



## Perspectives on applications of nanomaterials from shelled plankton

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### Abstract

Biom mineralization ubiquitously occurs in plankton, featuring hierarchically nanostructured shells that display several properties that benefit their host survival. Nanostructures' shapes and many of these properties are tunable through in vitro or in vivo modification of microorganisms, making their shells very appealing for applications in materials sciences. Despite the abundance of shell-forming species, research has focused mainly on diatoms and coccolithophores microalgae, with current scientific literature mostly targeting the development of photonic, biomedical and energy storage/conversion devices. This prospective article aims to critically overview potentialities of nanomaterials from biomineralizing plankton, possible outcomes and technological impact relevant to this technology.

### Introduction

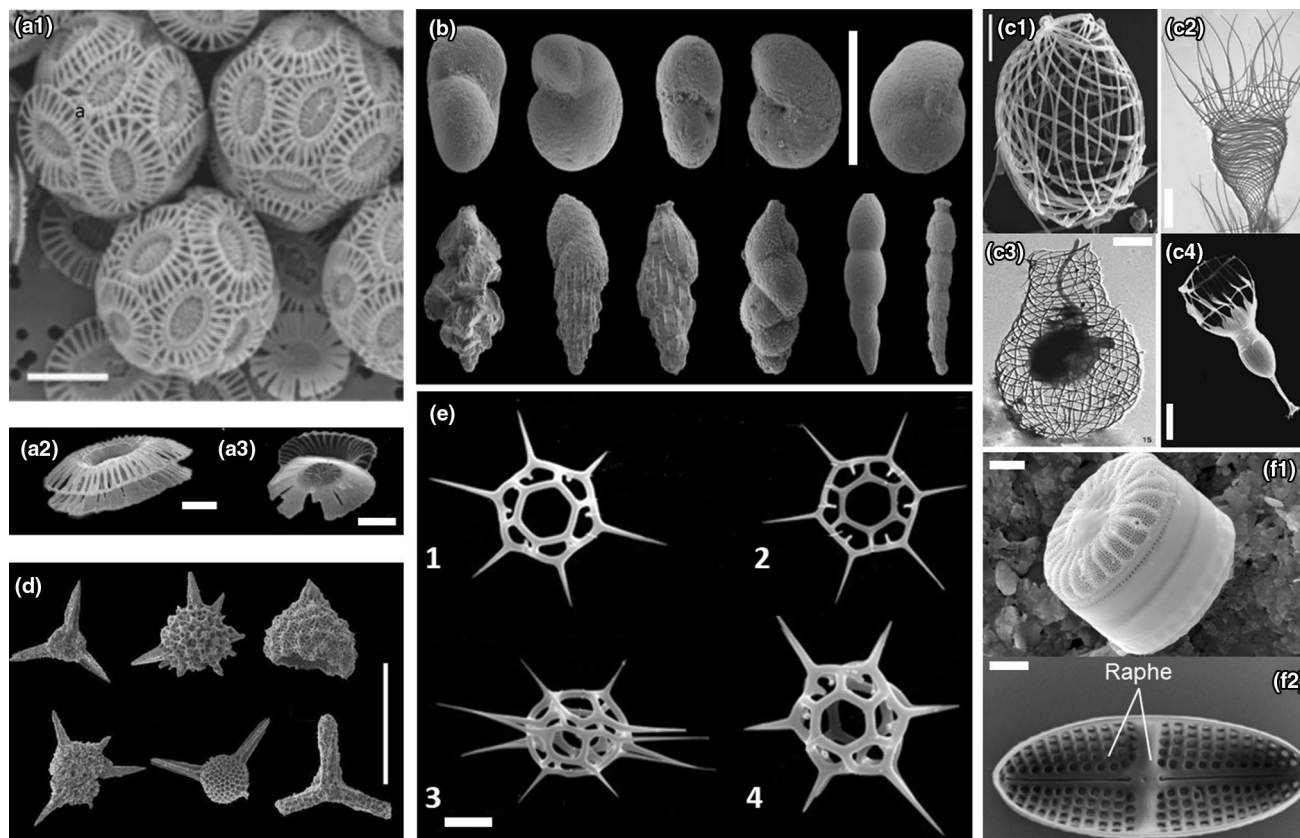
Plankton is a general term that refers to microscopic organisms living in watery environments, both salty and fresh. This word encloses many taxonomic groups since also bacteria, protists and single-celled plants belong to plankton. Phytoplankton biosynthesizes chlorophyll and uses it to capture sunlight, and photosynthesis is the main process standing for converting sunlight into chemical energy. While phytoplankton consumes carbon dioxide, mainly releasing oxygen, zooplankton gets energy from organic compounds found in other organisms. In this wide group, it is common to find examples of shelled microorganisms capable to biomineralize inorganic salts producing endo- or exoskeletons that perform many functions useful for cell survival. The diversity between skeletons formed by these organisms comes at different levels: chemical composition, microarchitecture and nanoarchitecture. Regarding the chemical composition, there are two major forms of biomineralization in marine planktons: silicification and calcification.<sup>[1]</sup>

Some examples of silicifying organisms include *Choanoflagellates*,<sup>[2]</sup> *Radiolarians*,<sup>[3]</sup> *Silicoflagellates*<sup>[4]</sup> and *diatoms* [Fig. 1(c–f), respectively]. All these organisms are able to produce shells with specific silica microarchitectures with different sizes ranging from 3  $\mu\text{m}$  (choanoflagellates) to 10–150  $\mu\text{m}$  (diatoms) till 2 mm (radiolaria). Some representatives of the calcification process reported in the materials science literature are *Coccolithophores* [Fig. 1(a<sub>1</sub>)] and *Foraminifera* [Fig. 1(b)].

Figure 1 shows some examples of different microarchitecture shapes formed by marine microorganisms.

