Therapeutic Applications of Phytoplankton, with an Emphasis on Diatoms and Coccolithophores

Mihai Lomora,* David Shumate, Asrizal Abdul Rahman, and Abhay Pandit*

Phytoplankton are complex living organisms that have attracted significant interest in the field of biomedicine. One subclass of phytoplankton, the diatoms, produce elegant self-assembled siliceous architectural features with a complex 3D porous structure. Diatoms are characterized by a distinct 3D architecture of silica cell walls called frustules with a highly ordered nano-/micropore structure and pattern. Another phytoplankton subclass of interest is the coccolithophore, which produces unique calcium carbonate plates with distinct architectural features called coccoliths. The unique morphological characteristics of coccoliths resembling the shape of a wagon wheel allows a higher surface area, thus an increased amount of immobilized therapeutic agent on their surface compared to a synthetic calcium carbonate microparticle. This review offers a summary of phytoplankton (microalgae) and their potential for application in drug delivery, diagnostics, drug discovery, as molecular factories, and as scaffolds for various therapeutic applications.

1. Introduction

Phytoplankton are the autotrophic components of the plankton class of organisms. They are small organisms which live in marine environments, producing their own food through photosynthesis. Inhabiting the sunlit layers of almost all bodies of water, phytoplankton create organic compounds from carbon dioxide dissolved in water in a process called carbon fixing that sustains the aquatic food web and supports the diversity of marine life. They are one of the most abundant forms of life on the planet, and there is a wide diversity of phytoplankton species although the exact number is unknown as many remain undiscovered.[1]

They contribute an estimated 45% of the oceanic primary production of organic material.[7] The diatom contains its protoplasm within an upper valve (epitheca) positioned on top of a lower valve (hypotheca) similar to that in a Petri dish assembly.[8] The epitheca connects the hypotheca through the girdle bands, revealing an intricate nanopatterning linkage system.[9] The various levels of complexity in biosilica micro- and nanopatterning in diatoms is the result of a high level of precision and in a reproducible manner as a function of each particular diatom species.[8] At the early stage of the cell cycle division process, readily available monosilicic acid, Si(OH)₄, present in the marine environments is uptaken inside the epitheca and hypotheca valve silica deposition vesicles (SDV) via specialized transporters, where the silica biomineralization process originates.[10] During the subsequent cell cycle stages, enrichment with the monosilicic acid induces the growth of the valve SDV. Once the maturity is reached, the valve is deposited on the cell surface by SDV exocytosis. It is also believed that the frustules of the silica wall of the diatom is a result of complex intracellular events involving the transport of amorphous silica particles (approximately 1–10 nm in diameter) by the so-called silica transport vesicles (STVs) to the extremity of the SDV.[11,12] Once the silica particles have reached the SDV, they further diffuse to accumulate and thermodynamically restructure into a breeding aggregate with a final smooth surface, a process known as sintering or relocalization. The biosilicification in diatoms proceeds from the nanoscale through the mesoscale, to the microscale progression. Toward the final microscale progression, the cytoskeleton’s components such as microtubules and motor proteins participate in the formation of the valve and girdle bands. The shape of the
girdle bands has geometries which vary from split rings, circular patterns to scale like. Although girdle bands are less intricate than valves, their structure is still species dependent and these bands are also synthesized within SDV. A vast understanding of the diatom silica biomineralization process has been achieved up to date, but there are still many open questions to be addressed.\textsuperscript{[13]}

The diatom’s features which facilitate its use as a versatile candidate for therapeutic delivery include homogeneous pore size, high surface area, intricate structure, controllable microstructure, and chemical inertness. Diatoms are characterized by a large pore size which can accommodate a high release rate of biomacromolecules. This makes them attractive as a delivery platform. Diatoms loaded with drug or biomacromolecules can be efficiently targeted to the release site and eliminate cancer cells.\textsuperscript{[14]}

Additionally, some of the constituents of diatoms are long-chain polyamines (LCPAs), silaffins, or less often described sterol sulfates and sulfotransferases which provide a variety of biological activities and potential therapeutic applications.\textsuperscript{[15]}

Diatoms are of interest in the field of fabrication due to their reliable manufacture of microscale and nanoscale silica which could be artificially controlled to produce nanoscale components.\textsuperscript{[9,16]} Theranostic silicon nanocarriers from diatoms have been used for the sustained delivery of chemotherapeutics,\textsuperscript{[17]} and diatom microcapsules have also been surface functionalized for the delivery of water insoluble drugs.\textsuperscript{[18]}

Porous silica-based particles have demonstrated their potential as therapeutic carriers due to their extended drug release profiles and high efficacy in delivering an insoluble drug. The most intensively investigated silica materials for drug delivery are mesoporous silica\textsuperscript{[19]} and oxidized porous silicon.\textsuperscript{[20]} These nanoparticles provide tunable pore size, high surface area, chemical inertness, and thermal stability. However, silica nanoparticles (SiNPs) come with significant drawbacks as they require many time-consuming processes, and expensive and toxic reagents during synthesis.\textsuperscript{[21]}

As evidenced by the drawbacks of SiNPs, diatoms are a superior choice for use in biomaterial production. Therefore, with its multiple advantages and wide availability, modification of the diatom skeleton has been explored thoroughly by alterations in surface chemistry. For instance, the diatom frustule can be chemically tailored to artificially tether bioactive molecules such as antibodies and/or drug molecules, while maintaining the inherent biological activity of the attached (bio)molecule. The mechanism of in vivo modification of diatoms via metal incorporation and NPs synthesis, their toxicity toward the diatom, along with their possible applications has also recently been reported.\textsuperscript{[22]}

Another interesting subtype of phytoplankton that are candidates for biomedical applications are coccolithophores. Coccolithophores are unique in that they produce coccoliths which are calcium bicarbonate plates that have potential to be used as a template in drug delivery. Each unicellular coccolithophore is enclosed in its coccolith which make up its coccosphere, or exoskeleton.\textsuperscript{[23]} Coccoliths are produced through calcification in the presence of light within the Golgi complex of the coccolithophore cell. Once produced, a coccolith is added to the inner surface of the cell via a vesicle.\textsuperscript{[23,24]}

Though not yet fully understood, the skeletal structure of Emiliania huxleyi coccoliths to form the coccosphere shield is believed to conform to Euler’s polyhedron formula.\textsuperscript{[25]} The coccosphere is specifically designed to withstand high mechanical load and confer protection for the coccolithophore, outperforming technical ceramics in the strength to weight ratio.\textsuperscript{[26]} The \( \approx 5.5 \, \mu m \) averaged diameter of \textit{E. huxleyi} coccosphere is formed from one single interlocking layer comprising \( \approx 15 \) averaged coccoliths, with additional formed ones easily detaching from the coccosphere.\textsuperscript{[27]}

In general terms, a fully developed \textit{E. huxleyi} coccolith plate is characterized by an oval central area surrounded by almost parallel outer and inner shields containing angled ridges. Some species continuously produce and shed coccoliths. This property is advantageous as these structures can be produced in a high-throughput fashion with appropriate culture conditions.\textsuperscript{[28]} Extraction and concentration of diatoms and coccolith skeleton have been simplified over time and can now be easily extracted using commercial cleaning fluids.\textsuperscript{[29]}

Coccolithophores have been intensively investigated to reveal insights for the mechanism of calcification and formation of their unique morphologies,\textsuperscript{[29]} to genome identification,\textsuperscript{[30]} their interaction with solar light exposure in the ocean,\textsuperscript{[31]} and impact
on climate change.\cite{12} Though not as fully investigated as diatoms for therapeutic applications, studies have been recently reported to use coccoliths in biomedical applications. Due to their unique morphological characteristics, their envisaged applications as part of micro- and nanodevices, in microfluidics or microfiltration applications, optics, or even as micromachines can be realized.\cite{13} As with so many other areas of science, what was first considered science fiction has moved into the region of science fact.\cite{14} Artificial micromotors have already been demonstrated to achieve complex tasks in vivo.\cite{15} It is expected that coccoliths will play an important role in this context as well due to their resemblance to the wagon wheel model.\cite{16}

Phytoplankton, including diatoms and coccolithophores, represent an exceptional source of metabolites as natural product extracts.\cite{17} Several studies in the last decade have reported on the discovery of enzymes and new metabolites with high impact for therapeutic applications.\cite{18} Many more therapeutically relevant phytoplankton metabolites are still on the verge of discovery.\cite{19}

Although they are important players in the marine environment, phytoplankton allows symbiotic relationships with mammalian cells and, when embedded in scaffolds, also for tissue engineering applications (Figure 1). This review encompasses a variety of phytoplankton, including diatoms and coccolithophores, and their therapeutic potential (Table 1).

