3 UN Conference on Environment and Development (1992) *Convention on Biological Diversity*, reprinted in *Int. Legal Mater.* 31
4 Miller, H. I. *et al.* (1990) *Science* 250, 490–491
5 Miller, H. I. *et al.* (1995) *Biotechnology* 13, 955–959
6 Anon. (1994) *Biotechnology Risk Control*, Official Publications of the European Community
7 Miller, H. I. (1997) *Policy Controversy in Biotechnology: An Insider's View*, R. G. Landes/Academic Press
8 Anon. (1993) UNEP/Bio.Div/Panels/Inf. 1, United Nations Environment Program
9 Giddings, L. V. (1996) *Nat. Biotechnol.* 14, 1304–1305
10 Anon. (1995) 'Accord on Biosafety Protocol Reached', *Bur. Natl. Aff. Environ. Daily*, November 20
11 Masood, E. (1995) *Nature* 378, 326
12 Anon. (1995) *Report of the Global Consultation of Government-designated Experts on International Technical Guidelines for Safety in Biotechnology*, UNEP/Biosafety/Global Consultation 4, United Nations Environment Program
13 Organization for Economic Cooperation and Development (1995) *Safety Considerations for Biotechnology: Scale-up of Microorganisms as Biofertilizers*, Organization for Economic Cooperation and Development, Paris, France
14 A. Pollack (1999) US and Allies Block Treaty on Genetically Altered Goods, *New York Times*, 25 February
15 Jayaraman, K. S. and Masood, E. (1998) *Nature* 392, 640
16 Masood, E. (1998) *Nature* 393, 99–100
17 Rabkin, J. in *The Greening of US Foreign Policy* (Anderson, T. L. and Miller, H. I., eds), Hoover Press, Stanford, CA, USA (in press)

Beyond micromachining: the potential of diatoms

John Parkinson and Richard Gordon

Diatoms are microscopic, single-celled algae that possess rigid cell walls (frustules) composed of amorphous silica. Depending on the species of diatom and the growth conditions, these frustules can display a wide range of different morphologies. It is possible to design and produce specific frustule morphologies that have potential applications in nanotechnology.

There has recently been a great deal of interest centred around the design and manufacture of devices of nanometre proportions and this speculation has spawned a new industry, termed nanotechnology. Much has been written about the potential applications of nanometre-sized devices with electronic, optical and/or mechanical properties such as gears, motors and transistors[1–3]. However, at present, the tools for creating such devices are still in the early stages of development.

The two main premises for creating nanotechnological devices is that they should be cheap to manufacture and they should also possess order at the atomic level. At present, it is possible to use micromachining processes to create tiny gears and motors that are measured in tens of micrometres. In addition, ion-beam etching can be used in microelectronics to etch lines less than 1 μm wide on wafers of silicon[4]. We propose that directed self-assembly by growing cultures of single-celled diatoms (Bacillariophyceae) may provide a valuable means of providing order at a scale between that currently obtainable by the latest micromachining processes and the atomic level, while also providing a cheap alternative to both of these technologies[5].

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Introduction to diatoms

Diatoms are microscopic (~1–500 μm in length) single-celled algae with characteristic rigid cell walls (frustules) composed of amorphous silica. They are ubiquitous organisms found in a wide variety of habitats and are thought to be responsible for up to 25% of the world's net primary production of organic carbon[6]. There are currently estimated to be over 100 000 different species, classified by their unique frustule morphologies[7]. Diatoms are usually classified as one of two main groups depending upon the symmetry of their frustules (Fig. 1a,b).

Centric diatoms tend to be radially symmetrical, while pennate diatoms tend to be elongated and generally have parallel striae (furrows or rows of holes in the silica) arranged normal to the long axis. The spacing between adjacent striae is species specific and typically varies from ~0.3 to 2 μm. The lines of silica between the striae are called costae. Costae tend to be arranged in combs or other space-filling patterns such as honeycombs, which possess remarkably uniform pores. Aside from their symmetry, frustules display an unparalleled diversity in structure and morphology, and this may be exploitable in nanotechnological applications.

The diatom frustule

The diatom frustule consists of two almost equal halves that fit together like a petri dish, enclosing the bulk of the single cell within (Fig. 1c). Each half (theca) consists of a valve (which forms the larger outer

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surface) and a girdle (the circular band of silica attached to the edge of the valve). During diatom replication, the two halves of the frustule separate, and new valves and girdles are synthesized intracellularly within specialized organelles (silica-deposition vesicles, or SDVs).