Coccolithophores are small (0.25–30  $\mu\text{m}$ ) algae forming a calcareous spherical body<sup>[5]</sup> (coccosphere) composed of several individual disk-like plates [Fig. 1(a<sub>1</sub>–a<sub>3</sub>)] which usually breaks up into its individual extracellular coccoliths (CaCO<sub>3</sub> platelets), upon the death of the organisms.<sup>[6]</sup> Foraminifera [Fig. 1(b)] produces an external shell (called test) ranging from 250 to 1000  $\mu\text{m}$  in length and is organized in different chambers connected to each other by a series of holes, called foramen.<sup>[7]</sup> Choanoflagellates [Fig. 1(c<sub>1</sub>–c<sub>4</sub>)] fabricate a basket-shaped shell (lorica) of about 3–10  $\mu\text{m}$ . Silicoflagellates (Fig. 1(e<sub>1</sub>–e<sub>4</sub>)) and radiolarians [Fig. 1(d)] produce a similar shape composed of a network of bars, spines and spicules, needle-like pseudopods and nano-lattices<sup>[8,9]</sup> but display different sizes (20–100  $\mu\text{m}$ ) for the former and 100  $\mu\text{m}$  to very large species up to 2 mm<sup>[10]</sup> for the latter. Last, but not least, diatoms are a vast group of microalgae, comprising over 100,000 single-celled species. They produce siliceous structures sizing 10 to 200  $\mu\text{m}$ , (frustules) which are highly reproducible within the same species and each species is characterized by a distinct architecture of the silica cell walls with a highly ordered nano-/micropore structure and pattern. Frustules exhibit a high hierarchical level of mesoporosity. As general rule, silica shells from centric diatoms contain 2 or 3 mesoscaled pores ranging from a hundred to 2–5 nm, while pennates possess ornated nano-clasps, nanoroughness or indentations which start to spread from the raphe nuclei.

All the mentioned species display also a third level of diversity that can be found in the nanofeatures (pores, spicules, lattices, areolae, papillae, keels and spines) that ornate all the produced shells. All these features have evolved to improve the



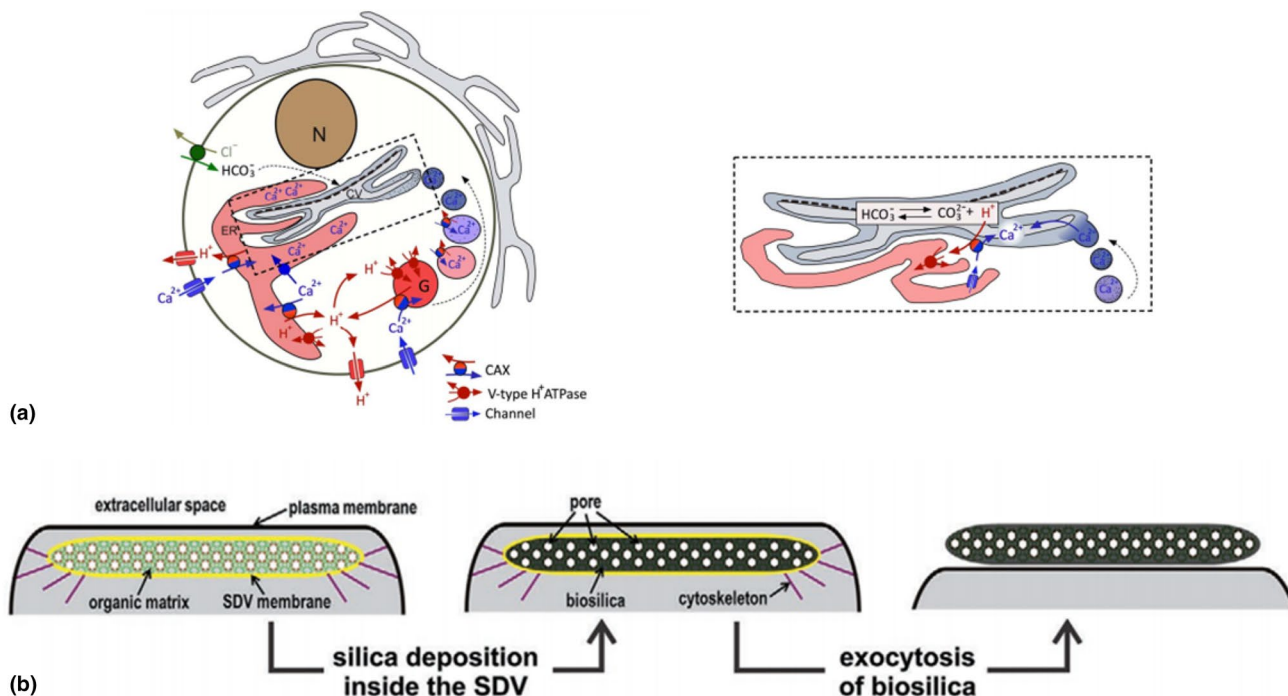
**Figure 1.** Examples of shelled forming planktons. (a<sub>1</sub>) Calcite coccoliths of *Emiliana huxleyi* (bar 2  $\mu\text{m}$ ) with permission of Ref. 12. (a<sub>2</sub>, a<sub>3</sub>) Isolated coccoliths in different views (bar 1  $\mu\text{m}$ ) with permission of Ref. 13. (b) Examples of foraminifera shells (bar 100  $\mu\text{m}$ ) with permission of Ref. 14. (c<sub>1</sub>) SEM micrographs of choanoflagellate *Savillea micropora* showing the construction of the lorica, and the helical and outer layer of longitudinal costae (bar 1  $\mu\text{m}$ ). (c<sub>2</sub>) TEM of lorica of *Acanthoea spectabilis* showing costae comprising the lorica chamber, and the individual helical costae with the costal strips with spines (bar 2  $\mu\text{m}$ ). (c<sub>3</sub>) *Savillea parva* cell forming the lorica (bar 2  $\mu\text{m}$ ). (c<sub>4</sub>) *Diplothea elongata* entire specimen (bars 5  $\mu\text{m}$ ). Picture (c) is reproduced with permission of Refs. 15–17. (d) Shells of radiolarians sp. (bar 100  $\mu\text{m}$ ) with permission of Ref. 18. (e<sub>1–4</sub>) SEM photographs of *Distephanus speculum* from apical, abapical, lateral and apical axis view (bar 10  $\mu\text{m}$ ), with permission of Ref. 19. (f<sub>1</sub>) *Cyclotella* sp. and (f<sub>2</sub>) *Achnanthes subsessilis* as a representative of centrate and pennate diatom cell walls (scale bar 2 and 1  $\mu\text{m}$  with permission of Refs. 20 and 21).

adaptability offered by the shell, helping the host in the defense from viruses, improve mechanical properties,<sup>[11]</sup> leading to buoyancy and confer specific photonic abilities (discussed in “[Photonic and electronic applications](#)”).

Unfortunately, besides the abundance of hierarchically organized structures retrievable in nature, not all these organisms are equally represented in the literature that has shown a preference toward diatoms and coccolithophores. There are just a few dated studies describing the nanofeatures of radiolarian<sup>[8]</sup> and silicoflagellates<sup>[9]</sup> but without any discussion about their possible function. For all these reasons, the current perspective work aims to create a natural relation between the different morphologies of the shells of marine microorganisms and their applications in photonics, electronics and biomedicine, aspects often highlighted in reviews on diatoms, less so on coccolithophores and other biomineralizing microorganisms.

