Review

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The Glass Menagerie: diatoms for novel applications in nanotechnology

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Diatoms are unicellular, eukaryotic, photosynthetic algae that are found in aquatic environments. Diatoms have enormous ecological importance on this planet and display a diversity of patterns and structures at the nano- to millimetre scale. Diatom nanotechnology, a new interdisciplinary area, has spawned collaborations in biology, biochemistry, biotechnology, physics, chemistry, material science and engineering. We survey diatom nanotechnology since 2005, emphasizing recent advances in diatom biomineralization, biophotonics, photoluminescence, microfluidics, compustat domestication, multiscale porosity, silica sequestering of proteins, detection of trace gases, controlled drug delivery and computer design. Diatoms might become the first organisms for which the gap in our knowledge of the relationship between genotype and phenotype is closed.

Why diatoms?

Why have grown men and women spent lifetimes, often unpaid and while pursuing other careers (Box 1), examining one division of single-celled algae over the course of more than two centuries? The answer lies in their inordinate beauty: the shells around each cell of Bacillariophyta [1], the diatoms, are made of amorphous, clear silica glass, more ornate [2] than the finest delicate crystal that human artisans have crafted [3]. Indeed, when designing buildings and aircraft, architects and engineers have applied the same structural principles in their work as diatoms use to create their shells [4–7], and now nanotechnologists are turning to diatoms to build a variety of devices [8]. There are ~250 living diatom genera with more than 200 000 estimated species classified by their unique morphologies [9] (Figure 1). Diatoms are also remarkable living creatures with significant biogeochemical [10] and ecological roles on this planet, including '~20–25% of the world net primary production' [11]. Their extraordinary diversity might be due to in part to rapid rates of horizontal gene transfer with many bacteria [12]. Here we will provide an update on our previous reviews published in this journal [13,14] and a compendium [15] that covered the status of the field as of 2005.

The basic advantage of diatoms for nanotechnology over standard photolithography methods (microelectromechanical systems [MEMS]) [16] is that diatoms grow in exponentially increasing numbers on surfaces [17] or in solution [18], whereas MEMS are manufactured in numbers that grow linearly with time. With MEMS, we build to our own design. With diatoms, we either select from available species or attempt to modify their morphogenesis. Doing the latter requires that we understand how diatoms build themselves. Generally, we expect industry to be utilizing basic research, but a counterintuitive consequence of the thrust to use diatoms industrially is an enormous industrially motivated growth in the basic science of diatoms [19]. For example, because diatoms are much like the rest of eukaryotic life in their fundamental biology, diatom nanotechnologists are inadvertently contributing to the solution of one of the major outstanding problems of biology, namely the possibly reciprocal relationship [20] between the one-dimensional, linear, sequenced genotype [12,19,21,22] and the chemistry and physics of the multidimensional phenotype. Diatoms provide a crucial testbed for the reductionist concept of 'specific gene products (proteins) guiding these biomineralization processes' [23]. Given the significant intraspecific variability of diatoms [24], the presumption that they are 'under precise genetic control' [25] might be an exaggeration, but a testable one.

Diatom silica structure

Diatoms are microscopic (2 μm to 2 mm [26], cf. Figure 1), and species are classified mostly by the shapes and patterns of their hard silica parts, so the foci of diatom taxonomists and nanotechnologists coincide. The silica shell, or 'frustule', consists of two overlapping valves joined with girdle bands [1], much like a Petri dish (Figure 2). There are two major groups that are separated based on valve symmetry [1]. The pennate diatoms are elongate, usually with bilateral symmetry. In the class of centrics, diatoms have radial symmetry (Figure 3a). A proper group theory analysis of diatom symmetries has yet to be done, but the centrics might be said to have n-fold two-dimensional (2D) rotational symmetry, with $n = 3$ on up, approaching full circular symmetry. The pennates are placed into two classes depending on whether or not they

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Box 1. Gentlemen diatomists of independent means

There was not a single paid professional 'diatomist' on Earth until ~1930. Diatom studies are the classic example of the gentleman amateur scientist. For instance, the leading British diatomist around 1855, William Smith [137], was a reverend. Adolf Schmidt, the man who started the world-famous gigantic Schmidt Atlas in 1874 [138], should be given the honour due to him by referring to him as 'the Archidiaconus [archdeacon] Schmidt' – certainly the most impressive title a diatomist could have.

Towering giants of the Victorian period, when the entire basis of diatom studies was founded, include the coauthors Albert Grunow (an Austrian naturalist and phycologist) and Per Teodor Cleve (a Swedish chemist) [139] and the brothers Hippolyte and Maurice Peragallo [140]. The latter two were 'anciens élèves de l' Ecole Polytechnique' – a famous institute, but not in any way connected to the life sciences.

But the most outstanding example is Henri van Heurck [141,142], a Belgian industrialist of the late Victorian era, who between 1860 and 1908 literally spent a fortune dabbling in diatoms. He was very wealthy indeed, so much so that he could ask Messrs. Zeiss to compute, design and construct a one-off special oil immersion objective for his diatom hobby. Also, he privately published books on microscopy and diatoms. Then he had a handy tool for his diatom studies: his own steam-yacht, completely fitted out as a laboratory. And finally, he spent astronomical (for that time) sums on acquiring materials and every new optical gadget that was being invented. His collections – far from intact, unfortunately – are now in Brussels.

Living examples of diatomists who are not paid to work specifically on diatoms include the NASA astrobiologist Richard B. Hoover [37] and three of the authors of this article: science consultant F.A.S.S. [2], psychiatrist S.S.N. [2,37,143,144] and 'armchair diatomist' R.G., who is a theoretical biologist in a medical school and has sought no grants for his hobby.

have slits in the valves called raphes [1] (Figure 2), which are involved in gliding motility [27,28].