Introductory Note: Within this review, the term “phytoplankton” was used interchangeably with the term “microalgae.” Coccolithophore is the organism, while the coccolith is the nonliving plate extracted and purified from the coccolithophore microalga. Similarly, the term “diatom” referred to the organism, while “diatom” is to be associated with the extracted silica skeleton after removal of the diatom cellular material. “Diatomite” or “diatomaceous earth” resembles fractions of diatom shell or broken frustules which are of irregular size and shapes. A general comparison of advantages and disadvantages between diatoms and diatomaceous earth related to price, availability, diversity, as well as future prospects can be found in Supporting Information Table 2.1 in ref. [40].
| Phytoplankton/mimic source | Species | Model drug/functional group/mimic | In vitro/In vivo modification | Cell line tested | Hydrogel/scaffold | In vitro/In vivo testing | Envisaged therapeutic application | Reference |
|---------------------------|---------|----------------------------------|-----------------------------|-----------------|-----------------|-------------------------|----------------------------------|----------|
| Diatom                    | Aulacoseira | Grafted oligo(ethylene glycol) methacrylates, levofloxacin | In vitro – – | – | – | In vitro | Antimicrobial, wound healing | [64] |
| Diatom                    | Coscinodiscus concinnus | Streptomycin | In vitro – – | – | – | In vitro | Oral drug delivery | [62] |
| Diatom                    | Coscinodiscus walesii | Amino groups (APTES) reacted with GA to bind protein A for the orientation of MoAb UN1 or rho-MoAb UN1 | In vitro – – | – | – | In vitro | Biosensing, drug delivery | [44,45] |
| Diatom                    | Pseudonitzchia multisiliata | Gold layer coating | In vitro RBC, REH B-leukemia cells – | – | – | In vitro | Chemical analysis of cellular membranes | [55] |
| Diatom                    | Thalassiosira weissflogii | Triethoxysilane-functionalized π-conjugated fluorophore | In vitro – – | – | – | In vitro | Photonic therapeutics | [7] |
| Diatom                    | Thalassiosira weissflogii | SBDF | In vitro – – | – | – | In vitro | Photonic therapeutics | [7] |
| Diatom                    | Thalassiosira weissflogii | –SH, –NH₂ | In vitro Dermal fibroblasts, osteosarcoma Saos-2 cells | – | – | In vitro | Delivery of therapeutics | [76] |
| Diatom                    | – | Scaffold incorporation and release | In vitro Human osteosarcoma cell line MG63 | – | – | In vitro | Bone mineralization | [107] |
| Diatom                    | – | HSA-iron oxide nanoparticles, rhodamine B | In vitro 4T1 murine breast cancer cells | – | – | In vivo | Chemotherapy | [79] |
| Diatom                    | Thalassiosira pseudonana | Antibodies⁵, CPT-loaded DOTAP liposomes, SN38-loaded cationic micelles⁴¹ | In vitro P38, HR 1 K acute B-cell leukemia, Jurkat acute T-cell leukemia, BSR⁵, SH-SYS⁵⁶ | – | – | In vivo | Targeted delivery of poorly water-soluble anticancer drugs to tumor sites | [14] |
| Diatom                    | Thalassiosira weissflogii | TiO₂ | In vivo – | – | – | In vitro | Antibacterial, photocatalysis | [74] |
| Diatom                    | Thalassiosira weissflogii | –SH, PDMP | In vitro – | – | – | In vitro | Functionalization with therapeutics | [75] |
| Diatom                    | Thalassiosira pseudonana | IgG-binding domain of protein G | In vivo – | – | – | In vitro | Targeted delivery | [14] |
| Diatom                    | Thalassiosira weissflogii | tPhA-Silane⁴ | In vivo – | – | – | In vitro | Photonic therapeutics | [45] |
| Diatom                    | Aulacoseira sp., Cyclotella sp., Ulnaria fenestraformis Kulikovsky, Stephanodiscus meyerii Genkal et Popovsk | NBD-CI, LCPA | In vitro – | – | – | In vitro | Liquid flow tracers | [87] |
| Diatom                    | Aulacoseira sp. | Frustules coated with gold | In vitro – | – | – | In vitro | Biosensing | [54] |
| Diatom                    | Aulacoseira | SiO₂ conversion to MgO with shape preservation | In vitro – | – | – | In vitro | Delivery of therapeutics | [149] |
| Diatom                    | Aulacoseira sp. | SiO₂ conversion to MgO, APTES silanization, addition of BS3 cross-linker followed by attachment of protein A (with or without FITC) and conjugation with anti-His-tagged monoclonal antibody | In vitro – | – | – | In vitro | Biosensing | [96] |
| Diatom                    | Coscinodiscus walesii | Graphene | In vitro – | – | – | In vitro | Biomedical applications | [106] |
| Diatom                    | Melosira preicelanica | Gold nanoparticles | In vitro – | – | – | In vitro | Detection of biological analytes | [40] |

(Continued)
Table 1. Continued.

| Phytoplankton/mimic source | Species | Model drug/functional group/mimic | In vitro/In vivo modification | Cell line tested | Hydrogel/scaffold | In vitro/In vivo testing | Envisaged therapeutic application | Reference |
|---------------------------|---------|----------------------------------|-------------------------------|------------------|-------------------|-------------------------|----------------------------------|-----------|
| Diatomite                 | –       | Gold nanoparticles               | In vitro                      | –                | –                 | In vitro                | Detection of illegal drugs in blood plasma | [57]      |
| Diatomite                 | –       | Gold nanoparticles               | In vitro                      | –                | –                 | In vitro                | Harmful contaminants in food products | [58]      |
| Diatomite                 | –       | Indomethacin, 3-aminopropyl/trimethoxy silane, N-(3-(trimethoxysilyl)propyl) ethylene diamine, 2-carboxyethyl-phosphonic acid, 16-phosphono-hexadecanoic acid | In vitro                      | –                | –                 | In vitro                | Bone and orthopedic                | [59]      |
| Diatomite                 | –       | Amino groups, gold nanoparticles | In vitro                      | –                | –                 | In vitro                | Trace components in latent fingerprints | [60]      |
| Diatomite                 | –       | Amino groups (APTES) and complexed with dimethyl suberimidate | In vitro                      | –                | –                 | In vitro                | Detection of human pathogen          | [61]      |
| Diatomite                 | –       | NO-releasing SNAP                 | In vitro                      | –                | –                 | In vitro                | Anti-thromogenic and antimicrobial biomaterials | [62]      |
| Diatomite                 | –       | (PS, PVAc) epoxy and polyurethane binders, alkyltrimethoxysilane, (PPFS, PSMA, PDMSMA) fluorosilanes | In vitro                      | –                | –                 | In vitro                | Superhydrophobic surfaces           | [63–73]   |
| Diatomite                 | –       | Amino groups (APTES) reacted with TRITC | In vitro                      | H1355 human epidermoid cancer cells | –                 | In vitro                | Drug delivery, cancer treatment      | [64]      |
| Diatomite                 | –       | Amino groups (APTES) followed by PEGylation and bioconjugation with CPP, Alexa Fluor 488 dye | In vitro                      | MCF-7 and MDA-MB-231 human breast cancer cells, RBC | –                 | In vitro                | Drug delivery, cancer treatment      | [65]      |
| Diatomite                 | –       | APTES silanization followed by PEGylation, gold nanoparticles, Alexa Fluor 488 | In vitro                      | HeLa cells       | –                 | In vitro                | Bioimaging                        | [66]      |
| Diatomite                 | –       | siRNA                             | In vitro                      | H1355 human epidermoid cancer cells | –                 | In vitro                | Cancer treatment                   | [67]      |
| Diatomite                 | –       | Enhancement of hydroxy groups (Piranha solution), amino groups (APTES), Sulf-GMBS<sup>13</sup>, Alexa Fluor 488, polyArg-siRNA complex | In vitro                      | H1355 human epidermoid cancer cells | –                 | In vitro                | Drug delivery, cancer treatment      | [68]      |
| Diatomite                 | –       | Mesalamine, prednisone            | In vitro                      | Colon cancer cells (Caco-2, HT-29, and HCT-116) | –                 | In vitro                | Gastrointestinal (GI) diseases      | [69]      |
| Diatomite                 | –       | As reinforcement composite into scaffold | In vitro                      | 3T3, MG 63, Saos-2, hFOB cell lines | Chitosan scaffold | In vitro                | Bone tissue engineering              | [70]      |
| Diatomite mimic Coscinodiscus wailesii | Graphene | In vitro | – | – | In vitro | Biomedical applications (biosensing) | [71] |
| Diatomite mimic Coscinodiscus | Gold nanoparticles | In vitro | – | – | In vitro | Templating | [72] |
| Diatomite mimic Thalassiosira weissflogii | PolyDMAEMA-co-EGDMA | In vitro | – | – | In vitro | Delivery of therapeutics | [73] |
| Diatomite mimic – | Silica nanospheres with hollow silica nanoparticles doped on the surface | In vitro | – | – | In vitro | Nanomedicine | [74] |
| Phytoplankton/ mimicsource | Species | Model drug/functional group/mimic | In vitro/ In vivo modification | Cell line tested | Hydrogel/ scaffold | In vitro/ In vivo testing | Envisaged therapeutic application | Reference |
|---------------------------|---------|----------------------------------|-------------------------------|----------------|---------------------|--------------------------|---------------------------------|-----------|
| Diatom mimic              | pGlcNAc | In vitro                         | -                            | -              | -                   | -                        | In vitro Degenerating intervertebral disc | [140]    |
| Diatom mimic              | Cylindrotheca fusiformis | Multilayer of R5 protein-MAP with SiNPs on titanium surface | In vitro Mouse preosteoblast MC3T3-E1 cells | -              | -                   | -                        | In vivo Dental and orthopedic implants | [145]    |
| Coccolithophore           | Emiliana huxleyi, Pleurochrysis carterae | Glucose oxidase, uricase | In vitro | -              | -                   | -                        | In vitro Therapeutic carrier | [87]     |
| Dinoflagellate coccosphere mimics | Thracosphaera heimii | Aragonite shells | In vitro | -              | -                   | -                        | In vitro Biomedical implants | [152]    |
| Green microalgae          | Chlorella sp. KR-1 | Encapsulation inside sacrificial CaCO₃ crystals coated PAH/PSS | In vivo | -              | -                   | -                        | In vitro Biological applications | [131]    |
| Green microalgae          | Chlamydomonas reinhardtii 1132b and Chlorella sorokiniana UTEX1230 | - | In vivo | Human cell line SaOS-2 | Alginated hydrogel | In vitro | Therapeutic microcarriers | [159]    |
| Green microalgae          | Chlamydomonas reinhardtii | Co-culture with mammalian cells | In vivo | Ca(II)-alginated and prodriven alginated silica hydrogels | In vitro | Tissue engineering | - | - | |
| Microalgae                | Chlorella vulgaris | Encapsulation inside hydrogels | In vivo | -              | -                   | -                        | In vitro Therapeutic microcarriers | [159]    |
| Green microalgae          | Dunaliella salina | Self-synthesized NPs with containing β-carotene | In vivo | C29 human melanoma cell | -                   | -                        | In vitro Cancer therapy | [122]    |
| Green microalgae          | Spirulina | Scaffold incorporation | In vivo | CS7/16N mice kidney extracted MSCs | PDLLA scaffold | In vitro | Tissue engineering | [155]    |
| Microalgae                | Pseudokirchneriella subcapitata | Co-encapsulation with Daphnia magna zooplankton inside matrix | In vivo | -              | -                   | -                        | In vitro Biosensing | [159]    |
| Microalgae                | Chlamydomonas reinhardtii | Co-culture with mammalian cells | In vivo | NIH-3T3 fibroblasts | Integra matrix bilayer skin | In vitro | Tissue engineering | [160]    |
| Microalgae                | Chlamydomonas reinhardtii | Mixed with fibrinogen prior to scaffold entrapment | In vivo | -              | -                   | -                        | In vitro Hyposia in tissues | [162]    |
| Green microalgae          | Chlorella vulgaris | Co-culture with mammalian cells | In vivo | C2C12 mouse myoblasts, rat cardiac cells | Microalgae-mammalian cells 3D cardiac tissue | In vitro | Tissue regeneration | [164]    |
| Microalgae                | Chlorosarcina littorale | Co-culture with mammalian cells | In vivo | -              | -                   | -                        | In vitro | - | |