## Biomaterialization process

The range of control over microfabrication employed by the microorganisms is beyond what can be achieved with contemporary laboratory and industrial methods and it is of great interest for materials design, offering new ways to develop hierarchical micro/nanostructures with enhanced functionality. Nanoscale biomineralization involves the molecular construction of self-assembled organic supramolecular systems (vesicles, charged peptides networks and so on) that are used as pre-organized environments for controlling the formation of finely divided inorganic materials, 1–100 nm in size. The fabrication of fully structured shells also involves the construction of templating organic frameworks, but the length scale is greater (micrometers) and the matrix is often polymeric.<sup>[22]</sup> The biomineralization process is appealing also for developing new bioinspired synthetic procedures for nanomaterials and has been characterized for diatoms and coccolithophores.



**Figure 2.** Scheme of biomineralization process in cocolithophores and diatoms. (a) The intracellular nucleation of calcite crystals on an organic scaffold within Golgi-derived vesicles in cocolithophores, and the formation of the endomembranes<sup>[24]</sup> as processes related to  $\text{Ca}^{2+}$  and  $\text{H}^+$  flux inside cells, adapted with permission.<sup>[26]</sup> (b) Biomineralization process in the diatom *Thalassiosira pseudonana*: left, Intracellular silica deposition vesicle (SDV) (silicalemma in yellow) kept by the cytoskeleton (purple); middle, generation of porosity in the precipitated silica with the organic matrix trapped inside; right: exocytosis of the cell wall. Adapted with permission.<sup>[27]</sup>

Biosilicification in diatoms [Fig. 2(b)] starts from sodium metasilicate uptake from waterish sources; the further conversion of this inorganic silicate into organic compounds is orchestrated by several classes of specific biomolecules, such as positively charged peptides, polyamines and saccharides associated with silicalemma. Silaffin, a complex post-translationally modified peptide, and the membrane protein silicanin-1, which is highly conserved throughout all diatom species, play both important roles in biosilica deposition. Overall, the ensemble of biomolecules involved in the process yields the biomineral properties of the formed frustule.<sup>[23]</sup> After the assembly of the organic scaffold, silica vesicle precursors appear inside cells and generate new valves that can be composed and extruded, shaping to the classical form of a Petri dish-like structure characteristic of frustules. According to the polymerization orientation of biosilica around nuclei or raphe, and the related frustule shape, diatoms can be classified in *centrales* and *pennates* [Fig. 1(f<sub>1</sub>, f<sub>2</sub>)].

Biocalcification in cocolithophores starts with intercellular nucleation of calcite crystals in Golgi-derived vesicles that, on maturation, are released and molded on the cell surface via exocytosis.<sup>[24]</sup> This process is finely controlled by the calcium and proton uptake and trafficking inside cells [Fig. 2(a)]. Considering the model cocolithophore organism *E. huxleyi*, the diameter of its coccosphere is around 5.5  $\mu\text{m}$  and is formed from one single interlocking layer comprising 15 averaged

cocoliths, with additional formed ones easily detaching from the coccosphere.<sup>[25]</sup> More generally, a fully developed *E. huxleyi* cocolith plate is characterized by an oval central area surrounded by almost parallel outer and inner shields containing angled ridges.

### Applications in materials science

The production of inorganic skeletons represents an undebatable evolutionary advantage for survival since most microorganisms have evolved and perfected the construction of their “house” in an independent way.<sup>[28]</sup> Indeed, biomineralized materials display several properties useful for the defense of the host microorganism or photosynthesis, since their porosity actively enhances solar energy harvesting efficiency<sup>[29]</sup> and their physical resistance to stress or cut is remarkably high if compared to synthetic materials with the same chemical composition.

It follows that they have attracted great interest in materials science both for bio-driven production of nanostructured materials or for the development of new synthetic techniques inspired by the biomineralization process. Both cases offer new possibilities to overcome some issues related to most traditional approaches employed in the synthesis of nanoscale materials such as energy inefficiency, the requirement of stringent synthesis conditions (e.g., high temperature,

pressure, pH), low yields, production of toxic byproducts and irreproducibility because of the difficulty of control of agglomeration.<sup>[30]</sup> Moreover, there are additional advantages to employing microorganisms for the synthesis of nanostructured materials, compared to synthetic approaches, since morphology and size of produced nanostructured materials may be controlled through genetic or culture media modifications, responding to different purposes. Furthermore, microorganisms need only salts dissolved in aqueous media, often sunlight and mild temperature and pressure conditions to produce their nanostructures, instead of the complex and sometimes environmentally unfriendly artificial processes for their production. Finally, due to the mild conditions required and the absence of toxic compounds, biominerals produced by microbes are in principle more amenable to be used in biomedical applications because of their biocompatibility. In this context, marine microorganisms seem the best candidates for improving the general health of marine ecosystems. Microalgae, marine plants and bacteria show enhanced physiological and biochemical adaptations to some specific physical and chemical conditions (*e.g.*, alkaline pH, salty composition) of the ocean environment, concerning the other terrestrial microorganisms. Also, their peculiar metabolisms, like biomineralization is, closely couple with sun irradiation, carbon dioxide depletion and toxic chemicals reduction. This paves their use as bioremediating living agents or as ecological sensors.

Due to their diversity, abundance and ability to produce complex nanostructured morphologies, these organisms are all in principle exploitable for the production of new generation materials for manifold applications. However, there are no reports on materials derived from radiolaria or silicoflagellates, while foraminifera-derived materials investigation is described only in a few articles. The last microorganisms have been studied to produce stable composites for the biomedical field as precursors to calcium phosphates<sup>[31]</sup> and delivery of bisphosphonate pamidronate and Gentamicin antibiotic.<sup>[32]</sup> On the contrary, diatoms<sup>[33]</sup> and coccolithophores<sup>[34]</sup> have been intensively investigated for the development of new strategies in nanotechnology and molecular assembly and, in general, for the development of new materials in different fields, with more than 1000 reports concerning material science on diatoms and around 100 for coccolithophores.

The concept of using marine microorganisms instead of industrial materials relies on the obtaining of similar material properties (high surface area, tunable pore size at the nanometer range, adaptable surface chemistry, biocompatibility) over several and simple biochemical processes that do not use solvents, toxic chemicals and high temperature, and exploit the only salty environment and water/ions equilibria. From an applicative point of view, biomimicry can be seen as a complex concept with 2 two principal objectives: (i) using synthetic methods to reproduce the ingenious architectures by microorganisms using the biological structures as a source of inspiration; (ii)

exploiting the biomineralizing organisms as biofactories in a biotechnological fashion.