The general structure of a valve can be summarized as follows: lines of silica called costae diverge and occasionally branch (Figure 2, Figure 3b) from a nucleation site, the linear midrib in pennate diatoms or the circular midring in centric diatoms [1,29]. As we shall see, this scenario might include honeycomb structures (Figure 3b). Each valve possesses a three-dimensional (3D) and hierarchical organization of porous plates and solid walls with pore diameters that range from nanometres to micrometres (Figure 3) and with enormous structural diversity of their patterns and shapes. Recent studies using high resolution atomic force microscopy, scanning electron microscopy and time-lapse light microscopy have revealed a diversity of new nano- and meso-scale silica morphologies of diatoms, including the presence of 50 nm spherical silica particles (cf. 'colloidal silica' [29]), which allow better understanding of biosilica formation and valve morphogenesis [30–32]. The general assumption is that the silica itself remains amorphous in all these detailed structures, despite their organic components and 'templating' surrounds [33], but this needs to be directly tested by electron diffraction (cf. [34]).

Silica biomineralization and diatom genomics

Diatom structures are presumed to be replicated from generation to generation by a genetically controlled biomineralization process that takes place at levels from the molecular to the nano- and micro-scale. However, cytoplasmic inheritance, such as occurs in the also hierarchically patterned surfaces of ciliates [35,36], might have a role, considering the existence of pennate diatoms with significantly differing valves (heterovalvy) despite there being just one cell nucleus, as well as the transmission of shape aberrations or complementary geometry of valves of daughter cells [1]. We can anticipate a role of gravity because microtubules (MTs) are undoubtedly involved [37], and manipulations of pattern via environmental changes have begun in earnest [38–40].

There are two basic forms of morphogenesis: (i) patterns that form spontaneously as symmetry-breaking phenomena and (ii) patterns that are guided by prepatterns, so-called 'structure-directing templates' or 'scaffolds' [41]. Prepatterns lead to an infinite explanatory regress, as in the long-defunct idea of Leeuwenhoek of the homunculus in sperm [42], the inflatable 'little man' who contains sperm that each contain another smaller homunculus with sperm, etc., back to Adam and Eve. At this point we have several pieces to the puzzle of diatom shell morphogenesis, but not the whole picture. The morphological evidence suggests that silica precipitates in at least five stages: (i) formation of small silica spheres of 30 to 50 nm diameter [29], perhaps inside membrane-bound silica transport vesicles (STVs) (Figure 4); (ii) transport of these vesicles to the periphery of a flat membrane bag called the silicalemma (SL), into which the silica spheres are released; (iii) 2D precipitation of the spheres onto the growing valve inside the SL, starting from a nucleating structure and taking but a few minutes [23,43]; (iv) pore formation [44–46]; and (v) thickening of the valve, taking hours and often accompanied by further pattern formation in the third dimension [29,43,44,47]. These steps are generally not distinguished in the current literature on silica biomineralization, giving the impression that the molecular key(s) to morphogenesis have been found, without accounting for the multiple physical and time scales at which it actually occurs, let alone integrating them into one coherent theory. Of course, we need not follow nature's steps in synthesis of diatom-like structures [48,49], although a deliberate effort to do so might prove rewarding. Ultimately, the proof of a theory of morphogenesis will be its step by step quantitative matching to a computer simulation and/or real-world synthesis, rather than the current approach of shopping for diatoms whose mature valves roughly match a given simulation [29,44].

Seventy-five genes have been shown to be involved in silica metabolism [50], so there might soon be a basis for correlating their functions with these steps of morphogenesis. With full sequencing of a few species and the success of genetic transformation [51,52], molecular genetics and bioinformatics can now be brought to bear [51]. Silica-sphere formation has been shown to be enhanced by polyamines associated by specific polypeptides named silacidins [53]. Green fluorescent protein (GFP) fused via genetic transformation to a silaffin protein (perhaps involved in nucleation of silica precipitation [23,54]) has been shown to be incorporated into diatom silica, providing a new means of functional protein immobilization [55]. *In vitro*, similar fusion proteins can be used to make fibrous precipitates of silica [56]. Many enzymes have now been immobilized in silica [41], and old observations of organic components of diatom silica, which we at first dismissed as contaminants

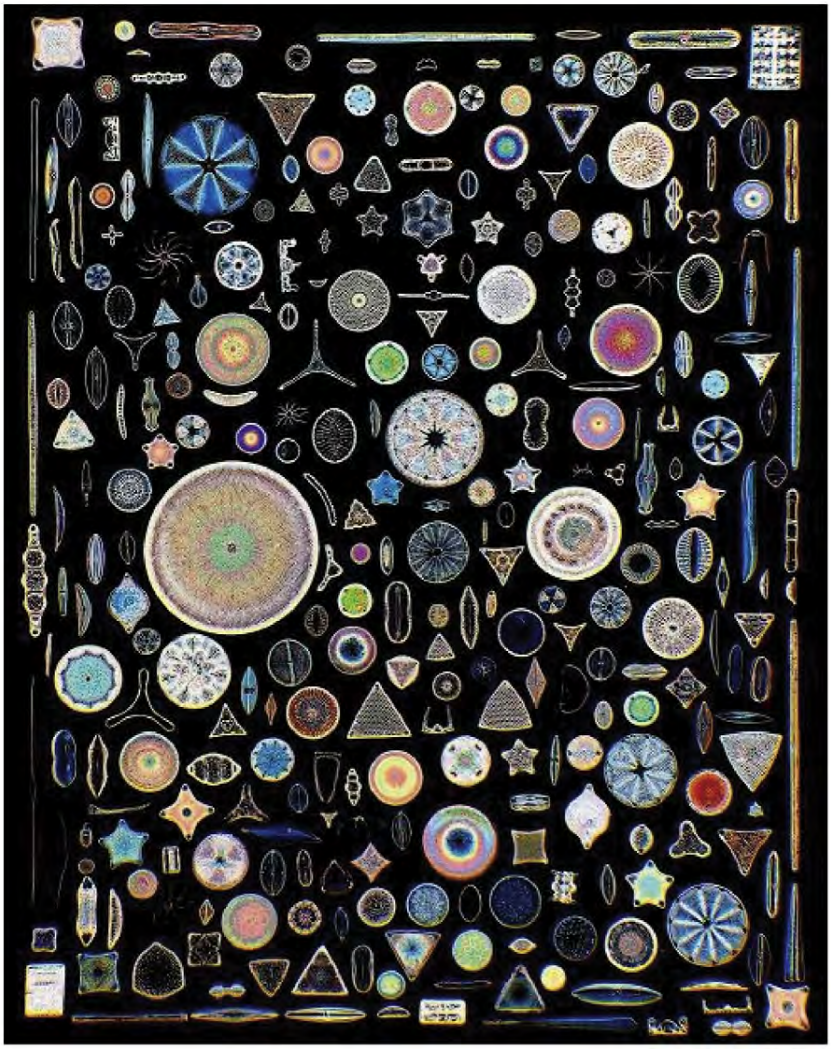

Figure 1. Three hundred diatoms mounted individually by hand. The examples shown include recent and fossil and freshwater and marine diatoms. They originate from the UK, Holland, France, New Zealand, Sulawesi, Caribbean, Indian Ocean, Florida, Maryland, Oregon, Montana, Nevada, British Columbia, California, Alaska, Honolulu and Russia. The array is 1.78 × 2.30 mm.