APTES, (3-aminopropyl)triethoxysilane; BS3, bis(sulfosuccinimidyl) suberate; CPP, (aminooxy)acetyl-Lys-(Arg)₉-COOH cell penetrating peptide; CPT-loaded DOTAP liposomes, camptothecin-loaded 1,2-dioleoyl-3-trimethylammonium-propane liposomes; FITC, fluorescein isothiocyanate; GA, glutaraldehyde; HSA, human serum albumin; HeLa cells, human cervical epithelioid cells; LCPA, long-chain polyamine; MAP, mussel recombinant adhesive protein; MoAb UN1, murine monoclonal antibody UN1; MSCs, mesenchymal cells; NBD-CI, 4-Chloro-7-nitrobenzofurazan; NO-releasing SNAP, nitric oxide-releasing 5-nitro-o-N-acetyl-l-phenylalanine; NPs, nanoparticles; PAH, poly(allylamine hydrochloride); PDLLA, poly-D,L-lactic acid; PDMPO, [2-(4-pyridyl)-5-((4-(2-dimethylaminoethylamino-carbamoyle)methoxy)phenyl)oxazol] fluorescent dye; PDM PO, poly-D-Arg-siRNA complex; poly-Arg-siRNA complex; poly-D-Arg peptide complexed with siRNA; polyDMAEMA-co-EGDMA, poly(2-(dimethylamino)ethyl methacrylate-co-ethylene glycol dimethacrylate); PFPs, poly(pentafluorostyrene); PS, polystyrene; PSMA, poly(stearyl methacrylate); PSS, poly(sodium 4-styrenesulfonate); PVAc, poly(vinyl acetate); RBC, red blood cells; rho-MoAb UN1, murine monoclonal antibody UN1 fluorescently labeled with rhodamine; SBDF, 7-fluorobenzo-2-oxa1,3-diazole-4-sulphonic acid fluorescent dye; SiNPs, silica nanoparticles; siRNA, small interfering ribonucleic acid; TRITC: tetramethylrhodamine isothiocyanate; α Antibodies: anti-rabbit IgG-HRP, DyLight 549-conjugated mouse anti-human IgG, anti-CD20 antibody, anti-p75NTR mouse monoclonal antibody, anti-rat IgG conjugated to a 10-nm gold nanoparticle; βSN38-loaded cationic micelles: 7-ethyl-10-hydroxy-camptothecin-loaded cetytrimethylammonium bromide micelles; β-BSR: clone of baby hamster kidney fibroblast cells; β′SH-SYSY: human neuroblastoma cells; γPAA-Silane: two-photon red emitting triphenylamine-based fluorescent dye containing a triethoxysilyl functional group; γPEGylation: heterobifunctionalized poly(ethylene glycol) with carboxyl and amino groups (HOOC-PEG-NH₂); γ⁺Sulfu-CMBS: N⁺-maleimidobutryloxy-sulfosuccinimide ester; δIntegra epidermis: single collagen layer (in vitro) or double-layer collagen + silicon (in vivo).
2. Diatoms and Their Applications in Diagnostics and Therapeutics

Fossilized diatoms, as well as diatomaceous earth (DE), enable various modification possibilities for the design of superior therapeutic devices, thus addressing limitations of its silica-based synthetic counterparts. The phytoplankton could be modified in vivo (during the growth process), or by processing of the diatom silica skeleton in vitro (after the removal of the phytoplankton organic matter).

2.1. Diatoms in Diagnostics

Precise manipulation of diatoms is challenging, but the complexity of the various micro- and nanostructuring and their functional roles, along with their surface chemistry makes the diatom an attractive candidate for biosensing and photonic applications. Thalassiosira weissflogii diatom frustules were covalently conjugated for the first time in vitro with a thiopehene-benzothiadiazole-thiophene (TBT)-based fluorophore, resulting in a biohybrid luminescent material with high photoluminescence quantum yields which has applications in bioimaging. Following the report in which TBT was covalently attached to diatom frustules, diatoms were recently modified in vivo to incorporate a two-photon absorption triethoxy derivative of a red emitting dipolar dye (tPhA-Silane) which can be used for photodynamic therapy applications. This modification involved incorporation of the dye inside the diatom skeleton inducing light scattering events leading to a loss in the fluorescent signal. This loss of signal is unlikely if multiphoton absorbing dyes with emission in the red or NIR region are used. Therefore, T. weissflogii diatoms were fed with a specifically synthesized highly hydrophobic two-photon red emitting triphenylamine-based fluorescent dye containing a triethosilanyl functional group (tPhA-Silane). Feeding the diatoms with the synthesized fluorescent dye had no influence in the modification of diatom architecture. The incorporated fluorescent dye allowed monitoring of the in vivo growth of diatom cultures up to 24 h and the diatom skeleton induced a protective effect for effuged dye: separation of diatoms from the organic matter after 4 days feeding resulted in strong emission, illustrating the successful in vivo incorporation of the specifically synthesized fluorophore inside the diatom skeleton.

As an alternative to fluorophore attachment, the feasibility of the diatoms’ frustules to act as optical biosensors could also be demonstrated by covalently attaching monoclonal antibodies on the frustules of diatoms to specifically bind their antigens. Polyamines naturally occur in living diatoms, therefore a high binding efficiency is expected for LCPAs to silica. LCPA can be obtained by condensation of short-chain polyamines with 1,3-dibromopropane. Additionally, 4-chloro-7-nitrobenzofurazan (NBD-CI) is a nonfluorescent reagent which becomes fluorescent upon reacting with primary or secondary amines. The combination of LCPA bearing more than six nitrogen atoms with the highly sensitive chromogenic and fluorogenic reagent NBD-CI resulted in NBD-tagged LCPA. Extracted siliceous frustules of Cyclotella sp., Ulnaria ferruginosa Kulikovskiy, and Stephanodiscus meyerii Genkal et Popovsk diatoms can be mixed with NBD-LCPA to produce fluorescently labeled diatoms. NBD-LCPA fluorescently labeled diatoms showed higher staining activity than that of the previously described non-LCPA-based fluorophores.

Rapid diagnosis of pathogens with high sensitivity within a single-tube assay has been demonstrated by in vitro modifications of DE. Various pathogens such as Brucella, Salmonella, and Escherichia coli from infected urine can be concentrated on the surface of amine-functionalized DE (using APTES) via electrostatic and physical absorption thereby enhancing the pathogen detection limit. Moreover, amine-functionalized DE forms a complex with dimethyl suberimidate to extract nucleic acid from the enriched pathogen. The pathogen extracted DNA was detected within 20 min using an in-house real-time, label-free isothermal solid-phase amplification/detection (ISAD) device. The single-tube diatomaceous-earth-based assay allowed enrichment of Brucella in human urine of up to eightfold and up to single pathogen colony detection, thus being 100 times more sensitive than a commercially available kit.

Recent functionalization of diatomite particles (irregular shape, approximately 350 nm in size) with a peptide-siRNA complex has recently been shown to be efficiently uptaken by human lung epidermoid carcinoma cell line (H1355). No cell cytotoxicity or alteration in the morphology of the cancer cells was detected in the presence of the modified diatomite nanoparticles (DNPs) for up to 72 h. In addition, the uptake of the modified DNPs within the components of the cancer cells could be efficiently monitored using Raman imaging with their corresponding Raman spectra without the requirements of a labeling strategy. The results of Raman imaging could be validated by confocal microscopy and photoluminescence measurements during the uptake of fluorescently labeled DNPs containing the peptide-siRNA complex inside the H1355 carcinoma cell line. In contrast with possible artefacts arising due to the use of fluorophores (photobleaching, modification of size, charge, chemical properties of DNPs), the label-free Raman technique allowed a clear position identification of the peptide-siRNA complex modified DNPs within the cancer cells. Successful uptake of tetrathiomethylrhodamine isothiocyanate (TRITC) fluorescently labeled DNPs by human lung epidermoid carcinoma cell line has also been achieved. Similarly, human cervix epithelioid carcinoma (HeLa) allowed internalization of PEGylated DNPs having gold nanoparticles attached on their surface. Despite the shape irregularity and broad size distribution of the DNPs, these studies reflect the versatility of the diatom-based materials as promising nanodevices for possible cancer diagnostic.