Considering the first approach, shell-forming plankton organisms are used just as a form of inspiration<sup>[35]</sup> for the construction of well-defined hierarchical organizations, and many research groups have reported the synthesis of a great variety of highly organized bioinspired multiscale microstructures.<sup>[36,37]</sup> Furthermore, these metamaterials can attain exceptionally high strength that may offer a new class of damage-tolerant lightweight engineering materials.<sup>[38]</sup>

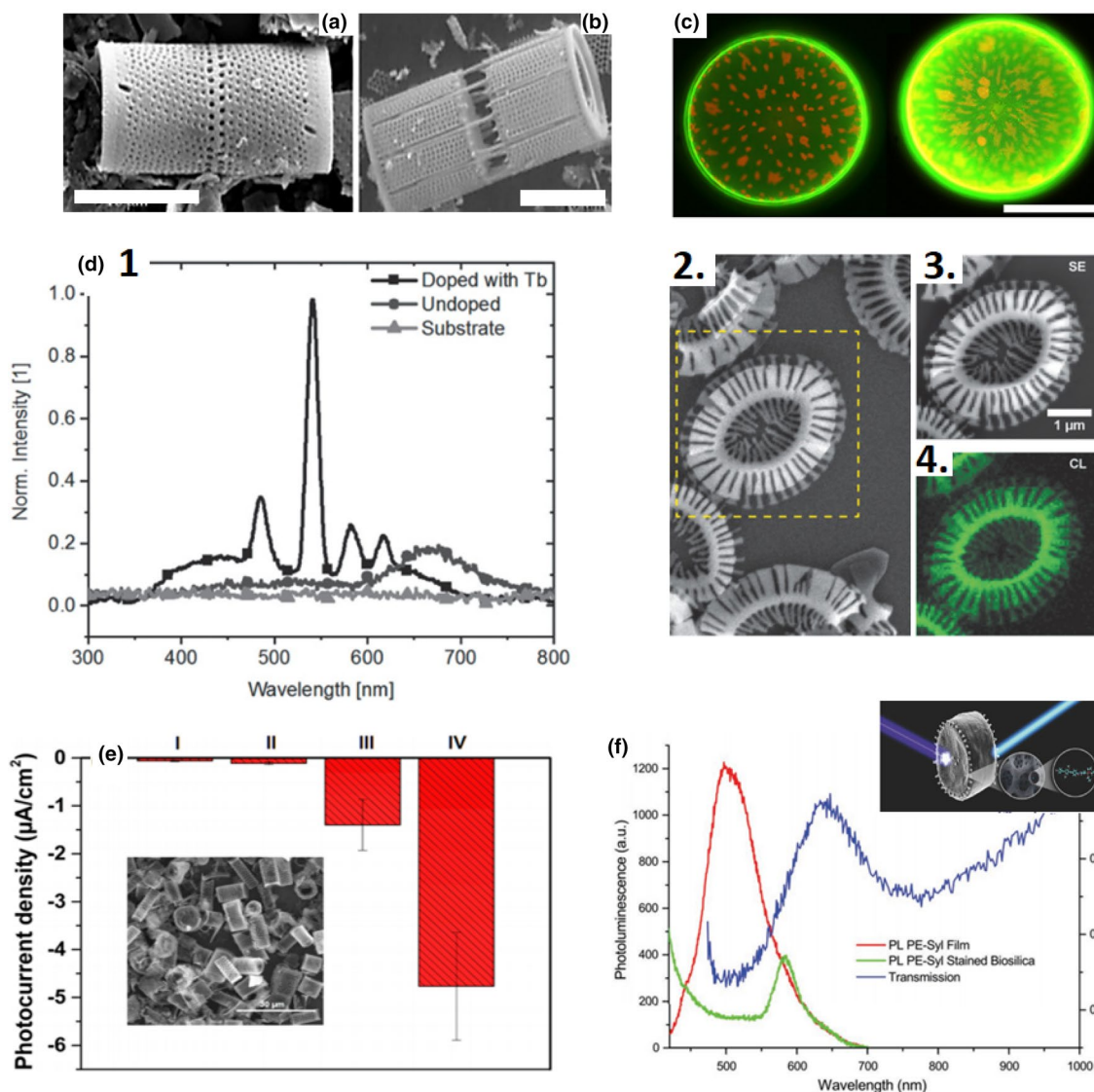
Looking at the second approach, the nanostructured material is usually extracted directly from the organism and subsequently modified by a variety of possible functionalization strategies reported so far, including amino-silylation,<sup>[39]</sup> thiol-silylation,<sup>[40]</sup> drugs<sup>[41]</sup> [Fig. 5(h)], antibodies,<sup>[42]</sup> enzymes,<sup>[43]</sup> DNA,<sup>[44]</sup> or coating with polymers.<sup>[45]</sup> Noteworthy, starting from the extracted nanostructure, it is even possible to completely change the inorganic matrix unaltering the bulk architecture as reported by Bao *et al.*<sup>[46]</sup> who converted 3D nanostructured silica diatom micro-assemblies into nanocrystalline silicon or silicon/magnesia composites through a bioclastic conversion at low temperature, deeply extending their possible applications in electronics. Figure 3(a, b) shows examples of cylindrical shells extracted from *Aulacoseira* spp. Diatom species suitable for the material conversion.

Taking advantage of the evolution of these organisms and their building ability, many noticeable procedures have been reported involving modifications of shape or composition of nanostructures carried out by the microorganism itself, achieved through genetic modifications or simple optimization to the culture medium. More specifically, it is possible to hack the biomineralizing process that both in coccolithophores and diatoms<sup>[47]</sup> relies on an organic matrix scaffold, by introducing target molecules or metal ions thus offering the possibility to introduce both inorganic<sup>[48]</sup> and organic compounds<sup>[49,50]</sup> inside the nanostructured matrix. Adopting this strategy, it is even possible to select the desired pore size simply by adding dopants to cultures thus affecting their optical properties.<sup>[51]</sup>

Given all potentialities provided by these organisms in developing advanced materials, here we overview the most recent impactful achievements reported so far, outlining the field of application in which diatoms and coccolithophores are addressed, respectively, and predicting possible future for this technology, but also we want to point out a general carelessness for studies on applications about all biomineralizing organisms (not only the over-cited diatoms and coccolithophores), hence encouraging the scientific community to fill this gap.