[29], are now confirmed by NMR [57] and are obviously at minimum catalytic for silica precipitation into spheres.

Three models have been proposed for the rapid 2D precipitation phase of valve morphogenesis:

- Diffusion limited aggregation (DLA), or precipitation of silica spheres, initially onto a nucleating structure [29], involves a solid phase that grows but does not move except for sintering [29] and a liquid phase, or mother liquor, that concentrates the organic matter in it, potentially changing details of the precipitation, such as keeping pores open, as the concentration of non-silica material increases [29,58] and that is capable of flowing (although flow has been ignored in DLA modelling so far). The DLA model was of limited success when sintering was not invoked, imitating irregular costae patterns of some centric diatoms and aberrant pennate diatoms [29]. The addition of radially organized MTs, presumed to carry silica spheres to the perimeter of the SL, increased the range of diatom patterns simulated [58]. Perhaps a new approach using 'slippery' DLA [59] specific for colloids in water would lead to more realistic patterns.
- In a two-liquid model, the pattern is explained as a phase separation that occurs between them [44].
- It has been suggested that the solid silica forms only within the silica deposition vesicle (SDV) by 'aquaporin-induced syneresis' (extrusion of water from a silica gel) after transport by 'STVs filled with the soluble complex of oligosilicates with polyamines... [that] by means of fusion, discharge their content into the SDV', with long-range order attributed to the cytoskeleton and 'branching due to arrival of new microtubules' [26]. This model has only been simulated for a cross section of an SL, which does not permit detailed comparison with diatom valves. In this proposal, the STVs contain no silica, and are '... simply transport vesicles (TVs) that deliver constituents of cellular origin; most probably membrane parts for the expanding SDV and polypeptides (silaffins,

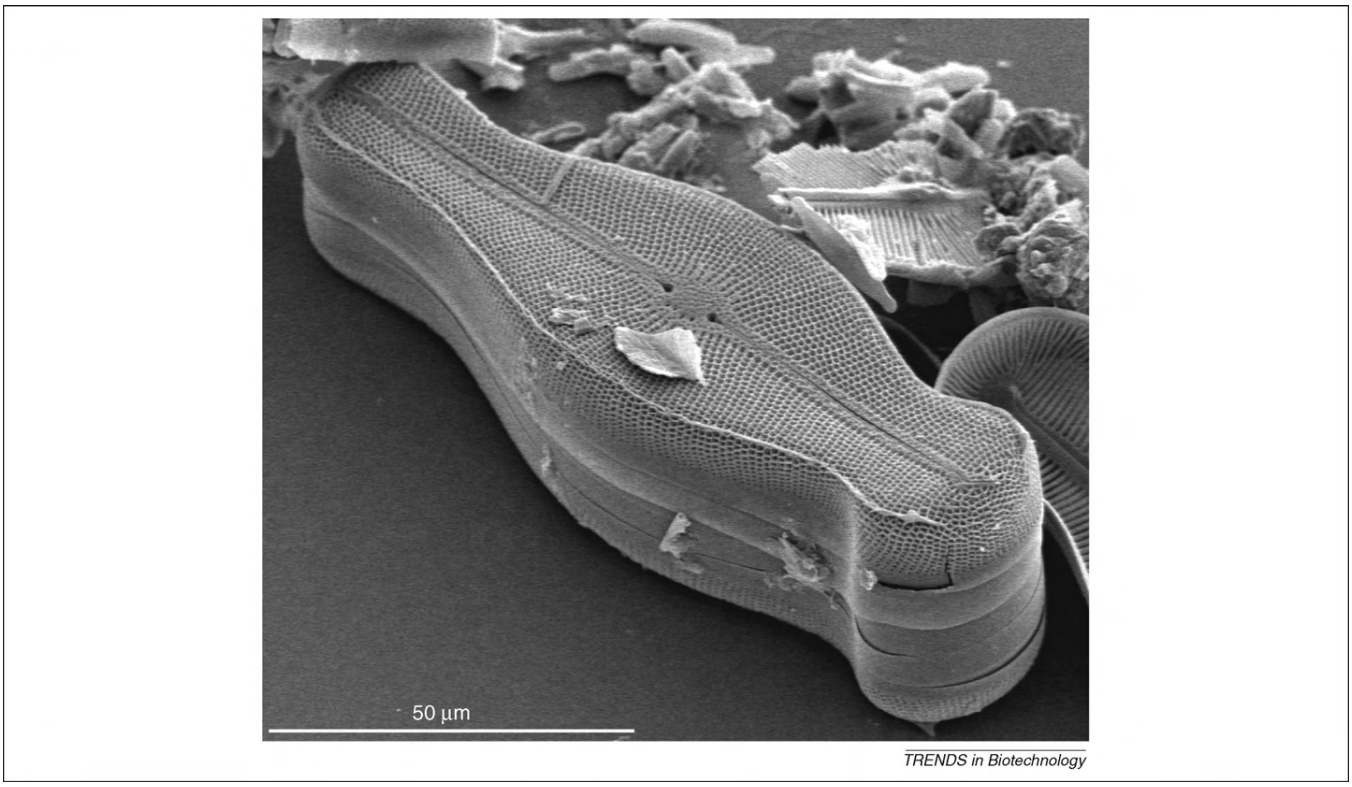

Figure 2. Scanning electron micrograph (SEM) of a pennate diatom, *Didymosphenia geminata* (Lyngbye) Schmidt from Cache la Poudre River in Colorado, USA. The two slits along the midline are the raphes, which are involved in motility. The branching silica precipitation of costae proceeded from this midline to the periphery and down the sides; this is more clearly visible on the inner view of the valve on the right. Girdle bands can be seen between the two valves. The scale bar represents 50 µm.