Diatoms appear to be an ideal biological scaffold not only due to their ability to capture, localize, sequestrate biological entities but also due to their ease of functionalization. If the surface is coated with gold nanoparticles (Au NPs), then the diatom becomes an ideal ultrasensitive biosensor, as Au NPs amplify the signal in surface-enhanced Raman spectroscopy (SERS), one of the most sensitive technologies for the identification of relevant biomarkers from complex clinical samples, with detection limits down to the subfemtomolar level (<10−15 mol L−1). The high surface area of ultra-high-purity fossil diatomaceous earth can be modified with amino groups and the citrate-stabilized Au NPs could be allowed to evenly distribute, followed by pressing the composite material (diatomaceous earth functionalized with Au nanoparticles) on the surface of amine-functionalized DE. The modified DE can be used to detect pathogens in a single-tube assay. This assay allows for the detection of up to eightfold enrichments of Brucella in human urine.
NPs) into button-like portable tablets.\textsuperscript{[53]} Diatom-based button-like portable tablets were afterward used to identify the biochemical composition of eccrine sweat in latent fingerprints by SERS analysis (Figure 2). Compared with the same mass-equivalent amount of Au NPs attached on a glass surface, diatom-based tablets containing Au NPs revealed a higher enhancement factor, a key parameter for assessing the performance of SERS substrates. The detection tablets showed good signal reproducibility and long-term stability. The signal was slightly less than 50% SERS enhancement and was obtained after adding a drop of a dye probe molecule (rhodamine 6G) and diatom-based tablets storage for 3 months in sterile Petri dishes. Advancement in diatom modification with Au NPs as SERS substrates has made possible the detection of p-mercaptoaniline (Raman signal enhancement factor of 10\textsuperscript{5}),\textsuperscript{[54]} bovine serum albumin in the sub-femtomolar range (10\textsuperscript{-16} mol L\textsuperscript{-1})\textsuperscript{[40]} and chemical analysis of red blood cells and REH-leukemia cell membranes for pathological assessment.\textsuperscript{[55]}

Microfluidic chips allow the design of biochemical reactions at cellular reaction volume levels and thus translation of large-scale reactions into lab-on-a-chip platforms.\textsuperscript{[56]} A diatom-based lab-on-a-chip analytical device has been designed for sensing ppb levels of drugs such as cocaine in human blood samples.\textsuperscript{[57]} The microfluidic channels containing diatomaceous earth were obtained using an adhesive-tape-based pre-formed channel template on the surface of a glass slide. This was followed by spin coating of the templated channels with diatomaceous-earth- and tape-based template removal (Figure 3). This resulted in a glass slide with a 400 \times 30 \mu m\textsuperscript{2} in cross-section diatomite channel array that can be partially immersed in the solution of interest to allow the liquid migration into the diatomite channels based on capillary forces and separation of the analyte spots of interest. The position of the spots can be identified by UV (380 nm) and iodine colorimetry to enable precise deposition of Au NPs and their detection by SERS. Again, the large surface area and pore volume made diatomaceous earth an ideal material to be used in microfluidics. This enabled the microfluidic diatomite analytical device to reach a new level of sensitivity, that is up to 1000 times greater than typical chromatography plates. A similar approach was also used to identify analytes from seafood.\textsuperscript{[58]}

Encapsulating magnetic nanoparticles (MNPs) and small molecule drugs inside diatoms has been reported.\textsuperscript{[59]} Diatoms are bigger (10 \mu m in diameter, with \approx 500 nm pores) than SiNPs (50–100 nm in diameter), leading to higher amounts of MNPs and drug entrapment. The diatom is thus conferred with superior magnetic properties which can be harnessed by applying an external magnetic field to target drug release at the site of a tumor (Figure 4). When diatoms are incubated for 1 week in a body fluid mimic, a significant amount is partly degraded, offering insights on their biodegradability.\textsuperscript{[59]} And, while directing in vivo MNPs- and drug-loaded diatoms toward tumors is an attractive concept, caution in interpreting this data should be exercised, as no evidence for the statistical analysis of the presented results is provided. Moreover, the entrapped molecule inside the diatom is a fluorescent dye used for visualization purposes in an in vivo animal model. The dye could be easily replaced by a drug for allowing the transition of the modified diatom from a diagnostic to a therapeutic device.

2.2. Diatoms in Therapeutics

Modification of the diatom skeleton has been thoroughly explored because of its multiple advantages and its ready availability. A wide range of various chemical alterations have been
Figure 3. A) Graphical concept of the microfluidic channel array containing diatomaceous earth for ppb analyte detection from complex samples via on-chip chromatography and SERS. B) Optical image of a channel containing C) diatomite particles as visualized under SEM. D) UV visualization of the microfluidic channel array containing diatomaceous earth during analyte migration. Reproduced with permission. Copyright 2018, Elsevier.

Figure 4. Diatom encapsulating magnetic nanoparticles (MNPs) and small molecules is directed via a magnet to accumulate inside tumor in vivo. A) SEM micrograph of diatom particle. B) diatom containing entrapped NPs are attracted in solution toward the magnet (left), while the diatom alone remains suspended in solution in the presence of the magnet (right). C) By using the magnet, higher fluorescence intensity of the diatom encapsulating MNPs and small molecule biodistribution were observed at the tumor site, correlated with more than six times higher accumulation in the presence (left) versus absence (right) of the magnet. Reproduced and adapted with permission. Copyright 2013, Royal Society of Chemistry.

made on the surface to fabricate therapeutic diatom-based reservoirs as previously noted. Some notable examples include direct surface modification of diatoms and diatom-based materials (diatomite) to control the release of both hydrophilic and hydrophobic drugs. As an illustration, fabrication of stimuli-responsive diatoms using aqueous silica electron-transfer-based atom transfer radical polymerization (Si-ARGET-ATRP) was demonstrated as a controlled hydrophobic drug delivery device. The surface of cylindrical frustules of Aulacoseira sp. Diatoms was grafted with a thermo-responsive mixture of oligo(ethylene glycol) methacrylate (O(EG)2MA/O(EG)4–5MA) and used to entrap the hydrophobic antibiotic levofloxacin. Once the O(EG)2MA/O(EG)4–5MA copolymer is heated (45 °C) above its lower critical solution temperature (LCST) and then cooled down (25 °C) below its LCST, it behaves as an actuator, transitioning between a rigid (below LCST) and collapsed state (above LCST), thus keeping the drug entrapped (below LCST) or releasing it (above LCST). In response to temperature modulation, the stimuli-responsive diatom microparticles showed controlled antimicrobial action against two common wound pathogens, namely Staphylococcus aureus and Pseudomonas aeruginosa.

Diatomaceous earth (DE) was also functionalized with three amino-silylation agents: (3-aminopropyl)triethoxysilane (APTES), N-(6-aminohexyl)aminomethyltriethoxysilane (AHAMTES), and 3-aminopropyltrimethoxysilane (APDMES), with the aim to covalently tether N-acetyl-d-penicillamine (NAP) to the diatom surface. The final objective was to load the diatomaceous earth particles (10–15 μm in size) with nitric oxide and release it via nitrosation of NAP to S-nitroso-N-acetyl-penicillamine (SNAP) (Figure 5). The aminosilane self-polymerization of the three silylation agents showed the highest levels of amine content and NAP attachment in the case of APTES. Amine levels introduced on the surface of DE particles were up to ten times higher than those when using AHAMTES and APDMES. Interestingly, only up to 2.4 times NAP attachment was observed in the case of APTES functionalization over that of the other two amino-silylation agents. And although the authors offered a plausible explanation of this unexpected behavior, further optimization in increasing NAP attachment (reagent concentration, reaction time, and pH) is being envisaged. The release of NO from functionalized DE particles occurred under in vivo simulated conditions (0.01 M PBS with ethylenediaminetetraacetic acid [EDTA], 37 °C) and nontoxic amounts of NO were released over 24 h, as detected by chemiluminescence. NO release from the functionalized DE particles was demonstrated to have antibacterial properties against gram-positive bacteria S. aureus with more than 90% efficiency, and to being nontoxic toward 3T3 mouse fibroblast cell lines.

The lotus or self-cleaning effect due to the superhydrophobicity (water contact angle greater than 150° and sliding angle less than 10°) exhibited by the leaves of lotus plants (Nelumbo nucifera) can be emulated onto the surface of microparticles.
comprising diatomaceous earth. This enables diatomaceous earth to be suitable for various therapeutic applications that can be facilitated through superhydrophobic property of the surfaces such as the recently indicated reduction of thrombogenicity during blood contact of devices or implants, or to study the collective migration of mammalian cells, among many other related applications. Skeletons from diatoms, and in particular the highly hydrophilic diatomaceous earth, were treated with 3-(heptafluorosilopropoxy) propyltrimethoxysilane (HFIP-TMS) silane coupling agent, which allowed a polystyrene (PS) and poly(vinyl acetate) (PVAc) polymer coating systems to render them superhydrophobic. The presence of HFIP-TMS creates low surface energy which, when combined with the micro-/nanosurface topography of diatomaceous earth, leads to superhydrophobicity. A low amount of HFIP-TMS (approximately 2%) was sufficient to fully cover and to induce superhydrophobicity of the diatomaceous earth’s surface (correlated with a contact angle of 163°). Once a minimum loading amount of diatomaceous earth treated with HFIP-TMS exceeded a threshold of ~40% on the polymer coating, the contact angles of the coatings were independent of the polymer system in use. To be noted is that the minimum amount of silane coupling agent, such as HFIP-TMS, required to induce superhydrophobicity of the diatomaceous earth particles was a function of particle morphology. Therefore, the minimum loading amount of diatomaceous earth treated with HFIP-TMS inducing superhydrophobicity in polymer coatings might vary, as different amounts of fluorosilanes covers the pores and hence decreases the surface area of the particles. The superhydrophobicity of diatomaceous earth particles was also studied using grafted alkyltrimethoxysilane [CH₃(CH₂)ₙ(OCH₃)₃], with an increasing chain length (n = 3, 8, 12, 16, and 18). The morphology of diatomaceous earth, amount of grafted alkyltrimethoxysilane, and chain length played an important role in the formation of superhydrophobic materials. Modification of the diatomaceous earth particles with poly(pentafluorostyrene) (PPFS), poly(stearyl methacrylate) (PSMA), and poly(dimethyl siloxane methacrylate) (PDMSMA) was also attempted to render superhydrophobicity of the particles. This resulted in fully shaped water droplets to be covered with the modified diatomaceous earth particles (Figure 6). Moreover, superhydrophilic patterns can also be created on superhydrophobic fluorosilanized diatomaceous earth particle coated surfaces (glass and polystyrene) using Argon plasma treatment (Figure 6). The tunability of diatom composition in vivo provides an opportunity to confer additional functionality for the enhancement of the properties of the diatom scaffold. In this respect, the centric T. weissflogii diatom cultures were supplemented with titanium (IV) bis-(ammonium lactato)-dihydroxide (TiBALDH), a Ti-based precursor so that they can incorporate TiO₂ during their in vivo frustule synthesis. T. weissflogii diatom when exposed to TiBALDH concentrations up to 200 μM did not modify the architecture, as identified by atomic force microscopy. TiO₂ was successfully incorporated into the diatom, with the presence of Ti being detected using energy-dispersive X-ray analysis (EDX). The TiO₂-modified diatom led to the depletion of E. coli and the oxidative degradation of methylene upon exposure to UV light (Figure 7). Using in vitro/vivo specific modification strategies, the T. weissflogii diatom was also successfully modified with thiol groups. These results are highly significant, as they indicate a strong potential for diatoms to be conferred with therapeutic properties, as the photocatalytic ability of TiO₂ was reported to induce oxidative DNA strand breakage and cancer cell depletion, whilst the introduction of thiol groups allows further functionalization and enhancement in protein stability.

