and texture of the inorganic particles can be readily observed by transmission (TEM) and scanning (SEM) electron microscopies.[48] Note the organic fraction of the sample would be difficulty observed and may be damaged by the electron beam radiation. An additional possibility when using electron microscopy is including energy dispersion X-ray spectroscopy (EDS) for detecting the presence of elements of interest and estimating the chemical composition of the sample. If required, the elemental composition of the mineral solid can be quantitatively studied by inductively coupled plasma analysis (ICP).[85] In this regard, Thermal Gravimetric Analysis (TGA) might be a relevant technique in quantifying the ratio of organic matter in the sample and the decomposition temperature.[86] TGA is also a relevant technique in assessing the phase change of an inorganic material between amorphous and crystalline phases.[87]

Raman spectroscopy, especially in the confocal microscopy mode can be a valuable tool in obtaining local composition and distribution of the main constituents as discussed above.[55] An additional remarkable study illustrating the potential of the Confocal Raman Microscopy was reported by Faivre et al., on the role of the divalent cations Ca^{2+} and Mg^{2+} mediating the assembly of proteins in mineral-free teeth. [88] In that case, the mineral was not required for achieving functional teeth from biological origin. The Raman data provided detailed insights on protein conformation and location over the complete teeth structure.

Table 1. Summary of the most relevant experimental techniques for the study of biomineralization processes at fluid interfaces.

Technique	Attained information	Reference
In situ Experimental Techniques		
Surface pressure-molecular area isotherms	Thermodynamic state of the organic layer. Presence and surface area of the inorganic matter at the interface	[48]
Surface potential-molecular area isotherms	Electrostatic properties of the organic/inorganic interface	[56]
Sum Frequency Generation spectroscopy	Ordering of organic molecules and water molecules at the organic/inorganic interface	[35]
Grazing Incidence X-ray diffraction (GIXD)	Crystalline phases of inorganic and organic matter: ordering of Langmuir monolayers	[80]
InfraRed Absorption Reflection Spectroscopy	Crystalline phases of inorganic matter. Mechanistic information on the interaction between the Langmuir monolayer and the inorganic material	[81]
Brewster Angle Microscopy (BAM)	Direct visualization of inorganic particles on the micrometer range. Assessment of domains in lipid and peptide molecules in the Langmuir monolayers	[89]
Ex situ Experimental Techniques		
Electron Microscopy	Imaging of the inorganic particles in the nanometer range. Diffraction patterning for assessing crystallinity	[48]
FTIR and Raman spectroscopy	Crystalline phases of inorganic matter. Identification of organic compounds interacting with the inorganic material. Local composition in microscopy mode	[55]
Inductively coupled plasma analysis (ICP)	Quantitative elemental composition	[85]
Thermal Gravimetric Analysis (TGA)	Phase change of crystalline and amorphous particles. Content of organic matter	[86]

CONCLUSIONS AND OUTLOOK

Biomineralization is a lively and expanding field of research. Basic understanding of the in vivo biomineralization processes is far from complete and constitutes a great challenge. Functional nanostructures based on biogenic materials are being explored for biomedical applications. The Colloid and Interface Science is undoubtedly contributing to a great extent to both approaches in biomineralization research. The Langmuir technique stands out as an advanced platform for obtaining unique insights in the organic/inorganic interface during

biomineralization. Information with molecular detail on the dynamics of the mutual influence between the organic surface and the mineral can be obtained using a Langmuir setup. In summary, fluid interfaces is a rich field for understanding and applying biogenic minerals and related structures.

Given Biomineralization is one of the most vibrating and challenging fields on this century, thus to anticipate the new directions of research remains quite speculative. Integration between the knowledge at the nano-, micro- and macro-scales into a coherent and holistic description will be a long-standing frontier. New medical treatments avoiding the use of surgery might appear, e.g., removal of harmful osteogenic material and growth of bone material with suitable microstructure for vascularization aimed at limb regeneration. On a global scale, the carbon dioxide uptake and the acidification of the ocean is balanced by the biomineralization of marine microorganisms: a global remediation for global warming might be designed based on biomineralization. Albeit challenging, the field of biomineralization will surely continue being a rich source of scientific answers and technological solutions of foremost relevance.