For the majority of species, the petri-dish nature of the frustule leads to a reduction in size during successive divisions in one of the daughter cells (Fig. 1d); regeneration of the original cell size subsequently occurs via a sexual-reproduction phase. Interestingly, the reduction in overall size leads to a reduction in the numbers of costae, with their spacing remaining constant. The rate of division is determined by environmental and genetic factors, but can be as high as eight divisions per day[8].

Frustule formation

The process of frustule formation is not well understood but is thought to involve the diffusion-limited precipitation of silica[9,10]. Amorphous silica particles of relatively low molecular weight and ~1–10 nm in diameter are thought to be transported to the periphery of the SDV by silica-transport vesicles (STVs) (Fig. 1e). Once released inside the SDV, the particles diffuse until they encounter part of the growing aggregate, to which they adhere.

The surface of the particles is thought to consist mainly of silanol groups[11] [$Si(OH)_2$ or Si–OH], which enable them to diffuse over the surface of the aggregate in a process termed sintering. This surface migration allows the molecules to reorganize their positions towards thermodynamic equilibrium, usually resulting in a smoothing of the aggregate surface. Sintering has been shown to be affected by factors such as pH and temperature[12], which may explain the changes in frustule morphology observed when a single diatom species is grown under varying conditions[13,14]. After deposition and a period of surface relocalization, the silica morphology becomes stabilized in a process that may involve an inorganic cation such as aluminium[9,10].

Although little is known about how the silica is transported to the SDV, microtubules have been found to be associated with the developing SDV[15]. Recent computer simulations imply a role for these microtubules as carriers for the STVs (J. Parkinson and D. Gordon, unpublished); the arrangement of the microtubules may thus account for the gross morphological characteristics of the frustule (i.e. the micrometre level of order), each costa being associated with the site of release of STVs being transported by an individual microtubule. Finer morphological details, such as the width of the costae or the creation of pores, may then result from either thermodynamic properties associated with the medium in the SDV (e.g. surfactants, pH) or the presence of 'blocking agents' within the SDV[16].

Designing 'nanofactories'

Attempts to synthesize diatom-like forms from silica have been attempted chemically and have led to the formation of some characteristic patterns[17,18]. The approach uses both organic and inorganic compounds that organize themselves into modular patterns such as honeycombs. Although this approach may be useful for the creation of certain materials, it is somewhat limited by its inflexibility and inability to create different morphologies that may have wider applications. Such restrictions do not apply to diatoms, which may be manipulated genetically to create a wide range of morphologies with, potentially, a greater number of uses than may be obtained using a chemical approach. We therefore propose the use of diatoms to generate