An organic solvent/covalent cross-linking free strategy is a notable example of simultaneous attachment of antibodies and drug molecules to the surface of the diatom-based biosilica. Practically, the Thalassiosira pseudonana diatom was genetically engineered in vivo via live diatom silica immobilization to display an IgG-binding domain of protein G on the biosilica surface. This in vivo modification was followed by attachment of antibodies (Ab) and chemotherapeutic drugs (camptothecin and 7-ethyl-10-hydroxy-camptothecin). Due to their low water solubility, camptothecin (CPT) was loaded into 1,2-dioleoyl-3-trimethylammonium-propane (CPT-DOTAP) liposomes,
whilst 7-ethyl-10-hydroxy-camptothecin (SN38) was loaded into cetyltrimethylammonium bromide (SN38-CTAB) micelles. Both CPT-DOTAP and SN38-CTAB were positively charged, allowing their attachment to the negatively engineered Ab-diatom via electrostatic interactions. This attachment enabled a multi-faceted platform for the targeting and release of the encapsulated chemotherapeutic agents allowing the delivery of a toxic drug dose to tumor cells while leaving surrounding healthy cells intact (Figure 8).

This is not the first example of how diatoms could be used as an advanced cure for cancer. Diatom-based particles have already been shown to act as robust and efficient delivery nanocarriers of poorly soluble anticancer drugs. More importantly, genetically engineered diatoms have been widely explored as a factory of metabolites production. The T. pseudonana diatom displaying an IgG-binding domain of protein G on the biosilica surface has established a new pathway of how genetically engineered diatoms could be used as a new toolbox to develop the next generation of therapeutic devices.

3. Coccolithophores/Coccoliths and Their Therapeutic Potential

Coccolithophores produce protective coccolith plates which are a significant source of calcium carbonate in the ocean thus playing a crucial role in the marine carbon cycle. The potential use of coccoliths in a therapeutic strategy has not been investigated as much as in the case of diatoms, due to their smaller pore size (0.4 nm) than that of diatoms (200–900 nm). It is expected that particles having smaller pores also have a larger specific surface area, thus have a higher drug loading capability. A rough comparison between E. huxleyi coccoliths and hollow diatom frustules reveals a similar surface area of
approximately 19 m² g⁻¹. While porosity within the particle volume plays an important role in the final surface area, the limitation of the pore size has a crucial effect in the molecular attachment and mobility of biomacromolecules.

Nonetheless, as is the case for diatoms, it is also possible for coccolithophores/coccoliths to be modified in vivo/vitro, or coated with materials followed by the removal of the coccolith template to produce coccolith replicas/mimics.

The exploitation of coccoliths as therapeutic carriers offers essential advantages such as a high surface area and readily available intricate mineralized morphology. The surface coverage of coccoliths can be controlled in vitro by tuning the purification process. As a contrast to other similar composition-based particulate drug delivery systems (e.g., synthetic CaCO₃ microparticles), E. huxleyi extracted coccoliths and in vitro modification with enzymes provide almost five times higher surface area than the above mentioned systems, thus increasing the amount of immobilized enzymes (i.e., glucose oxidase and uricase) with preserved activity.

A possible pathway for introducing new therapeutic functionalities into coccolith plates can be achieved in vivo by specific optimization of coccolithophore growth parameters (light, pH, temperature, culture media composition). This enables the coccolithophore cell to produce coccoliths with specific ions or molecules in their composition, specific size, morphology, or porosity.

Sr enhancement of coccolithophore was obtained by controlling the Sr in the culture media which allowed the coccolithophore cell to facilitate the metal ion transfer from the interior of the cell into the final crystalized coccolith plate. Similarly, the growth media of the same coccolithophores was modified with different Zn/Ca ratios and the calcium and zinc uptake inside the cells and final coccolith was characterized.

This offers coccolithophores new functionality for detoxification processes. Interestingly, zinc concentration uptake inside coccolithophores was shown to be tuned with the presence of investigated weak organic ligands (L-cysteine, d-cysteine, l-histidine, l-glutathione, desferrioxamine-B, or phytochelatin) and chelating agents, such as EDTA. The incorporation and regulation in coccolithophore/coccoliths was also demonstrated for boron, barium, magnesium, rare earth elements (samarium, lanthanum, neodymium, terbium, praseodymium), and rare earth elements (samarium, lanthanum, neodymium, terbium, praseodymium). Surprisingly, a correlation between Cu, Cd, and Zn and release of thiol containing compounds (cysteine, glutathione, or γ-L-glutamyl-L-cysteine) from E. huxleyi coccolithophores was identified. A potential avenue for research is to assess whether any of these thiol-based compounds remain incorporated in the final extruded coccolith. New functionalities in the coccoliths using specific thiol post-functionalization can be initiated or harness these properties for molecular factories to initiate the production of relevant therapeutic compounds.

Controlling the growth rate and modulating CO₂ content in culture composition has led to variations in coccolith-associated polysaccharides. The presence of polysaccharides on the coccolith skeleton could allow for further interactions with biomacromolecules to be investigated due to the hydroxyl and carboxyl groups available in the polysaccharide backbone structure.

E. huxleyi coccolithophores are accessible to viral infection, leading to an increase in intracellular reactive oxygen species (ROS) production and termination of phytoplankton blooms. There are instances that have reported that E. huxleyi coccolithophores adapt escaping strategies to the viral susceptibility. The icosahedral giant lytic coccolithoviruses, that specifically infects E. huxleyi coccolithophores, are part of the Phycodnaviridae family with a diameter of approximately
150–200 nm and with functions similar to those observed in animal and plant cells.\(^{[105]}\) The relevance of novel viruses in their design and manipulation for fighting infectious diseases is of relevance in current fields of research in immunomodulation.

Additionally, one feature of the biogenic-based coccolith is that there is no known intricate morphology that can be obtained synthetically, although some design mimics have been attempted (Figure 9). As with other types of calcite (CaCO\(_3\)) such as chalk, natural calcite, or synthetic calcite, coccoliths present very similar infrared (IR) patterns, but with a slight content (below 1%) of polysaccharides as demonstrated by a combination of IR spectroscopy and density functional theory.\(^{[106]}\)

There are many studies that report on the use of CaCO\(_3\) in potential therapeutic applications,\(^{[110]}\) elucidating the route of administration and preclinical safety.\(^{[111]}\) These studies serve as an inspiration for the use of coccoliths, as these are far stable structures and with the added advantage of increasing the amount of delivered therapeutics. Coating of the coccolith surface with mussel-bioinspired polydopamine as has been recently demonstrated for diatoms\(^{[112]}\) will enable new routes of post-functionalization\(^{[113]}\) and facilitate identification of novel properties of the combined polydopamine-coated coccoliths.

4. Phytoplankton and Their Role in Drug Discovery

Natural products derived from marine organisms provide a unique structural diversity over that of synthetic chemistry compounds which presents opportunities for discovering novel bioactive molecular compounds. Biodiscoveries from phytoplankton is currently a very active and extensive area of research with many insightful and broad reviews covering the topic.\(^{[114]}\) Many (micro)algal and invertebrate species are found only in marine environments, thus possessing different biosynthetic routes from terrestrial counterparts which may potentially represent a source of novel metabolites.\(^{[115]}\) The marine organisms are known as efficient producers of bioactive secondary metabolites or active therapeutic compounds. Recent discoveries represent considerable experimental evidence that bioactive molecules isolated from marine organism such as sponges, (micro)algae, mollusks, and coral are produced by microbial symbionts.\(^{[116,117]}\) Among all the investigated marine organisms, marine phyla such as Porifera and Cnidaria have been recognized as the richest source of marine natural compounds.\(^{[117,118]}\) These natural products have been documented as a source of anticancer compounds resulting from sponge–microbe symbiotic associations.\(^{[119]}\) Multiple studies have demonstrated a wide range of biological activities for these compounds including anticancer, antibacterial, inflammatory, immunosuppressive, neurosuppressive, neuroprotective, and antifouling, amongst other bioactivities.\(^{[12]}\)

For instance, diatoms are well known to produce useful carotenoids, polysaccharides, and many other known or unknown metabolites with promising therapeutic applications (Figure 10).\(^{[120,121]}\)

Unfortunately, coccolithophores lag behind diatoms in terms of their potential for biodiscovery. Nevertheless, advances in
technology, improved molecular modeling, and sequencing have already opened doors to genetic engineering tools for modifying microalgae in the discovery of therapeutically active compounds.\textsuperscript{122}

5. Phytoplankton-Based Molecular Factories

Phytoplankton have been established as fast-growing sustainable green molecular factories due to their great potential for producing value-added metabolites with therapeutic applications.\textsuperscript{123} An interesting aspect of microalgae is their visualization as drug delivery devices and therapeutic molecular factories based on active biocompounds/particles synthesized and in situ released into the surrounding medium. Though some of the released compounds may be identified as toxins posing a threat for human health,\textsuperscript{124} others, conversely—such as polysaccharides—are found to have beneficial antioxidant properties.\textsuperscript{125}

Like exosomes, that come equipped with specific biomolecular information and proven “self-therapeutic” clinical applications,\textsuperscript{126} phytoplankton have recently been shown to produce vesicles inducing melanoma cancer cell apoptosis in vitro.\textsuperscript{127} Specifically, \( \approx 80 \)-nm-sized nanoparticles (NPs) were originated from \textit{Dunaliella salina} microalgae simply by 45 min microwave irradiation of the phytoplankton suspension. These phytoplanktonic NPs carried \( \beta \)-carotene, a vitamin A precursor and ubiquitously found in plants and fruits, has known anticancer effects. Only under ultraviolet irradiation (362-nm-UV lamp), the encapsulated carotenoid inside spherical NPs produced ROS such as single oxygen which was responsible for approximately 80% cell apoptosis due to the burst of ROS and phototoxicity. Interestingly, the authors noted that the levels of ROS were high even 44 h post-irradiation in the NPs-treated C32 human melanoma cells as compared with the irradiated cell in the absence of NPs. This overcomes one of the challenges of photodynamic therapeutic agents with limited efficiency of singlet oxygen production (Figure 11).