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FIGURE CAPTIONS

Figure 1. Representative examples of biomineralization. A) Striking colours from molluscan shells. Shells of the land snail Polymita picta from Cuba. Copyright from Cambridge Philosophical Society, 2016.[1] B) A single coccolithophore cell mastering the art of biomineralization. Specimens of Emiliania huxleyi, type O (lamella). The scale bar indicates 1 µm. Creative Commons Attribution 4.0 License.[2] C) Bones as highly hierarchical biomaterials. From trabecula and osteons to a cross section through a proximal femur. Copyright from Elsevier, 2011.[4]

Figure 2. Complexity in biogenic minerals directed by biomolecules. A) Scanning electron microscopy picture sea urchin skeletal elements, illustrating the striking complexity of biogenic minerals attained by a directing organic surface.[36] B) Optical microscopy pictures of the protein fraction from a biogenic mineral from Salmo salar.[37]

Figure 3. Fluid interfaces offer exceptional opportunities for biomineralization. A) Scanning electron microscopy picture of a calcium phosphate thin film templated by a Langmuir monolayer of stearic acid.[49] B) Microlens array of calcium carbonate particles formed at the air/liquid interface covered by a monolayer of Tween 20. [55] C) Experimental and calculated spectra sum frequency generation spectra of Langmuir monolayers of model short peptides LE10 and LLE10 before and after mineralization of calcium carbonate at the monolayer interface. [44]

Figure 4. Biomineral-based nanostructures for diverse applications in Biomedicine. A) In vivo fluorescence imaging of 4T1 tumor-bearing mice intravenously injected with nanocomposites based on calcium carbonate for delivery of the Ce6 photosensitizer. The fluorescence signal is produced by Ce6.[69] B) Optical pictures of dissected tumors demonstrating the superior performance of the biomineral-based nanocomposites.[71] C) Scanning electron microscopy pictures showing the morphology of ST2 cells on patterned substrates. Concave and convex surfaces can determine cell adhesion and migration.[75]