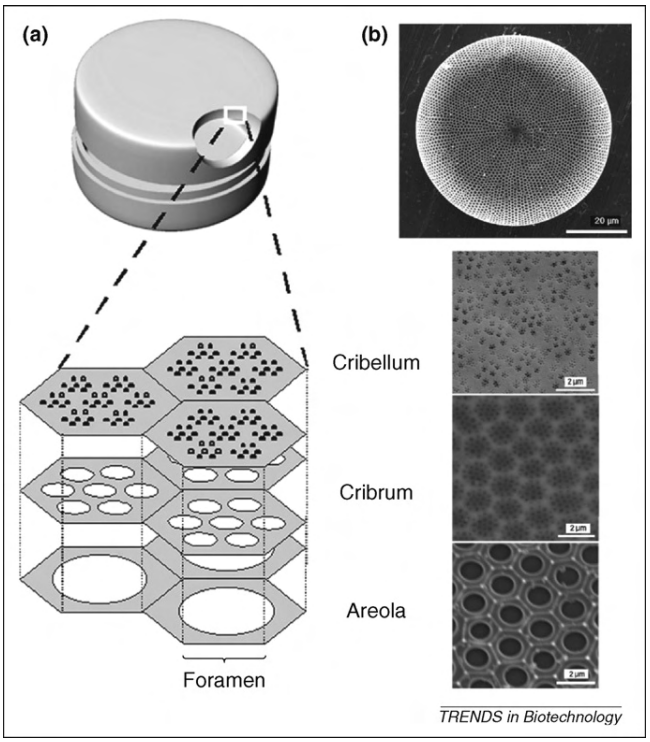

Figure 3. Diatom structure. **(a)** Schematic of a centric diatom frustule with cross-sectional three-dimensional (3D) profile of the silica wall based on SEM data. The inner layer contains honeycomb-like vertical chambers called areolae. The large hole in the floor of an areola is known as a foramen. The roof of the areolae is called the cribrum, which contains a regular pattern of pores. The layer over the cribrum is a thin siliceous membrane known as the cribellum, which consists of small pores. **(b)** SEM image of a *Coscinodiscus* sp. with corresponding layers [46,133,134]. Reproduced from [134] with permission.

long-chain polyamines) for which it is expected that they accelerate silica precipitation' [40], and pinocytosis of $Si(OH)_4$ occurs only via route C in Figure 4.

Note that none of these models invokes a prepattern of silica binding to supramolecular scaffolds, as has been presumed necessary to span the size gap from the 50 nm sphere to the whole diatom [53,60]. The first two models yield convincing patterns for a different range of diatom valve patterns, so they are being combined into a three-phase model: two liquids plus the solid silica precipitate (in collaboration with Philip J. Camp). These separation–precipitation patterns are uncannily similar to patterns generated by more complicated, but more popular, Turing reaction–diffusion equations [61], suggesting that solutions of both sets of equations share a fundamental mathematical topology [62]. We might find that all of the models discussed here 'work', and that step by step experimental analysis will prove necessary to get at the actual mechanism of diatom morphogenesis. Synthesis, both by experimental reproduction of diatom patterns [63], using nanotechnology to create some semblance of an artificial diatom cell with an SDV, and computer simulation is needed to confirm that any analysis of morphogenesis is sufficient [64].

Note that although purported STVs have not yet been shown to contain silica, contrary to earlier beliefs [1], present evidence suggests that the only detectable form of silica within diatom cells is precipitated silica [65]. Thus, transport within the cell is of solid silica, not $Si(OH)_4$ (Figure 4). This observation seems to contradict the