6. Progress Toward Phytoplankton-Based Artificial Organelle

We have recently reviewed various micro- and nanoreactors fabricated either using a sacrificial template or via a self-assembly process, for their therapeutic potential.\textsuperscript{128} A 3D micro-/nanoreactor consists of a polymer capsule with an inner core and an outer shell/ membrane. The inner core could be used to encapsulate a biomacromolecule, while the polymer shell or membrane can be engineered to tune up its permeability and control the reactants/products entering or exiting the capsule. Similarly, instead of encapsulating a biomacromolecule,\textsuperscript{129} live cells could alternatively be entrapped.\textsuperscript{130} Microalgae can be regarded as a live cell-based micro-bio-factory due to their rich source of released metabolites. Embedding them inside a polymer microcapsule is an elegant way of mimicking natural subcellular organelles, or even to act as tuneable cell mimics for therapeutic applications. Recently the \( \text{CO}_2 \)-tolerant microalga, \textit{Chlorella} sp. KR-1, was embedded inside the precipitated rectangular-shaped \( \text{CaCO}_3 \) crystals.\textsuperscript{131} This was followed by the coating of the microalgae-\( \text{CaCO}_3 \) crystals with six consecutive polyelectrolyte layers consisting of poly(allylamine hydrochloride) (PAH) (first positively charged layer)/poly(sodium 4-styrenesulfonate) (PSS) (last negatively charged layer). Once the coating was done via the layer-by-layer (LbL) technique, the sacrificial \( \text{CaCO}_3 \) template was solubilized using mild conditions in 0.2 M EDTA, 1-h incubation. This resulted in a polyelectrolyte layer shell with several microalgae freely suspended inside the empty core of the capsule (Figure 12). The shell of the capsule provided enough permeability to allow nutrients to access the encapsulated microalgae enabling their growth for up to 3 days, as demonstrated using light and fluorescence microscopy. Moreover, the size and shape of the sacrificial template can be controlled via the concentration of precursor (i.e., \( \text{CaCl}_2/\text{Na}_2\text{CO}_3 \)), while the polymer shell could be functionalized with carbon nanotubes (CNTs), Au nanoparticles, or Fe\(_3\)O\(_4\) nanoparticles.

Recognizing the versatility of this approach, it is possible to envisage the encapsulation of other types of microalgae (i.e., diatoms and coccolithophores), and their potential resulting stability, biocompatibility, or biodegradability, as well as their potential for cytotoxicity studies. Therefore, progress toward a real therapeutic application involving phytoplankton encapsulated inside polymer capsules is expected.

7. Phytoplankton-Derived Bioinspired Mimics

Identification of new strategies for mimicking the inorganic skeleton of marine-based intricate structures such as coccolithophores and other microalgae’s complex architecture might lead to synthetically produced morphologies adaptable to the application of interest such as biomedical implants or light-weight ceramics. The first attempt in this direction was made by Walsh and Mann in 1995, in which they demonstrated the fabrication
of micrometer-sized hollow porous spheres with honeycomb microstructures.\(^{132}\) The synthetic hollow porous spheres were obtained using polystyrene beads of micrometer size as a sacrificial template and an oil-water-surfactant emulsion approach. The final architectures had: i) a composition of calcium carbonate (aragonite) and magnesium traces; ii) irregular-shaped tubular pores interconnected via a crystalline wall network; iii) with tunable pore size as a function of water:oil ratio, to resemble the spherical calcisphere of calcareous dinoflagellate *Thoracosphaera heimi*.  

Synthetic CaCO\(_3\) structures stabilized by iminodiacetate-substituted organic ligands such as 2,2'-\(\text{H}_2\text{bza}^{\text{DA}}\) led to supramolecular coordination assemblies that self-organized into hierarchical calcite aggregates, some of these having partial similarity in morphology with *E. huxleyi* or *Gephyrocapsa oceanica* coccolithophores.\(^{133}\) The synthetic coccosphere (\(\approx 10 \mu\text{m}\) in diameter) comprised a shell of 7–11 specific hollow centered calcite discs similar to the morphology, structure, and configuration of a natural coccolithophore (Figure 13).

Complex architectures closely resembling the structure of diatom cells were investigated by employing the formation of micelles as a sacrificial template based on cetyltrimethylammonium bromide (CTAB) cationic surfactant and tetraethyl orthosilicate (TEOS) as silica precursor in a solvent mixture of water and alcohol containing ammonia as catalyst.\(^{135}\) Using this procedure, monodisperse raspberry-like mesoporous silica nanoparticles or diatom cell mimics (DMIMs) were obtained. DMIMs morphology, size (100–600 nm) and 2 nm hollow SiNPs doped on their surface were tuned as a function of ethanol:water ratio, CTAB, and TEOS concentration. Diatomite (*Coscinodiscus wailesii* diatom) could be used as a biogenic template to deposit graphene powders via a chemical vapor deposition (CVD) technique, thus generating graphene...
powders as a low cost and impurity free mass-produced biomorphic material.[136] The diatomite resisted to a temperature of around 1000 °C, necessary for the vapor chemical deposition of graphene. Upon wet-etching removal of the diatomite template, the deposited graphene perfectly preserved the original shape of the diatomite (Figure 14). Interestingly, compared with diatomite, the graphene diatomite mimic revealed a surface area that was more than 100 times larger. Also compared with previously reported solution-based counterparts, the graphene mimic showed better dispersibility in N-methyl-2-pyrrolidone solvent, exhibited blue photoluminescence and much higher electrical conductivity, among many other enhanced properties.

It is worth noting that graphene powders are widely investigated for biomedical applications, some relevant applications including chemically modified graphene for single-bacterium, DNA, pH, or protein adsorption sensing,[137] but also as a versatile therapeutic device in bioimaging, drug delivery, or tissue engineering applications.[138]

Mimicking silica polymerization of diatom cell walls with respect to polycationic peptides (sillafins) and LCPAs followed by their self-assembly behavior is another route for nano- and microdevices development with possible therapeutic applications.[139] In this regard, a novel deacetylated poly-N-acetyl glucosamine (pGlcNAc) which was derived from a marine diatom was used to form a hydrogel for treatment of the degenerating intervertebral disc.[140]

By creating a (bio)macromolecule layer on the frustule surface, followed by the diatom dissolution, the design of diatom mimics is allowed. As an illustration, polymer brushes such as grafting poly 2-(dimethylamino) ethyl methacrylate-co-ethylene glycol dimethacrylate (poly(DMAEMA-co-EGDMA)) polymer, using deactivation enhanced atom transfer radical polymerization (DE-ATRP), were grown from the centric diatom T. weissflogii.[141] The resulting polymer chains could be cross-linked due to the newly introduced vinyl groups present on the polymer chain inducing a rigid structure not breakable during dissolution under basic conditions (1 M, NaOH, 6 h) of the diatom. Diatom replicas, such as 3D polymer structures based on poly(DMAEMA-co-EGDMA), could lead to further functionalization, with potential applications in the delivery of therapeutics.[141]

Similarly, the use of diatom frustules can also serve as a template to fabricate complex nanostructures not attainable...
by conventional techniques such as a high-precision gold nanostructure.[142] Furthermore, silica-based diatom frustules could be turned into MgO-based diatom frustules through a shape-preserving displacement conversion process.[143] This enables diatom silica-based material to be transformed into new oxides or oxide-bearing composites while retaining their 3D morphology and nano-structure features.

The fragmentation of the diatom siliceous skeletons to “frustules” diatomite powder represents a source of silica-based DNs and use as nontoxic therapeutic agents. For instance, nanovektors comprising DNs of up to 450 nm in diameter with small interfering ribonucleic acid (siRNA) successfully transfected H1355 human epidermoid cancer cells leading to gene silencing.[144]

The presence of proteins and amine-derived diatoms could serve as new tools for the fabrication of therapeutic devices such as bone promoting medical implantable devices. A dual marine-bioinspired attempt was demonstrated in this sense in the engineering of a silica-protein glue, via genetic fusion of R5 peptide derived from *Cylindrotheca fusiformis* diatom and mussel recombinant adhesive protein (MAP).[145] Following bacterial expression and purification, the fused proteins were deposited on titanium surfaces and silificated, the proteins acting as glue spots for the formed SiNPs. Once coated, the roughness of the titanium surface increased as a function of the protein glue-SiNPs composites layer addition using the LbL approach, reaching a maximum roughness >4.0 μm (excessively rough) which is attributed to surface saturation. Mechanical testing revealed that increasing the number of up to six protein glue-SiNPs composite layers also imposed higher resistance to local elastic and plastic deformation than the monolayer assembly. Rougher titanium surfaces (~4.0 μm) led to an increase in osteoblast attachment but with a lower proliferation level and change in cell morphology detected on excessively rough surfaces (>4.0 μm) after 72 h. In vitro testing of preosteoblast behavior on titanium-modified surfaces in terms of focal adhesion polymerization and expression of focal adhesion-related genes (β-integrin, FAK, and Ras homolog gene family member A) and analysis of osteogenic differentiation revealed that the most appropriate candidate for in vivo implantation was the multilayered protein glue-SiNPs composite-modified titanium surfaces with a medium degree of roughness (between 1.0 and 2.0 μm). Medium rough protein glue-SiNPs composite-modified titanium surfaces were implanted in 8-weeks-old male Sprague-Dawley rats with critical-sized calvarial defect site which revealed an osteopromotive ability that was almost double that with a nonmodified titanium mesh surface (Figure 15), proving the therapeutic benefit of the diatom–mussel dual bioinspired medical implant.