## Photonic and electronic applications

As for other photosynthetic organisms, shell-forming phytoplankton has adapted their elaborate nanostructures for photonic modulation of light<sup>[52]</sup> achieving controllable absorption, reflection or transmission of the desired wavelength of the solar spectrum in response to environmental changes and



**Figure 3.** Plankton-based photonic and electronic materials. (a, b) SEM images showing silica diatom frustule examples extracted from *Aulacoseira* spp. with permission. Bar: 10 µm<sup>[74]</sup>; (c) fluorescence microscopy image of luminescent stained biosilica in living diatoms, with permission. Bar: 30 µm<sup>[75]</sup>; (d<sub>1-4</sub>) cathodoluminescence emission of doped coccoliths and SEM image of Tb<sup>3+</sup>-doped coccoliths, with permission of Ref. 66. (e) Photocurrent density measurements for boron-doped silicofructules at 0 V (I), with InP nanocrystals and catalyst at 0 V (II) and -0.5 V (III), and in measured in 0.1 M H<sub>2</sub>SO<sub>4</sub> electrolyte (IV). The inset shows the boron-doped silicon diatom frustules on gold, with permission of Ref. 68; (f) emission spectra of phenyleneethynylene (PE) dye (red) and PE-Syl stained biosilica (light green), together with characteristic transmission spectra of the valve center (blue). Inset: modulation of light transmission in a *Thalassiosira w.* valve, with permission.<sup>[62]</sup>

stimulations.<sup>[53]</sup> For example, the transmission of incident light through diatom frustule nanostructure has been demonstrated to be wavelength dependent, being more pronounced for red light rather than blue and green wavelengths.<sup>[54]</sup> This feature helps diatom in reflecting noxious UV and blue wavelengths (< 410 nm), thus protecting the inner protoplasm. Such protection can be found also in *holococcoliths* (i.e., haploid phase of many coccolithophores) from *Calcidiscus leptoporus* and *Helicosphaera carteri*, which display a periodic structure of calcite crystallites able to efficiently reflect UV light, thus expanding their survival space.<sup>[55]</sup> Moreover, it was found that coccoliths

exhibit structural color and, interestingly, this feature is affected by strong magnetic fields,<sup>[56]</sup> displaying a light-scattering anisotropy that reduces or enhances light penetration in the cell depending on the angle of the incident light.<sup>[57]</sup>

Diatoms are even more specialized than coccolithophores in photonic control by silica structures, and hence diatom bare frustules have been investigated as natural micro-lenses, optical filters, polarizers, waveguides,<sup>[58]</sup> beam splitters, couplers, laser cavities and angle-independent near-infrared reflectors.<sup>[59]</sup> Among diatom species, *Coscinodiscus* has been extensively investigated for photonic properties related to its frustule

nanostructure featured with three layers (*cribellum*, *cribrum*, internal plate) hierarchically arranged in hexagonal holes. This architecture confers diatoms a light-trapping ability generated by the constructive interference caused by the multiple scattering layers.<sup>[60]</sup> Furthermore, the organization with a regular disposition of pores in superimposed layers, in a hierarchically ordered periodic pattern of high and low refractive index, makes diatoms living photonic crystals,<sup>[61]</sup> a feature exploitable not only in light-trapping application, but also to develop photonic materials with photoluminescence tunability properties [Fig. 3(f)].<sup>[62]</sup> The utilization of silica shells as light traps is highly desirable and may be employed for large-scale fabrication of naturally designed nanostructured layers for photovoltaic devices. Following this concept, after shell extraction, together with characterization and functionalization, processes of biosilica integration into simple devices have been proposed in the literature to produce green energy. Sun et al. exploited diatom frustules to generate a solar harvesting layer on the surface of a light-absorbing (PTB7:PC71BM) layer of thin-film solar cells, thus achieving enhanced light absorption simply taking advantage of the silica nanostructures.<sup>[60]</sup> On this ground, Raston and co-workers coated diatoms with TiO<sub>2</sub> nanoparticles to produce biosilica-based photoanodes, reporting the enhancement in solar energy conversion efficiency up to 30%.<sup>[63]</sup> The technology to reach low-cost, tunable, hierarchically structured and nano-patterned inorganic materials could greatly empower the way we fabricate devices for photovoltaic,<sup>[64]</sup> optoelectronic, electroluminescent displays,<sup>[65]</sup> cathodoluminescent materials<sup>[66]</sup> [Fig. 3(d)], energy storage, and production. However, these application fields often require some conductivity or semi-conductivity properties of the materials. These features can be introduced into diatom frustules as their composition can be modified with metal oxides such as titanium or germanium dioxide.<sup>[67]</sup> Voelcker demonstrated a magnesiothermic conversion of boron-doped silica diatom frustules into nanostructured silicon that was used as a photocathode for water splitting leading to a significant photocurrent density [Fig. 3(e)].<sup>[68]</sup> Alternative ways for energy production from modified biosilica include diatom-inspired hierarchical carbon nitride array proposed by Liu et al. for photocatalytic mediated enzymatic reduction of formaldehyde to methanol and H<sub>2</sub> generation,<sup>[69]</sup> as well as Ag/diatomite for solar steam generation devices, with improved performances due to the combination of Localized Surface Plasmon Resonance (LSPR) effect of metal particles with the light-trapping effects of diatomite.<sup>[70]</sup> Many of these new fabrication strategies for energy-producing devices have already been patented<sup>[71,72]</sup> including the use of nanostructures on silicon-based solar cells to increase surface area.<sup>[73]</sup>