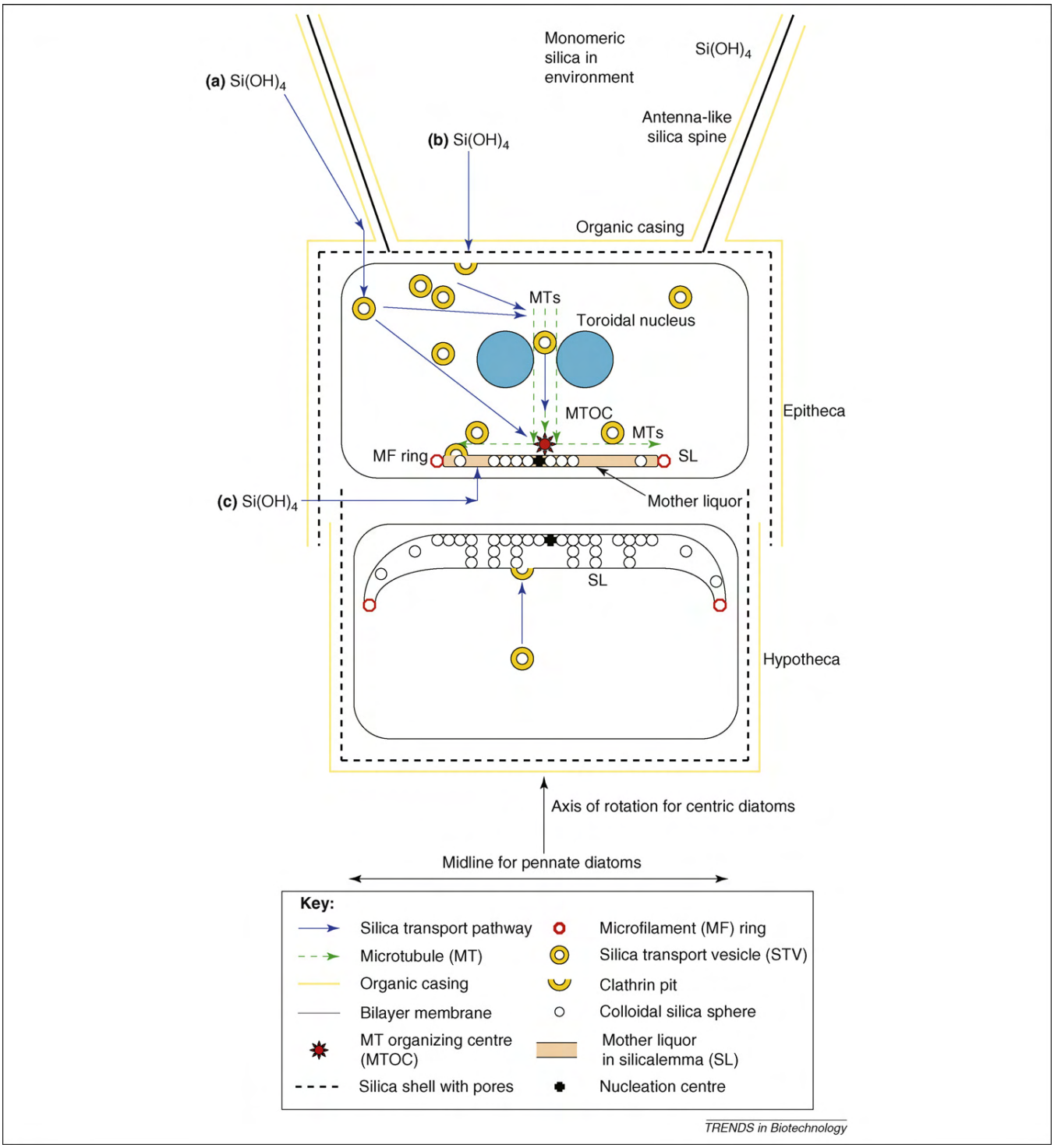

Figure 4. Diatom morphogenesis. Schematic view of a cross section of a pair of daughter diatom cells after cell division while the new valves are forming inside, each in a silica deposition vesicle (SDV) consisting of a bilayer membrane, called the silicalemma (SL), and its contents. The upper cell shows the rapid two-dimensional (2D) phase of valve formation, which only takes minutes. The cell nucleus might be torus-shaped, and bundles of microtubules (MTs) extend through the nuclear hole [85].. MTs emanating from a microtubule-organizing centre (MTOC) are on the inner face of the SL, which is a flat, membrane-bound bag at this stage. The SL contains a nucleating centre where silica precipitation starts. This centre is where colloidal silica spheres of ~50 nm diameter, which are probably the diffusing and precipitating entities, initially adhere. They then stick to the already precipitated silica spheres. The SL also contains the mother liquor, the fluid remaining after silica precipitation and sintering [29]. The mother liquor might have two or more immiscible liquid components [44–46]. The MTs on the inner face of the SL might mechanically counteract contraction of the microfilament (MF) ring around the perimeter of the SL [86]. This tensegrity structure presumably keeps the SL thin and flat, thereby allowing a 2D pattern of precipitated silica to form within it [29]. Three possible routes of entry of monomeric silica, $Si(OH)_4$, into the cell are shown: route **(a)** proceeds via adsorption to spines and migration along the organic casing [1]; route **(b)** is through the silica shell pores; and route **(c)** is via the gap that is formed between the daughter cells and that follows through or past the possibly more permeable girdle bands [31,135] (not shown here, but depicted in Figure 2). Transport of silica within the cell might involve hypothesized silica transport vesicles (STVs), which could be formed at the cell membrane by a clathrin mechanism, then labelled with a trafficking signal and transported to the SL margin by motor molecules on MTs [58]. At the SL, each deposits its membrane and silica sphere. It has been assumed that the silica within the STVs is solid (but cf. [26]). Silica-binding organic and inorganic molecules and proteins [23,53] that are present in the mother liquor, including nickel [38], germanium [39], H^+ [136] and salts [40], might influence the pattern of precipitation and might become incorporated into the silica. The daughter cell depicted in the lower half of the figure is at a later stage of cell division and shows

syneresis model [26]. We are equally ignorant of the state of organic matter inside the purported STVs, even whether any of the silica-binding proteins are in them. Such organic macromolecules could be trapped within the silica spheres during STV formation, or between them when they sinter inside the SDV, or both. There is a clear need for a definitive study of the contents of STVs using SIMS (secondary ion mass spectrometry) [37] or other modern analytical tools.

For centric diatoms with hexagonal patterns, once attributed to surface tension, vibrational or electromagnetic forces [66], a hierarchical phase transition model invoking liquid droplets of various polyamines (cf. 'condensed protein spheroids' [29]) undergoing sequential phase separations has been proposed for the pores-within-pores structure [45,46], perhaps enhanced or made species-specific by peptides named silacidins and silaffins [23]. This model has not yet been consolidated with observations that centric diatoms with hexagonal patterns instead have branching patterns emanating from the midring when silica-starved [29], nor with the 3D layered structure shown in Figure 3, and computer simulations are desirable because the varying diameters of the silica spheres obtained [60] do not seem to form any long-range order of closely packed structures, let alone result in 'self-assembly ... of structures or patterns at various length scales without external guidance' [60]. If close packing of silica nanospheres or liquid droplets is involved in the sometimes highly regular, long-range order hexagonal patterns of diatoms (Figure 3b), then a mechanism must be found, such as endocytosis via clathrin-coated pits [67,68] (Figure 4) or other means [49,69–71], by which their uptake size might become more monodisperse, and the actual spectrum of diameters of silica colloidal particles within live diatoms needs to be observed and quantified.

Little progress has been reported on the intracellular pathway by which silica makes its way from outside the cell into the SL [23,72], although tools for tracking silica are being developed, including ^{29}Si NMR spectroscopy with confocal laser fluorescence microscopy [65] and dyes [73]. An old standby is germanium (Ge, below Si on the periodic table), which is presently being used to alter photonic properties of diatom valves [39,74,75]. Viral particles of 100-nm diameter have been tracked in live cells [76], so similar work in diatoms should be possible. Perhaps silica-coated nanospheres containing luminescent nanocrystals [77], quantum dots [78,79] or gold [80] would be taken up by diatoms. It seems from earlier work [29] that silica is not stored to any significant extent [72] but rather is taken up from the environment as silicic acid [81] during valve formation, as shown by the time course of silica uptake [82]. Silica transporter (SIT) proteins can 'cycle between the plasma membrane and intracellular vesicles' [82], and an 'organic pentaoxo-azo-silicon complex' might be involved [83]. However, the intracellular silica is likely to be condensed [65], presumably in STVs, shortly after incorporation from the medium as monosilicic acid, $Si(OH)_4$, because the latter is not detected inside cells [23]. Perhaps the external surface of the shell is not only an 'antenna' for silica [29] but also a catalytic surface for condensation. This might be another role of the silica-embedded proteins, whose relationship to the 'organic casing, which coats all the siliceous components' [1] has yet to be investigated, although the casing was found to be chemically removable [60].