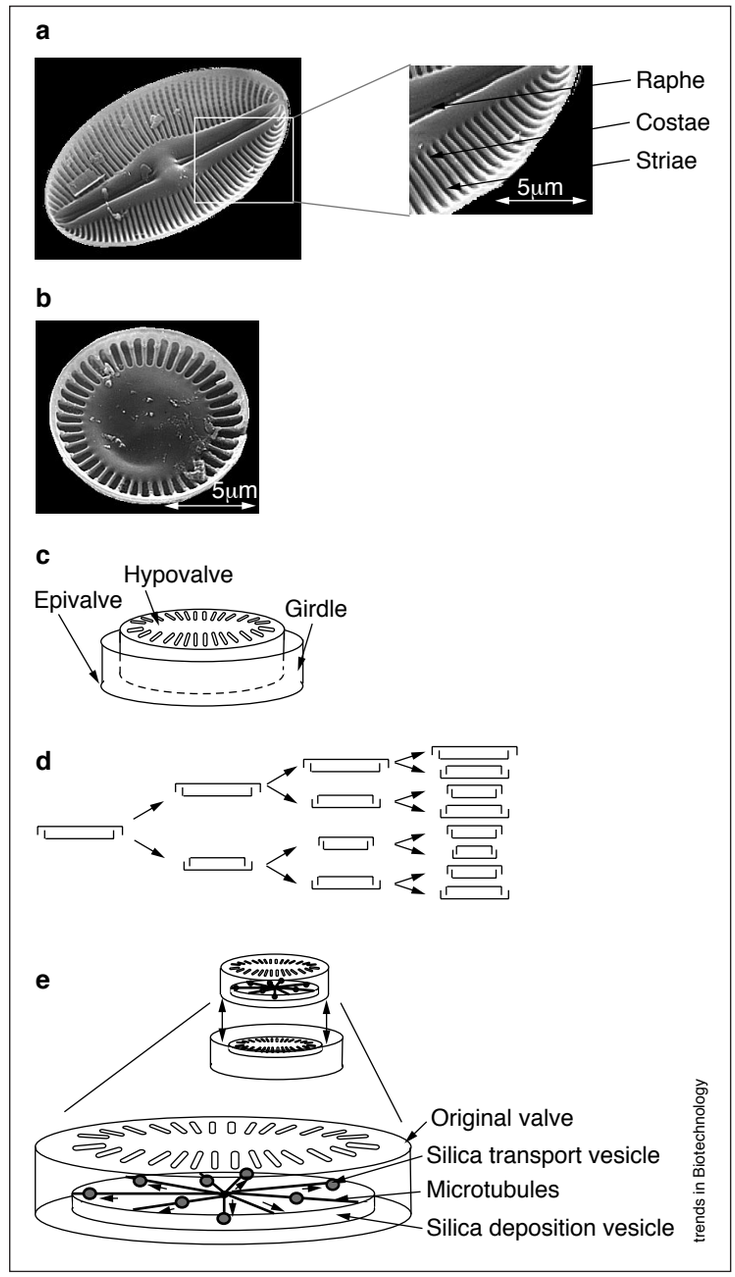

Figure 1

Diatom morphology. The two common forms of diatoms are: (**a**) the pennate form (here, a *Diploneis* sp.; the expanded section shows part of the frustule, detailing the central raphe, the costae and the striae); and (**b**) the centric form (here, a *Cyclotella* sp.). (**c**) Schematic diagram of a centric diatom showing the petri-dish-like nature of the frustule. The larger valve is termed the epivalve and the smaller valve the hypovalve; the girdle is the portion of the frustule between the two valves. (**d**) Successive replications involving the hypovalve lead to a reduction in size. (**e**) Proposed mechanism of diatom morphogenesis: upon diatom replication, a silica-deposition vesicle (SDV) is formed in each half; we propose that microtubules associated with the periphery of the SDV localize the deposition of new silica within the SDV via the silica-transport vesicles. (Micrographs courtesy of the Center for Algal Microscopy and Image Digitization, Bowling Green State University, OH, USA.)

frustule morphologies with potential applications in nanotechnology.

A method for the design and production of a specific frustule morphology has already been proposed and was termed a compustat[19]. Production of a specific frustule morphology starts with the selection of a diatom species that has a morphology close to that required by the application. The diatom would then be cultured in conditions leading to the creation of random mutations (e.g. by adding a chemical mutagen or subjecting the growing diatom culture to UV light). The majority of these mutations will be deleterious and/or have no effect on morphology. However, given a sufficient number of individuals undergoing mutagenesis, some diatoms may have an altered morphology that is closer to the design specification.

Such diatoms may be selected using the compustat: a computer with image-analysis and pattern-recognition software, and linked to a camera mounted on a microscope. Cultured diatoms are circulated or moved under the objective and their images sent to the computer. Those diatoms not approaching the requisite characteristics can be destroyed by shining a laser or a UV microbeam through the microscope[20]. In addition to the initial selection of the designed frustule, the process will also ensure that these evolved nanostructures do not revert back towards their original morphology by natural mutation. Diatoms attaining the desired characteristics could then be cloned.

The use of diatoms in the manufacture and design of materials of micrometre proportions has several advantages over the more-common chemical techniques[17,18]. Their greatest asset in terms of nanotechnological applications lies in their biological heritage. An innate ability to self-replicate and the possibility of genetic engineering permit the low-cost production of a flexible and programmable manufacturing system. Recently, genetic engineering was successful at expressing a new protein in the diatom *Cyclotella cryptica*[21]. As our knowledge of the genetic makeup of diatoms increases, it may be possible to design molecularly precise structures possessing both long-range (μm) and short-range (nm) order.

Nanotechnological applications of diatom frustules

The removal of organic material from diatoms leaves a single valve, a solid precipitate of silica preserving the detail seen in the valve of the living cell[22]. Later, we will show how other materials might be incorporated into this structure, leading to the possibility of moving parts. For now, we will examine the material properties of the inorganic diatom frustule and how its morphology may be utilized for a range of processes.

Filtration

Diatomaceous earth

Diatomaceous earth (DE) is a heterogeneous mixture of the fossilized remains of diatoms. It is classified into a number of grades according to its permeability. Owing to its potential in filtration, DE has been investigated for use in water purification[23,24]. As it is the size of the largest pores in the medium that determines its permeability, the filtration properties of DE are dependent upon the packing of the siliceous material. If we can identify and select for those properties of diatom frustules that lead to a decrease in the size of interfrustule pores, we should be able to design a range of diatom frustules capable of acting as filtration media with a potentially increased range of grades.