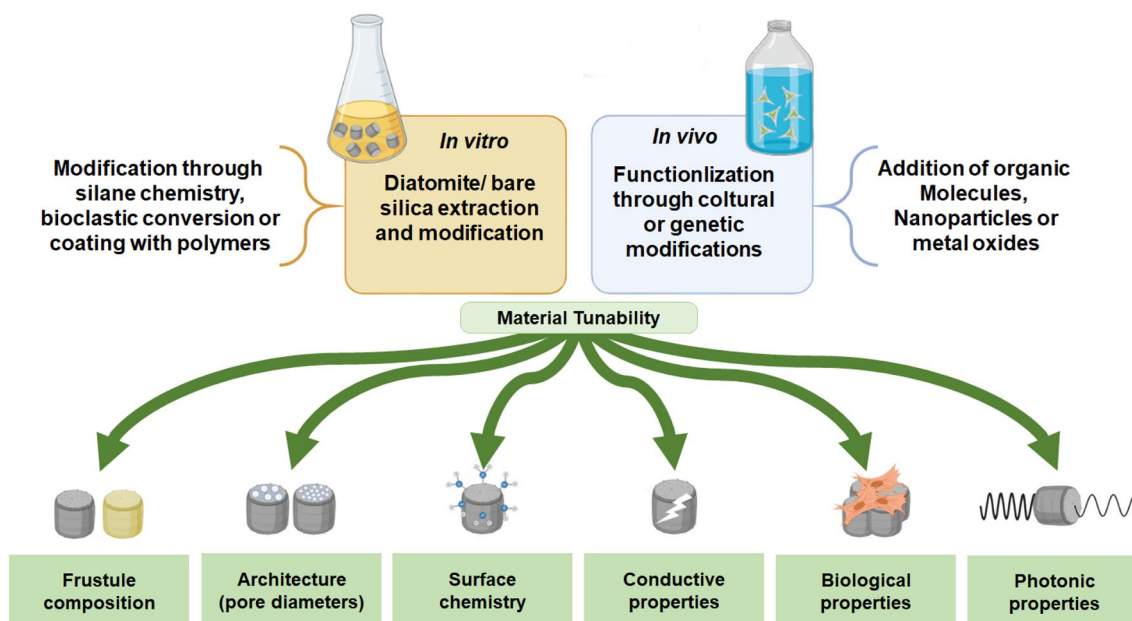
## Biomedical applications

The most extensively investigated biomineralized materials for medical applications are mesoporous silica nanoparticles<sup>[76]</sup> and nanostructured calcium carbonate composites.<sup>[77]</sup> For silica-derived materials, cellular uptake and toxicity depend on

particle's size, shape, surface charge and functional groups.<sup>[78,79]</sup> Small particles (200–300 nm) usually undergo an endocytotic process which ends with the fusion with intracellular acidic lysosomes. Inside these acidic (pH  $\approx$  4.5) organelles, silica's surface groups can be protonated facilitating the “proton sponge effect”, which leads to the endosomal escape of the uptaken particles.<sup>[80]</sup> This is particularly useful in drug delivery since it enables the membrane-impermeable cargoes such as hydrophilic drugs, DNA and siRNA molecules to be released from the membrane-bounded endosomes and travel to their effective sites.<sup>[76]</sup> The main advantages of CaCO<sub>3</sub> nanoparticles over biosilica concern their availability, low cost, longer biodegradation times, osteoconductivity and pH-dependent properties. The solubility of CaCO<sub>3</sub> increases as pH decreases.<sup>[81]</sup> Conversely, biosilica becomes more soluble at pH values set over 9.5. Indeed, slow degradation of CaCO<sub>3</sub> matrices allows longer times for drug release after administration, granting long-term sustained-release, performance and high stability.<sup>[82]</sup> Furthermore, CaCO<sub>3</sub> nanoparticles in aqueous conditions generally do not swell or change porosity.<sup>[83]</sup> On a biological viewpoint, toxicological tests in HeLa cells also showed biocompatibility of CaCO<sub>3</sub> particles both in the micrometer and nanometer ranges.<sup>[84]</sup> Finally, a delivery system with pH-sensitive characteristics can be a candidate for drug delivery to cancer tissues. Since the microenvironment in tumors is generally more acidic than in normal tissues, the pH sensitivity may thus delay the release of drugs from the carrier matrix in the blood (pH 7.4) and concentrate it in the targeted cancer tissues, thus offering an effective alternative for delivery and release of anticancer drugs.<sup>[85]</sup>

Both biosilica from diatoms and calcium carbonate from coccolithophores fulfill requirements of biocompatibility, clinical safety,<sup>[86]</sup> and the large surface area needed to act as therapeutic carriers and display extended drug release profiles especially in the case of water insoluble drugs. Opportunities offered by shell-forming plankton are widely recognized in this context, making bioderived hierarchical nanostructures a superior choice for the development of devices that benefit from the exclusive advantages such as high surface area ( $\sim 19 \text{ m}^2 \text{ g}^{-1}$ ) of diatom and coccolith skeletons.<sup>[87,88]</sup> For example, extracted coccoliths modified *in vitro* with enzymes showed almost five times higher surface area than synthetic calcium carbonate, thus increasing the amount of immobilized enzymes (*i.e.*, glucose oxidase and uricase) with preserved activity.<sup>[89]</sup> Moreover, due to their organic skeleton, coccoliths are more robust in calcium-free solution and pure water versus synthetic calcite particles.<sup>[90]</sup> On the other hand, the huge amount of modification protocols for diatom frustules makes biosilica a highly customizable choice. Figure 4 summarizes the main reported modification that can be performed on diatom frustules using *in vitro* or *in vivo* procedures showing a wide spectrum of material tunability.

Operating on the surface chemistry enables to control the release of both hydrophilic and hydrophobic drugs<sup>[91]</sup>, tether fluorophores<sup>[49]</sup>, antibodies<sup>[92]</sup> and/or drug molecules<sup>[93]</sup> [Fig. 5(*i*<sub>1-2</sub>)], thiols,<sup>[40]</sup> amines,<sup>[93]</sup> magnetic nanoparticles, peptide-siRNA for cancer treatment<sup>[94]</sup> and coating polymers.<sup>[45]</sup>



**Figure 4.** Diatom frustule modifiable properties operating in vitro or in vivo approaches.

Moreover, in vivo modification of diatoms has been deeply explored for biomedical and photonic applications offering several strategies for metal incorporation<sup>[95]</sup> and NPs synthesis,<sup>[96]</sup> insertion of thiol functionality,<sup>[97]</sup> the introduction of fluorophores for bioimaging [Fig. 3c, 3hf], the introduction of TiO<sub>2</sub><sup>[98]</sup> to add a photocatalytic ability exploitable to induce oxidative DNA strand breakage<sup>[99]</sup> and cancer cell depletion.<sup>[100]</sup>