Therefore, more studies are required to understand silica trafficking during the process of morphogenesis. It is not known: (i) whether colloidal size particles are ever taken in, which would seem to be more efficient when they are available in the environment; (ii) whether the valves and silica spines have roles in providing adsorption 'antennae' (another DLA problem) and surface migration 'funnels' to bring the external silica to the cell membrane, a form of dimensionality reduction analogous to the cell nucleus and its pores [84]; (iii) whether pinocytosis, clathrates, etc. are involved in taking up the silica, or where on the cell surface this occurs; (iv) whether silica is packaged into vesicles at the cell membrane or in the Golgi apparatus, or how STVs [23,29] are delivered to the surface of the SL; (v) whether STVs are deposited to spatiotemporally localized positions on the SL [58]; (vi) how their contents are exocytosed into the SDV through its membrane, the SL; and (vii) whether the growing surface area of the SL depends on the STV membranes for its increase as the nascent valve grows inside. There is a need to understand the roles of the cytoskeleton in all this [58], and intriguing hints have been provided by the MT bundles that extend through a hole in a torus-shaped nucleus [85] impinging on the SL to a microtubule-organizing centre (MTOC) on the external surface of the SL, as well as a microfilament ring that might be involved in keeping the SL flat as it grows [86] (Figure 4). The flatness of the SDV might be essential to keep the initial pattern formation confined to an essentially 2D space, as is required for pattern formation by bacterial colonies [87]. Basically, to link the fine molecular genetics that is being done [50] to the morphogenesis of the valve will require that we now turn our attention to the trafficking of silica inside diatoms.

Diatom biophotonics

Because of their similarity to opals, diatoms are often referred to as 'jewels of the sea' or 'living opals'. In fact, diatom shells are opal material made from silica nanoparticles, and diatoms can be described as a living cell inside a glass house [88]. Thus, it is not surprising that within the glass menagerie of diatoms, we can find outstanding examples of multifunctional structures based on interaction with light, so that the beauty of diatoms lies not

the slow, 3D, thickening phase of valve formation, which typically takes hours. Most cellular details that are the same, such as nucleus and mother liquor, are identical and are not shown for clarity. Instead, only events that differ from the earlier, rapid phase of valve formation are depicted. The MF ring and MTs are no longer in mechanical opposition, so the SL is free to thicken. Fusion of STVs to the face of the SL rather than its perimeter might occur at this valve-thickening stage [29]. Note that because the valves fit within one another, on exocytosis of the new valves, the bottom daughter cell will be smaller than the top one. In addition to this size difference, the epitheca and hypotheca might also have significantly different morphologies (heterovalvy [1]). Considerably less is known about morphogenesis of the girdle bands [1], spines [26] and other silica attachments.

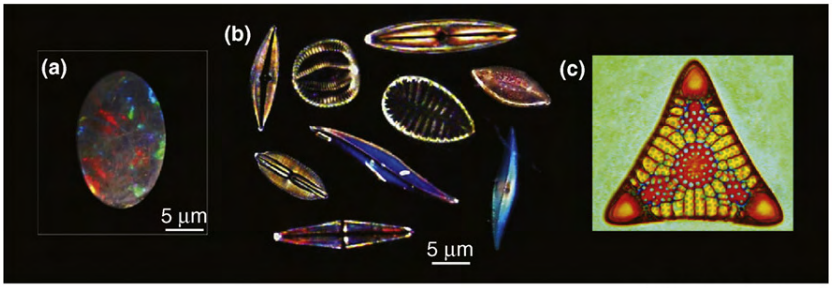

Figure 5. Optical and photonic properties of diatoms. **(a)** Opal is shown as an example of a photonic crystal with a characteristic play of colours. Reproduced with permission of S. Ely. **(b)** Light microscopy images of several pennate diatom species with characteristic colours as a result of light interference and diffraction from their silica structure. **(c)** Digitally enhanced Jamin-Lebedeff micrograph of the marine centric diatom fossil *Triceratium morlandii*. Cell width ~120 μm.

only in the artistry of their forms and structures but also in the equally valuable optical properties of their transparent silica structures. The strong interaction with light produces stunning structural colours with intense diffraction and interference effects when diatoms are observed under a light microscope (Figure 5). Displaying a play of lustrous colours like those of the rainbow, this phenomenon is caused by multiple reflections from multilayered, semi-transparent surfaces in which phase shift and interference of the reflections modulates the incident light by amplifying or attenuating some frequencies more than others: the precise interference effect depends on the angle at which light strikes the surface, hence the diatom seems to change colour as it or the observer moves position, as with a thin film of oil on water. Such iridescent effects [89] are used widely in colour cosmetics products and personal care packaging, and there is great potential for using diatoms in this industry [90].

Jamin-Lebedeff interferometric microscopy, using polarizing optics to separate specimen and reference beams, gives particularly spectacular colour effects involving transmitted light. Although the raw image is quite washed out, digital post-acquisition image processing allows reduction of haze, the intensification of colour saturation and additional sharpening (Figure 5c). Whereas in transmission mode light diffraction is more important, in reflectance mode the result is similar to iridescent colours. Further study is warranted, especially the possibility of inferring diatom structure via visible light computed tomography methods [91,92], which would then, for example, permit time-lapse of the 3D phase of valve morphogenesis *in vivo*.

Photonic crystals are materials with spatially ordered and periodic nanostructures that can control the propagation of light, only allowing certain wavelengths to pass through the crystal (similar to the propagation of electrons in a semiconductor crystal) [93]. They are able to control photons, producing remarkable effects that are impossible with conventional optics, and have the potential to revolutionize existing electronic and computing technologies [93]. The photonic crystal properties of diatoms' girdle band structures were recently confirmed [38,90,94], suggesting that diatoms are living photonic crystals. Diatom nanotechnology now allows us to grow a huge variety of biophotonic crystals. This extraordinary discovery raises questions about the biological relevance of the photonic properties of diatoms and their practical exploitation. Butterflies, beetles and many other organisms have been using photonic crystals for ages [89,95]. Their function varies from communication, camouflag, and thermal exchange to UV protection. Diatoms' photosynthetic receptors are located in chloroplasts close to the silica wall, and the light-channelling and -focussing [96] properties of their silica structure could help the transmission and collection of more light into the photoreceptors to improve their photosynthetic efficiency [90].