FIGURES

Figure 1

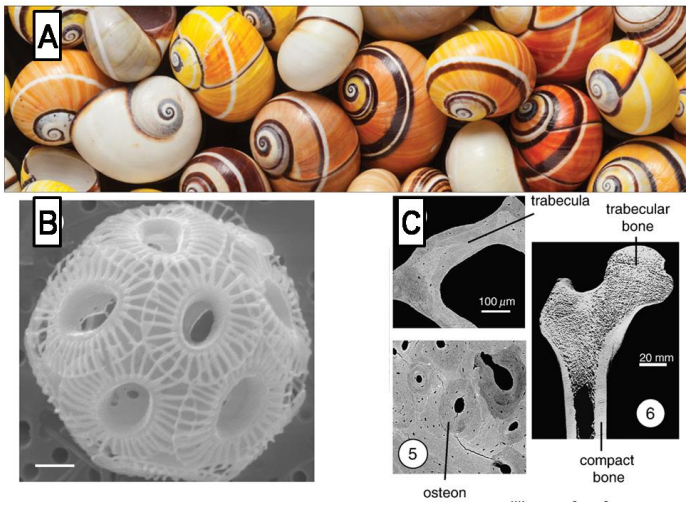


Figure 2

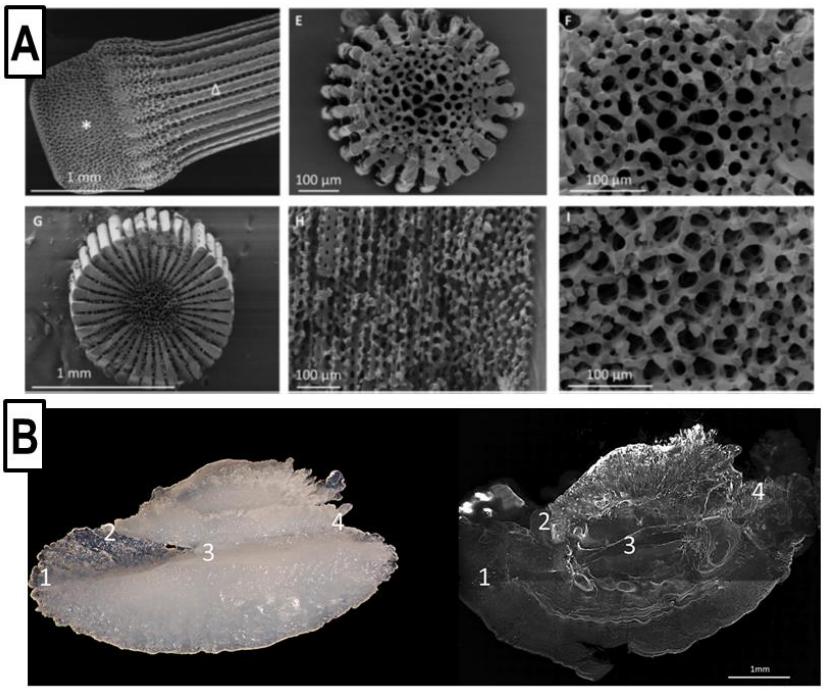


Figure 3

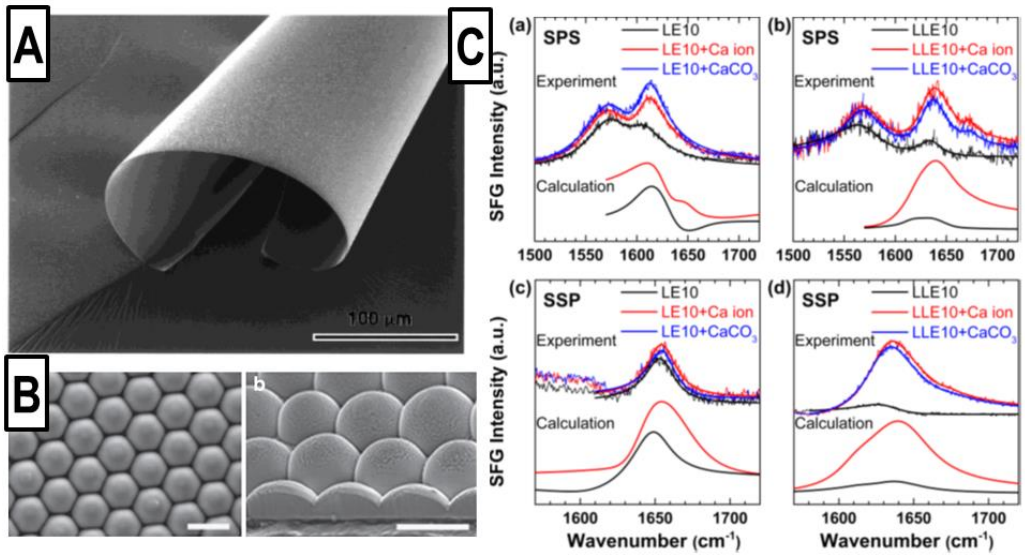
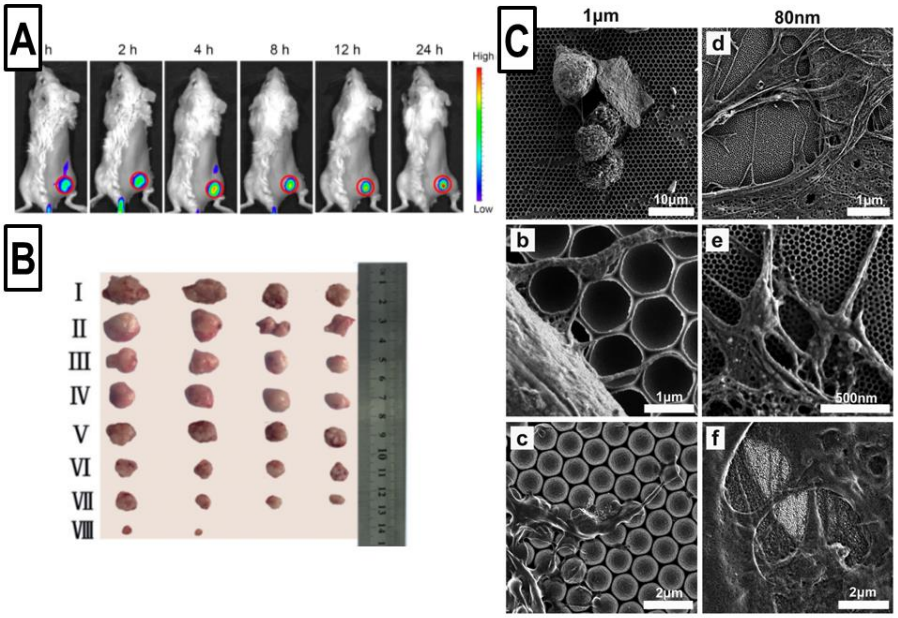


Figure 4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