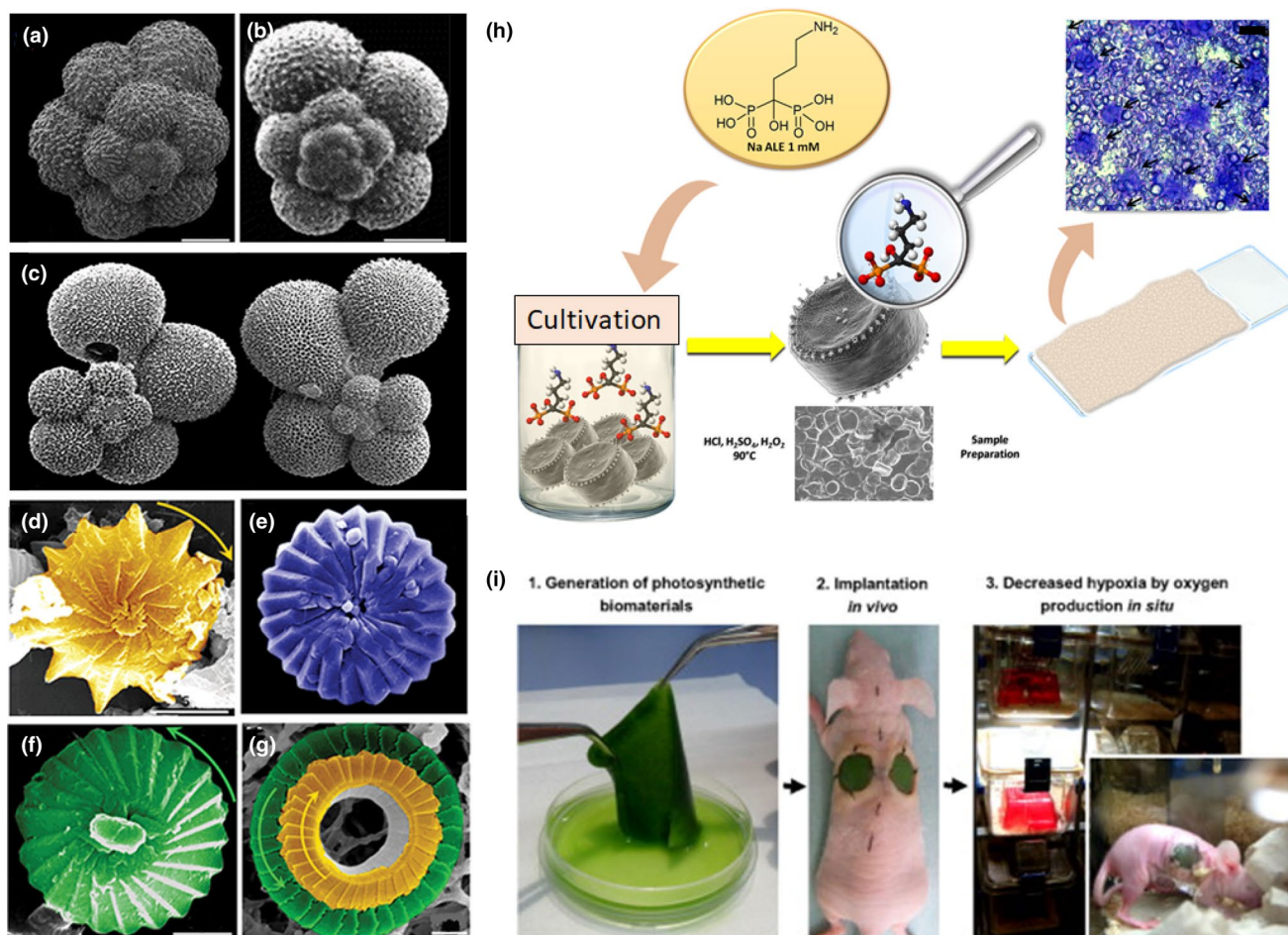
Nonetheless, as is the case for diatoms, materials derived from coccolithophores can be modified as well, both in vivo/ in vitro or coated subsequently to adapt the biogenic nanostructure to different functions.<sup>[89]</sup> A possible pathway to introduce new therapeutic functionalities into coccolith plates can be achieved in vivo by specific optimization of coccolithophore growth parameters (light, pH, temperature, culture media composition).<sup>[101,102]</sup> This enables the coccolithophore cell to produce coccolith bearing specific ions or molecules in their composition, specific size, morphology or porosity.<sup>[103]</sup> For example, strontium enhancement of coccolithophore was obtained by controlling the Sr content in culture media, thus allowing the coccolithophore cell to transfer the metal ion from the interior of the cell into the final crystalized coccolith plates.<sup>[104]</sup>

Similarly, the growth media of *E. huxleyi* coccolithophores was modified with different Zn/Ca ratios, elements both uptaken by cells and influencing the coccolith composition.<sup>[105]</sup> Interestingly, zinc concentration inside coccolithophores has been tuned by the presence of biomolecules (L-cysteine, D-cysteine, L-histidine, L-glutathione, desferrioxamine-B or phytochelatins) and chelating agents, such as ethylenediaminetetraacetic acid (EDTA).<sup>[106]</sup> Noteworthy, a correlation between Cu, Cd and Zn and release of thiol-containing compounds (cysteine, glutathione or  $\gamma$ -L-glutamyl-L-cysteine) from *E. huxleyi* coccolithophores was identified,<sup>[107]</sup> suggesting an alternative way of

modification by addition of bio-chelating agents. The incorporation and regulation in coccolithophore/coccoliths were also reported for boron,<sup>[108]</sup> barium,<sup>[109]</sup> magnesium,<sup>[110]</sup> and rare earth elements (samarium, lanthanum, neodymium, terbium, praseodymium).<sup>[111]</sup>

A further appealing property that can be found in coccolithophores and foraminifera-derived materials, which can be adapted as a powerful technological tool, is the chirality of the shell [Fig. 5(a–g)]. Calcium carbonate skeletons produced by planktonic foraminifera are found to be homochiral with clockwise [Fig. 5(a)] or counterclockwise [Fig. 5(b)] spiraling structures, but there are some species where different chirality can also co-exist in different individuals [Fig. 5(c)].<sup>[112]</sup> Among the coccolithophores species, clockwise enantiomorph (*Discoaster barbadiensis*) [Fig. 5(d)], symmetric achiral (*Discoaster barbadiensis*) [Fig. 5(e)] and counterclockwise (*Discoaster lenticularis*) [Fig. 5(f)] enantiomorph skeletons can be produced.<sup>[113]</sup> It is even possible to find species that produce clockwise and counterclockwise orientations in two adjacent layers within the same coccolith skeleton [Fig. 5(g)].<sup>[114]</sup> Considering that some species are able to chiral switching the skeleton,<sup>[115]</sup> it is envisaged to control the chirality of materials derived from foraminifera and coccolithophores which can be useful both for photonic and biomedical devices.<sup>[116,117]</sup> Moreover, a few reports are showing the possibility to perform the biomineralization of calcium carbonate in vitro using the organic network extracted from coccolithophores<sup>[118]</sup> overcoming production issues related to culturing time [Fig. 5(h)].