8. Phytoplankton-Mammalian Cell Co-Cultures Inside 3D Scaffolds

Creating a 3D tissue requires a specified thickness, uniformity of tissue architecture, and maintained functionality.[146] Simultaneously, levels of nutrients and, most crucially, oxygen, must be controlled throughout the construct volume for clinical relevance. The stacking of multiple monolayer cell-sheets on top of the other results in a 3D cell culture resembling a real-life tissue.[147] This strategy allows the formation of thick 3D tissues with enough stability to support the cells and without the need for designing an extra scaffold. The viability of such a scaffold is more probable when proper oxygenation and nutrient removal is achieved. A solution is to mix mammalian cells with microalgae, the latter acting as an oxygen source provider. For instance, *Chlorococcum littorale* microalgae were able to produce oxygen in a cell culture media with increased oxygen concentrations at 30 °C. This was enough for the survival of a C2C12 or a rat cardiac single-layer cell sheet. Furthermore, when microalgae were co-cultured with a rat cardiac 160 μm cell sheet, a significant decrease in glucose consumption and lactate production was observed which correlated with favorable ATP production in aerobic cell respiration. Interestingly, the histological examination of the 3D tissue layer revealed damaged tissue in the absence of microalgae in the co-culture and intact layer sheets in the presence of the microalgae. One-fifth less creatine kinase—an indicator for the cardiac tissue damage—and attachment of microalgae co-culture with the thick cardiac cell sheet layer for six days was observed, thus confirming the benefit of microalgae in the production of 3D clinically relevant thick healthy tissue constructs (Figure 16).

Living cells such as mammalian cells require highly porous extracellular-based 3D scaffolds to grow and generate tissue matrix.[148] Therefore, entrapment of microalgae living cells in 3D structures, or active immobilization,[149] is a strategy that should generate intense research interest for tissue engineering and regenerative medicine. Among phytoplankton, *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* are two main microalgae with therapeutic benefits and are well known as cellular factories for engineering protein therapeutics or having anti-tumor effects.[150] By using 3D bioprinting of cells into scaffolds, *C. reinhardtii* and *C. sorokiniana* microalgae were imbedded in a highly structural organization alginate-based hydrogel scaffold.[151] 3D bioprinting has emerged as a viable technique to design and create scaffolds embedded with cells for tissue and organ growth with clinically relevant size, shape, and structural integrity.[152] This involves a computer-controlled 3D printer able to transform a computer aided design into rapid and high-precision construction of 3D biological structures using an appropriate bioink (Figure 17A).[153] Bioprinted microalgae into alginate-based hydrogels were subjected to various cultivation temperatures and modes of illumination and compared with the same microalgae suspension cultures under similar conditions.[154] During the photoautotrophic cultivation, the physiological state (i.e., viability) of microalgae populations was monitored using single-cell flow cytometry analysis (microalgae suspension cultures) and, based on chlorophyll autofluorescence and fluorescent-dye-based membrane staining, fluorescence image analysis (microalgae imbedded in hydrogel). Data analysis revealed that viability of *C. reinhardtii* microalgae increased approximately 30% when immobilized in the alginate microgel, irrespective of the temperature and illumination conditions. As a contrast, *C. sorokiniana* microalgae showed a viability increase of around 23% when immobilized in the alginate-based hydrogel as compared with the suspension culture, only under specific temperature and illumination optimized parameters (37 °C, 24/0 h light/dark continuous illumination) (Figure 17B,C). These results, illustrating that the scaffold assists microalgae support and growth, were published almost 1 month after the first ever study (2015) in which mammalian cells such as human...
cell line SaOS-2 were embedded along with the C. reinhardtii microalgae in alginate/methylcellulose scaffold using a green bioprinting technique (Figure 17). Also, in this case, the viability of the microalgae was found to be high and the scaffold was found to be providing a fertile growth environment for the microalgae even after 12 days. The methylcellulose from the printing composition of the scaffold is removed during the cross-linking process forming pores into the final scaffold that act as spaces to accommodate the microalgae and support their development. Following this, mammalian cells were co-printed along with the microalgae within the alginate-based scaffold. Although the role of microalgae is to provide a source of oxygen and metabolites for a long-lasting delivery of the mammalian cells within the scaffold, the viability was only confirmed after 12 h post-printing and incubation, with longer time being required for further optimization (Figure 17D–H).

Instead of using bioprinting, microalga Spirulina (Arthrospira) was incorporated within poly-γ-d,l-lactic acid (PDLLA) scaffolds fabricated by the electrospinning method. Spirulina-PDLLA scaffolds promoted the adherence and proliferation of mesenchymal stem cells (MSCs) extracted from C57/B16N mice liver.

Properties of the inexpensive and abundant silica diatomite particles (SiO₂·nH₂O) such as high porosity and wettability, mechanical enhancement when combined with other biomaterials (e.g., chitosan), and promoter of cell proliferation makes it a candidate for the design of therapeutic devices. For instance, silica diatomite particles were recently combined with marine cartilaginous extra cellular matrix chitosan to serve as a scaffold material for bone tissue engineering applications. Overall, the amount of diatomite influenced the porosity and pore morphology of the final chitosan/diatomite scaffold, thus allowing the tuning of the mechanical properties, porosity within the limits of trabecular bone (80–90%), pore size range (218–319 μm), wettability, protein adsorption, scaffold degradability, (bio)mineralization, osteoblast adherence, and proliferation. Similarly, silicon-releasing diatom particles (DPs) were combined with silk fibroin scaffolds to improve the osteogenic properties of osteoblast-like cells.
Figure 16. A) Microscope image of *Chlorococcum littorale* microalgae (left) and the schematic drawing of culturing the microalgae, or co-culturing the microalgae with mammalian cells. Oxygen production is controlled via a microalgae light-induced photosynthesis process (right) and serves as a source of survival for the mammalian cells within the 3D tissue construct: an increase in dissolved oxygen is observed when the mammalian cells (C2C12 or rat cardiac cell sheet) are B) co-cultured in the presence of microalgae (grey bar) in contrast to the low amount of dissolved oxygen in the absence of microalgae (black bar). C) Histological observation of the 3D tissue constructs shows they are intact when microalgae are present in the system (right) and damaged in the absence of microalgae (left). D) Almost 80% of the tissue cell sheet without microalgae is detached (left), while the presence of microalgae preserved the attachment and thus the survival of the tissue construct for up to 6 days (right). Data in (B) is expressed as means ± standard deviation (in three different points, n = 2). Two group comparison: unpaired Student t-test. Multiple group comparison: Ryan’s method. *p < 0.05. Representative images are shown in (C) (n = 3). Reproduced under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0).

Co-entrapment of phytoplankton along with zooplankter (i.e., *Daphnia magna*) calcium alginate capsules embedded in a silica matrix has also been reported which could be developed into a biosensing device. In addition, microalgae encapsulated inside silica hydrogels via proton driven alginate gelation were shown to act as microreactors for the biosynthesis of gold nanoparticles. Microalgae seem to be promising in providing oxygen and nutrients for damaged, hypoxic tissue which is still a major limiting factor of regenerative therapies based on cell transplantation for wound healing. In this regard, Egaña et al. propose the use of photosynthetic materials such as microalgae to serve as a source of oxygen during tissue engineering. For this, *C. reinhardtii* microalgae were directly added and cultured inside the porous structure of an FDA-approved, commercially available, cross-linked bovine tendon collagen and glycosaminoglycan matrix dressing wound. It appeared that by delivering the microalgae with a fibrin-based hydrogel, an improved distribution and integration of the microalgae within the collagen scaffold was achieved, along with a lower possible immunogenicity coming from the microalgae. Furthermore, implantation of the microalgae-fibrin dressing wound containing an additional silicon layer on top as a temporary epidermis in the back of athymic bilateral full skin defect nude mice, revealed viability and allowed vascularization for 5 days. Unlike the implantation of the wound dressing in the absence of microalgae (control group), no significant difference was observed in the size of organ weight (spleen and lymph nodes), immunoglobulin M (IgM) and G (IgG), or inflammatory cytokines systemic inflammatory effects. This was corroborated with a lower number of macrophages identified when the microalgae-fibrin dressing wound was injected into the transgenic zebrafish larvae in vivo-established model, expressing green fluorescent protein (GFP) in macrophages, in contrast with the macrophage activity where only microalgae medium or infectious *E. coli* bacteria cell suspension expressing red fluorescent protein (RFP) was injected. This confirmed the low local and systemic inflammatory response of the *C. reinhardtii* microalgae in vivo. Though diatoms proved to be nontoxic toward normal human cells as previously noted, evidence for cytotoxicity of using microalgae for therapeutic purposes is still scarce and ethical issues regarding the bioprinting process remain under debate, depending on the specificity of the clinical case. While these therapeutic approaches seem feasible, open questions remain as to how much oxygen is provided by the microalgae once used as an implant, since oxygen dosing appears to be critical for the degree of wound healing. This opens the path for further exploration and optimization.

Nevertheless, the combination of diatoms and coccolithophores/coccoliths with or without functionalization as therapeutic reservoirs along with mammalian cells inside extracellular-matrix-based scaffolds should be strongly encouraged.
Figure 17. A) Bioprinting process exemplified with an integrated tissue and organ printing device. This system comprises a closed chamber with four injection pressure-controlled cartridges able to move on a stage via an XYZ-stage controller to pattern various cells and biomaterials. In this illustration, poly(ε-caprolactone) (PCL) polymer (temperature controlled) and a sacrificial Pluronic F-127 hydrogel are used to obtain the 3D printed construct of a multiple cell-laden composite hydrogel. A 3D CAD model is obtained from the medical imaging (MRI, CT) data. This is converted to a visualized motion program with the necessary instructions for XYZ-stage movements and actuating pneumatic pressure to obtain the printed 3D shape imitating target tissue or organ. Growth rate of B) *Chlamydomonas reinhardtii* and C) *Chlorella sorokiniana* microalgae in suspension versus immobilized under different temperature and illumination conditions. D) A 3D model of alginate/methylcellulose scaffold containing *C. reinhardtii* microalgae and human cell line SaOS-2 and E–H) post-printing scaffold visualization via fluorescence microscopy. The bar graphs in (B) and (C) are represented with errors bars meaning the standard deviation (*n = 2*). A) Reproduced and modified with permission. Copyright 2016, Springer Nature. B,C) Reproduced and modified with permission. Copyright 2015, John Wiley & Sons. D–H) Reproduced and modified with permission. Copyright 2015, John Wiley & Sons.