Another optical surprise that comes from diatoms is photoluminescence [38,97,98]. A visual luminescence effect from diatoms is clearly seen after exposing the silica structures to UV light with a broad blue luminescence peak in the visible region (450 nm). This effect was found to be similar to the photoluminescence of artificially fabricated porous silicon. Diatom photoluminescence is strongly species dependent, and it is based on both their frustule structure and the surrounding environment. These characteristics were elegantly exploited [98] to create the first photoluminescence gas-sensing devices based on diatoms [99]. Ultra-sensitive detection (sub ppm) of a series of organic vapours (ethanol, acetone, xylene and pyridine) and gases (nitrogen dioxide, methane, carbon monoxide) has been demonstrated [100]. Based on these findings and the diversity of diatoms available with different photoluminescence characteristics toward different gases, one can predict the development of a universal photoluminescence gas-sensing platform with an array of different diatoms for toxic gas detection or air-pollution monitoring.

Microfluidics within diatoms

The gliding motility of pennate diatoms is intriguing because the cell does not change shape and there are no moving parts, contrary to our common experience with amoeboid, ciliate and bacterial motility. Two models for the role of microfilaments parallel to the raphes have long been in contention, without definitive resolution: one model identifies the microfilaments as the motor [28] and the other identifies them as controllers of the direction of motility [101]. A fibrous fluid, the 'diatom trail', is left on the surface a diatom traverses.

Biophotonics and motility might be intertwined in the colonial diatom *Bacillaria paradoxa*, in which the chained cells move back and forth against one another [102,103].

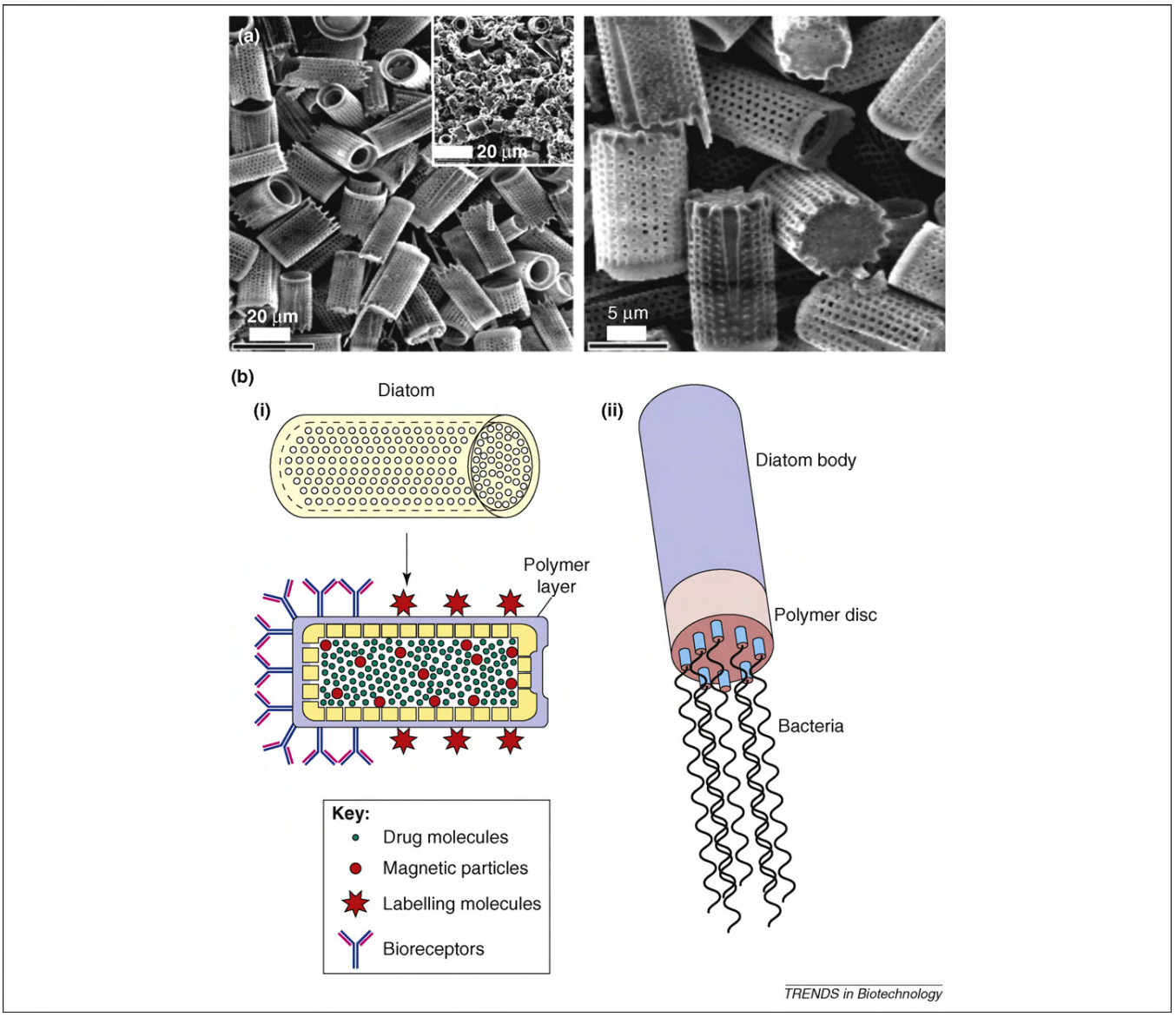

Figure 6. Diatoms for drug delivery. Panel **(a)** shows exemplary SEM images of purified diatoms with whole, fraction-free frustules in comparison with raw diatomaceous earth (inset image). Panel **(b)** shows a multifunctional diatom-based drug-delivery system (i) and and a model of a self-propelled drug carrier with diatom and attached bacterial biomotors (ii).