Despite potentialities proposed so far, the application of coccoliths in biomedical devices has not been explored as much as in the case of diatoms, likely due to their pore size (0.4 nm)<sup>[87]</sup> smaller than those ones of diatoms (200–900 nm),<sup>[119]</sup> or likely



**Figure 5.** Applications and perspectives for plankton-derived materials in biomedical applications. (a, b and c) SEM micrographs representing examples of Chiral diversity in foraminifera: (a) *Fingeria kingi*; (b) *Tenuitellinata angustumbilicata* (bar 50  $\mu\text{m}$ ); (c) *Globigerinella calida* (bar 100  $\mu\text{m}$ ); (d, e, f and g) colored SEM micrographs showing chirality in coccolithophore skeletons: (d) clockwise *Discoaster barbadiensis* (bar 5  $\mu\text{m}$ ); (e) achiral *Discoaster barbadiensis*, (f) counterclockwise *Discoaster lenticularis* (bar 3  $\mu\text{m}$ ); (g) clockwise (yellow) and counterclockwise (green) layers in *Umblicosphaera foliosa* (bar 1  $\mu\text{m}$ ), (a–g) adapted with permission.<sup>[128]</sup> (h) in vivo incorporation of alendronate drug into diatom biosilica for producing 2D biosilica-based scaffold for bone tissue applications<sup>[41]</sup> (bar 25  $\mu\text{m}$ ); (i) (left) bioartificial scaffolds loaded the photosynthetic microalgae *Chlamydomonas reinhardtii*, (middle) implantation in vivo, (right) living mouse after 5 days from the transplant with reduced hypoxic conditions, with permission of Ref. 129.

because silica exhibits higher chemical stability than calcium carbonate.

Foraminifera shells have been poorly exploited as biomedicine tools. It can be considered challenging the recent fabrication of various 3D structures bearing nanostructures from foraminiferal shells as templates.<sup>[120]</sup> Interestingly, the bone regeneration property of a beta-tricalcium phosphate hydrothermally converted from foraminifera carbonate was investigated, underlining that these natural materials possess peculiar interconnected porous networks with adequate pore size distribution. These beta-tricalcium phosphate systems were able to be osteogenically active, and they exhibited full closure of the defect site induced in living samples.<sup>[121]</sup> Similar results were obtained investigating the therapeutic efficacy of zinc-containing tricalcium phosphate (ZnTCP) produced by hydrothermally converted calcium carbonate

exoskeletons from foraminifera, exploited for the treatment of osteoporotic mice.<sup>[122]</sup>

Last but not least, a truly innovative approach involves the use of living microalgae for the development of artificial tissues<sup>[123,124]</sup> whose technology currently is limited due to hypoxia [Fig. 5(i)]. Although such therapeutic approaches seem feasible, further studies are required to establish how much oxygen could be provided by microalgae when used as an implant, since its dosing is critical for most applications.<sup>[125]</sup> Moreover, though diatoms demonstrated their safety toward normal human cells,<sup>[126]</sup> studies of their cytotoxicity are still rare and ethical issues concerning the bio-printing process remain under debate,<sup>[127]</sup> making this ground an open path for further exploration and optimization of diatoms and coccolithophores.



## Other applications

Considering the available procedures<sup>[130]</sup>, easiness of extraction<sup>[131]</sup> and functionalization of materials derived from diatoms and coccolithophores, it is not surprising that their applications can be found in several and sometimes unexpected fields. For example, diatom frustules coated with gold nanoparticles have been proposed as ultrasensitive biosensor since they benefit from the amplification of the signal in surface-enhanced Raman spectroscopy, one of the most sensitive technologies for identifying biomarkers from complex samples, with detection limits down to the sub-femtomolar level ( $< 10^{-15}$  mol L<sup>-1</sup>)<sup>[132]</sup> and a diatom-based lab-on-a-chip analytical device able to detect ppb levels of drugs such as cocaine in human blood samples has been recently proposed.<sup>[130]</sup> More in general, the exploitation of natural nanostructures for sensing applications is well represented in the literature for the detection of antibodies,<sup>[133]</sup> NO,<sup>[46]</sup> H<sub>2</sub>,<sup>[134]</sup> pyridine, xylene, acetone, ethanol and NO<sub>2</sub> gasses.<sup>[135]</sup> Other possible pioneering applications that can be foreseen for these materials concern the development of micromachines/micromotors and which have already been surprisingly proposed both for diatoms<sup>[136]</sup> and coccolithophores,<sup>[89]</sup> opening a new field in biomedical research of devices able to fulfill complex tasks *in vivo*.<sup>[137]</sup>

## Conclusions

Here we have reported a critical overview of the possible development of materials and devices that can be made from biomineralizing organisms. The beauty and elegant perfection of phytoplankton and phytoplankton-derived constructs have provided a high level of bioinspiration in our history and culture, from which technological advances can largely benefit. Although there are many interesting organisms, research has focused mainly on diatoms and coccolithophores and, despite they display several common features, diatoms are far more represented probably for their similarity with silica mesoporous materials.

Precise manipulation of diatoms is challenging, but the complexity of the various micro- and nanostructuring and their functional roles, along with their surface chemistry and the intriguing photonic properties, makes the diatoms attractive candidates for developing photonic materials. Unfortunately, *in vitro* modification procedures for coccoliths are almost inexistent and most of the studies focused on the *in vivo* modification of coccolithophores can be considered quite at an early stage, if compared to the number of protocols available for diatoms. Moreover, modification of coccolith composition should be investigated to afford materials not restricted only to biomedical applications since they share a lot of photonic features with diatoms.

Strategies for energy generation devices are also still in their infancy stage and there are no reports for

coccolithophores or other calcifying organisms for these applications. This is probably because, contrariwise for diatoms, there are no procedures for the conversion of nanostructured calcite into more conductive materials. For the other silicifying organisms, instead, the biosilicification process is not studied, and hence it is unknown if *in vivo* modifications are feasible as for diatoms, but general procedures like bioclastic conversion should be able to introduce these alternative architectures that could be performing and innovative for electrochemical or energy-related applications. Moreover, cultivation procedures for biomineralizing plankton different from diatoms and coccolithophores are not readily available, and this is very likely to be the reason for the slowdown in their representation.

In the biomedical field, diatoms are widely reported and praised for their features such as homogeneous pore size, high surface area, intricate structure, controllable microstructure, high release rate of biomacromolecules and chemical inertness. While coccolithophores, though they display complementary features like pH-responsive ability and long-term biodegradability which can widen the possible applications, are still lagging compared to diatom's proposals. Furthermore, both for diatoms and coccolithophores, more studies related to their cytotoxicity, *in vivo* biodistribution and biodegradability are required leaving room for further exploration and improvement.

Finally, a critical bottleneck in this kind of applied biotechnologies resides in harnessing the benefits of plankton-mediated mineralization, often performed in the laboratory scale, and translating them into advances in scientific knowledge and tangible technological applications that have to be produced on the industrial scale. In this context, the scale-up is complicated because the level of control exerted at the single-object level tends to wane with scale-up attempts to address a large number of objects. On the other hand, considering the abundance of protocols and patents available for culturing and modifying these living biomaterials, we expect that this technology will proceed to explore new applications and, possibly shortly, moving to industry and large-scale production of the first materials, based on the already established knowledge.

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## Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

## Declarations

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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