Figure 18. Implantation of the microalgae-fibrin dressing wound in vivo: A) athymic bilateral full skin defect nude mice or F) transgenic zebrafish larvae. The photosynthetic scaffold A) remained green for 5 days, B,C) there were no signs of infection inflammation at the wound area, and D,E) promoted vascularization. F,G) Inflammatory response of microalgae (*Chlamydomonas reinhardtii*, green) was negligible as compared with the presence of microalgae culture medium only (control), or *Escherichia coli* (red). Scale bars: 1 cm (A), 4 mm (B–D), 1 mm (E), 500 μm (F, upper), and 100 μm (F, lower). Representative results in A–E (*n ≥ 8*). Representative data in F (*n ≥ 3*). Data in G is expressed as mean ± SEM (*n ≥ 3*), two-tailed Student’s t-test (*p ≤ 0.05, ***p ≤ 0.005). Reproduced with permission. Copyright 2014, Acta Materialia. Published by Elsevier.
9. Conclusions

The aim of this review is to broadly cover recent therapeutic applications of the unique morphologies derived from phytoplankton. The main focus is directed toward diatoms and coccoliths, in terms of in vitro or in vivo surface functionalization as carriers for drug delivery, diagnostics, or combination of microalgae-based mimics. While phytoplankton also serve as reservoirs per se, there are known and yet to be discovered metabolites with attractive therapeutic efficacy.

Therapeutic carriers using synthetic particles from calcium and porous silica have been used extensively to overcome the limitations of insoluble drugs, especially in chemotherapy. However, the synthesis of these particles, especially mesoporous silica particles, presents many challenges such as high cost, use of toxic materials that have potential environmental impact, or lack of scalability. In contrast, calcium carbonate microparticles require an easy fabrication process, but their stability and the precision in controlling their morphological features remain questionable. Nature, on the other hand, provides an attractive option by which to address some of these challenges with remarkable and intricate micro-/nanoscale pores of phytoplankton-derived architectures.

The diatom is a unique template for drug delivery applications specially due to its species-specific architecture (highly ordered pores, large surface area) and flexibility for surface/composition in vitro or in vivo modifications. Diatoms have been intensively investigated for a variety of biomedical applications, and their potential as the next generation of therapeutic devices has yet to be fully exploited. There are still particular challenges in tailoring specially to enable surface functionality of the diatom to achieve the required drug loading and release within the range of specific therapeutic applications. Nevertheless, the use of diatoms for biomedical purposes requires a more detailed investigation related to their cytotoxicity, in vivo biodistribution, and biodegradability studies.

The diatom surface can be coated with various materials followed by the removal of the diatom sacrificial template to create diatom-derived mimics. These mimics allows a preserved architecture and morphology of the initial diatom template providing novel properties of the coated material. This opens a new area of research and more effort should be invested, due to the multitude of materials available to be coated on the surface of the diatoms.

As well, the in vivo modification of phytoplankton provides a vast realm for bioprospecting. With the latest advances in genetic engineering, phytoplankton promise a stage for the identification or design of novel therapeutic compounds which have a potential in treatment of current incurable diseases.

The use of microalgae within scaffolds as a source of oxygen and genetically engineered providers of therapeutic metabolites in hypoxic conditions for tissue regeneration represent a smart strategy. Unfortunately, there are still challenges to overcome in this direction, such as controlling the amount of light versus survival rate of microalgae. It would also be interesting if this strategy could be used as a future application in chronic wounds.

Unfortunately, in vitro modification of coccoliths is almost inexistent and more research outcome in this direction is envisaged. Most of the studies focused on the in vivo modification of coccolithophores, while their mineralization process is still under debate and remains to be fully understood. Modification of coccolith composition is expected to induce a scaffold with tunable and intricate composition compatible with specific therapeutic applications.

The beauty and elegant perfection of phytoplankton and phytoplankton-derived constructs has provided a high level of bioinspiration from which technological advances have been largely profited. Phytoplankton are great candidates for use in biomimicry and the design of a new era of biomedical devices, though they are still in their early stage of investigation leaving room for further exploration and improvement.

Acknowledgements

This publication has emanated through the financial support of Science Foundation Ireland (SFI) co-funded under the European Regional Development Programme, Grant Number 13/RC/2073. M.L. is supported through a Swiss National Science Foundation Early Postdoc Mobility Fellowship (P2BSP3_174974). The authors would like to thank Maciej Doczyk (http://doczykdesign.com) for his support in the preparation of the concept figure and to Anthony Sloan for his help with English corrections.

Conflict of Interest

The authors declare no conflict of interest.

Keywords
coccolith, diatom, phytoplankton, therapeutics

Received: July 15, 2018
Revised: September 24, 2018
Published online: October 17, 2018
Adv. Therap. 2019, 2, 1800099

1800099 (22 of 24) © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

M. Mishra, A. P. Arukha, T. Bashir, D. Yadav, G. B. K. S. Prasad, Front. Microbiol. 2017, https://doi.org/10.3389/fmicb.2017.01239

M. Hildebrand, S. J. L. Lerch, R. P. Shrestha, Front. Mar. Sci. 2018, https://doi.org/10.3389/fmars.2018.00125

B. Delat, V. C. Sheppard, S. R. Ghaemi, S. Rao, C. A. Prestidge, G. McPhee, M.-L. Rogers, J. F. Donoghue, V. Pillay, T. G. Johns, N. Kröger, N. H. Voelcker, Nat. Commun. 2015, https://doi.org/10.1038/ncomms9791

C. Gallo, G. Nuzzo, G. d’Ippolito, E. Manzo, A. Sardo, A. Fontana, in Marine Enzymes and Specialized Metabolism, Vol. 605 (Eds: B. S. Moore), Academic Press, Cambridge, MA 2018, Ch. 5.

R. W. Drum, R. Gordon, Trends Biotechnol. 2003, 21, 325.

S. Maher, T. Kumeria, Y. Wang, G. Kaur, F. Fetih, A. Santos, F. Habib, A. Evdokiou, D. Losic, Adv. Healthcare Mater. 2016, 5, 2667.

M. S. Aw, M. Bariana, Y. Yu, J. Addai-Mensah, D. Losic, J. Biomater. Appl. 2013, 28, 163.

A. Watermann, J. Brieger, Nanomaterials 2017, 7, 189.

J. Chhablani, A. Nieto, H. Hou, E. C. Wu, W. R. Freeman, M. J. Sailor, L. Cheng, Invest. Ophthalmol. Vis. Sci. 2013, 54, 1268.

S. Murugadoss, D. Lison, L. Godderis, S. van den Brule, J. Mast, F. Brassinne, N. Sebaihi, P. H. Hoet, Arch. Toxicol. 2017, 91, 2967.

N. Ptylik, E. Brunner, MRS Commun. 2018, 8, 322.

C. de Vargas, M.-P. Aubry, I. A. N. Probert, J. Young, in Omics Technologies and Bio-Engineering: Towards Improving Quality of Life, Vol. 1 (Eds: D. Barh, V. Azevedo), Academic Press, Elsevier, London 2018, Ch. 12.

J. R. Young, K. Henriksen, Rev. Mineral. Geochem. 2003, 54, 189.

K. Xu, D. Hutchins, K. Gao, PeerJ 2018, https://doi.org/10.7717/peerj.4608

B. N. Jaya, R. Hoffmann, C. Kirchlechner, I. A. N. Probert, J. Young, in Omics Technologies and Bio-Engineering: Towards Improving Quality of Life, Vol. 1 (Eds: D. Barh, V. Azevedo), Academic Press, Elsevier, London 2018, Ch. 12.

H. Tsutsubi, S. R. W. Jordan, J. Microscopica Ultrastruct. 2018, 37, 249.

a) C. Brownlee, G. L. Wheeler, A. R. Taylor, Semin. Cell Dev. Biol. 2015, 46, 11; b) A. B. Read, T. M. Wahlund, in Handbook of Biominalization, Vol. 1 (Ed: E. Bäuerlein), Wiley-VCH, Weinheim, Germany 2008, Ch. 13; c) L. Berry, A. R. Taylor, U. Lucken, K. P. Ryan, C. Brownlee, Funct. Plant Biol. 2002, 29, 239; d) M. E. Marsh, Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol. 2003, 136, 743.

B. A. Read, J. Kegel, M. J. Klute, A. Kuo, S. C. Lefebvre, F. Maumus, A. I. Ianora, in Grand Challenges in Marine Biotechnology, Vol. 1 (Eds: P. H. Rampelotto, A. Trincone), Springer, Switzerland 2018, Ch. 11.

W. Gao, R. Dong, S. Thanphhiwatana, J. Li, W. Gao, L. Zhang, J. Wang, ACS Nano 2015, 9, 117.

a) U. Riebesell, I. Zondervan, B. Rost, P. D. Tortell, R. E. Zeebe, F. M. M. Morel, Nature 2000, 407, 364; b) C. Salaviale, B. Gollain, E. Mattioli, Palaeoecogr., Palaeoclimatol., Palaeoecol. 2018, 490, 240.

A. W. Skeffington, A. Scheffel, Curr. Opin. Biotechnol. 2018, 49, 57.

S. Sengupta, M. E. Ibele, A. Sen, Angew. Chem., Int. Ed. 2012, 51, 8434.

W. Gao, R. Dong, S. Thanphhiwatana, J. Li, W. Gao, L. Zhang, J. Wang, ACS Nano 2015, 9, 117.

P.-W. Zhai, Y. Hu, C. R. Trepte, D. M. Winker, D. B. Josset, P. L.ucker, G. W. Kattawar, Opt. Express 2013, 21, 17623.

J. W. Blunt, A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers, M. R. Prinsep, Nat. Prod. Rep. 2018, 35, 8.

C. Lauritano, A. Ianora, in Grand Challenges in Marine Biotechnology, Vol. 1 (Eds: P. H. Rampelotto, A. Trincone), Springer, Switzerland 2018, Ch. 11.