Whether the coupling of these autonomously oscillating [104] *B. paradoxa* cells is local, or global via the elasticity or anomalous viscosity [8] of the diatom trail slime, remains to be determined. The resting stage involves alignment of all the cells in a stack with no obvious mechanical stop [103]. Given that there is a photosensitive region at the distal ends of a pennate diatom, which causes the diatom to respond to a 'light wall' by reversing direction [105], we would like to hypothesize that a light pipe forms between these photosensitive regions when the cells are stacked. It might be responsible not only for the aligned resting stage but also for the partial synchrony of movement of the cells.

Diatom motility utilizes active flow of an adhesive fluid through a narrow slit, the raphe (Figure 2), which suggests a new branch of nanotechnology that might be called 'self-propelled microfluidics', compared to so-called 'active' microfluidics, in which the liquid is passively moved by an external force [106]. (Cytoplasm is a more complex self-propelled fluid.) A downside of diatoms is their adhesion to man-made surfaces under water, leading to biofouling [107], although this might lead to new commercially or medically important bioadhesives [108].

Diatoms for drug delivery

Nanotechnology is currently opening new therapeutic opportunities for agents that cannot be used effectively as conventional drug formulations owing to poor bioavailability or drug instability. The diatom silica shell possesses a combination of structural, mechanical, chemical and optical features that might both overcome challenges associated with conventional delivery of therapeutic agents and have advantages over existing microparticle delivery systems. The pill-box structures, micro- and nanoscale porosity, enormous surface area (100 m^2/g for

unheated, fresh diatom shells [29,109–112]) and biocompatibility and biodegradability of amorphous diatom silica make them a promising biomaterial for drug-delivery applications. They can be easily functionalized, protected and designed for controlled drug release through nano-sized pores or by embedding in the silica [55]. Even though diatoms can be easily cultivated, a large and even less expensive source of diatom silica is diatomite or diatomaceous earth, which is formed by the fossil siliceous frustules of diatoms. The preparation of the ultra-high-purity and fraction-free silica capsules from raw diatomite material (diatomaceous earth) is possible using simple separation procedures (Figure 6). These diatom microcapsules are proposed as excellent natural porous materials for drug-delivery applications. Diatom structure provides flexibility for the design of complex drug-delivery vehicles through functionalization with sensing biomolecules or immuno-targeting bioreceptors, optically active dyes (for imaging) and/or magnetic nanoparticles (for controlled movement to target diseased tissue or cancer cells) (Figure 6).

More sophisticated drug-delivery systems, such as self-propelled swimming microrobots, could also benefit from the unique properties of diatoms' frustule structure. Although not quite like science fiction [113], scientists have talked for quite some time about microdevices that can travel inside the human body and carry out a range of complex medical procedures, such as monitoring, drug delivery and cell repair [114]. Recent developments in micro- and nanoscale engineering have led to the realization of various miniature mobile robots, but we have an intriguing opportunity to integrate whole biological organisms or their parts [115,116]. In regards to their physical and structural properties, porous silica capsules of diatoms are ideal microscale bodies for designing these future robotic devices for medical applications. However, the self-propelled function is missing here, and to introduce mobility, we could attach bacteria to the diatom (Figure 6) in addition to, or instead of, the gliding motility of diatoms themselves. Many bacteria propel themselves along in a fluid by rotating their corkscrew-like tails, called flagella, at relatively high speeds, and as robust machines, such flagella can easily be integrated with other microscopic components and do not need to be purified or reconstituted [116–118]. The bacteria motors work using a simple chemical energy source (glucose) and are naturally sensitive to the environment (e.g. metal ions, ethylene diamine tetra-acetic acid [EDTA]), which means that nanobot movement could be controlled. Of course, in the dark recesses of our bodies, we might want to use motile apochlorotic diatoms [119].

Selective breeding of diatoms using a compustat

It might be possible to manipulate the morphology of diatoms by use of a compustat [120], which functions like a chemostat, except that the criteria for survival are morphological rather than nutritional. While this still has not been done for diatoms, somewhat similar devices have been constructed for bacteria [121]. With a compustat, one could try to select for several visual criteria, such as costal spacing, pore sizes, shell shape and biophotonic properties. Curiosity questions, such as whether we can, through such artificial selection, make one species of diatom look like another, might be worth pursuing. Selection could also be based on oil droplet sizes and number (T.V. Ramachandra *et al.*, unpublished) or detection, via absorption or fluorescence spectra, of other important bioactive materials.

Computing with diatoms

Perhaps the most sophisticated dream for diatoms to date is the hope to grow large numbers of 3D nanocomputers or computer components from them (M.R. Sussman [2008] 'In diatom, scientists find genes that may level engineering hurdle', http://www.eurekalert.org/pub_releases/2008-01/uow-ids011808.php). The idea of growing a computer goes back at least to the experiments of toy maker Robert Stewart on iron dendrites in nitric acid [122,123], which he derived from Lillie's iron wire model for nerve cell signal propagation [20,124]. The achievement so far is to transform the 3D shape of the amorphous silica of a diatom into silicon, preserving the morphology of the original diatom [125]. A combination of 3D diatom nanotechnology with 3D DNA nanotechnology [126,127] and the electronic properties of DNA [128] might be particularly rewarding. DNA binding directly or indirectly to silica [129] and silicon [130,131] has already been demonstrated, and one should keep in mind the silica-dependent nature of DNA replication in diatoms [132].

Conclusions

Diatom research is rapidly moving from the underappreciated domain of taxonomists and skilled amateur scientists into high nanotechnology and big business. Diatom bionanotechnology, a new interdisciplinary area, has successfully emerged over the past several years into a dynamic and productive research area with hundreds of papers to date. We have witnessed significant progress in understanding diatom structural, mechanical, genomic, optical and photonic properties and the silica biomineralization process, leading to the nanofabrication and engineering of new materials and devices based on diatom silica. Biofuels, food, cosmetics and pharmaceutical products might soon be in the offing. In basic research, diatoms are likely to contribute to the solution of one of the major unsolved biological problems: how the genome is involved in creation of form, and how form evolves. We now have a few competing hypotheses for diatoms, and there is a great need for intracellular observation to resolve what is going on. Their beauty inspires everyone who works with them.

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