The use of diatoms would have two main advantages over DE. First, owing to the homogeneous nature of the culture used to obtain the siliceous material, the permeability will be constant and will depend upon the species and clone of diatom used to generate the material and how the frustules pack together. Second, the fact that diatoms are biological means that they can be shipped in relatively small numbers, which reduces their cost; after shipping, they may then be grown to provide an almost limitless supply of filtration media. This latter property would make diatoms ideal for industrial processes.

In addition to interfrustule pores, certain centric-diatom species possess pores over the surface of their frustules, which may also be exploited for filtration purposes. These intrafrustule pores appear to be uniform in diameter, their size being species specific: *Melosira nyassensis* typically has 0.5–0.6 pores μm^{-1}, while the related species *Melosira nummuloides* has >2.5 pores μm^{-1} (Ref. 25). Although it has been suggested that pores may arise from the presence of blocking agents within the SDV[16], our simulations show that pore size could be a function of temperature and of the surface properties of the precipitating molecules (J. Parkinson and D. Gordon, unpublished). It is thus possible that pore size could be controlled by altering these parameters, and perhaps others, such as salt[26] and germanium[27,28] concentrations. The design of such frustules combined with their relative chemical inertness would have important uses in a number of specialized filtration procedures.

Gel filtration

Gel filtration is a technique commonly employed in the purification of materials such as proteins. The method relies on the use of dextran-based materials (e.g. Sephadex™) in the form of porous beads; the pores' sizes mean that they are inaccessible to large molecules and so material passing through these media is filtered in such a way that the larger molecules emerge first.

With the aid of the compustat, diatom frustules could be designed to possess pores of a constant, specific size, which could then be used as the filtration medium (Fig. 2a). As mentioned previously, the biological nature of the diatom would enable the production of large quantities of relatively low-cost material. In addition, unlike the dextran-based materials, diatom pores are not limited to a set number of sizes but may be varied according to the growth conditions.

Biosensors

The filtration properties of diatom frustules with pores of discrete size also has implications for biosensor design. Biosensors are devices incorporating a biological molecular-recognition component connected to a transducer capable of outputting a signal proportional to the concentration of the molecule being sensed[29]. Biosensors are gaining increasing amounts of interest in nanotechnology, with the production of

arrays of many hundreds of biosensors on a platform the size of a microchip[30,31]. It is envisaged that, for certain applications (e.g. monitoring changes in chemical levels in blood flow), aggregation of proteins around the sensor may interfere with the signal.

Frustules would allow a filtration step to be incorporated into the biosensor, with an individual frustule being associated with each sensor to form a chamber (Fig. 2b). The use of frustules would allow close control over the size of molecules allowed into the biosensing chamber. In addition, owing to their highly refractive nature, the use of frustules may lead to an increase in the signal obtained from those sensors that utilize light emission as a means of detection (e.g. fluorescent probes). Further, because the scale of frustule details such as pores and striae are comparable to the wavelength of visible light, it might also be possible to use them as light pipes.

Immunoisolation

Immunoisolating bioencapsulation is another area that could benefit from the filtration and encapsulation properties of diatom frustules. Recently, investigators using a combination of UV lithography, silicon thin-film deposition and selective etching created a biocapsule capable of immunoisolating transplanted cells[32]. The key feature of such capsules is their ability to protect the enclosed tissue from immune rejection while allowing an adequate supply of nutrients and oxygen.

The natural occurrence of pores in diatom frustules make them ideal vehicles for providing enclosed cells with nutrients. To protect their cells from immune rejection, however, the frustule must be capable of filtering molecules such as immunoglobulins and components of the complement system[33]. This can be achieved by restricting the size of pores to below the dimensions of these molecules (two of the most important molecules involved in the immune response, C1q and IgM, have a smallest dimension of 30 nm).

In addition to controlling the size of the pores, we can also alter the overall dimensions of a frustule, so that large biocapsules capable of containing several mammalian cells could be created. One possible drawback could be the immunoreactivity of the silica itself: it has been well documented that silica particles can cause fibrosis of the lung[34]. However, acute reactions tend to arise from prolonged exposure to large amounts of silica. If these problems do occur, there still remains the possibility of either coating the surface of the diatom with an immunologically unreactive material or growing frustules using material other than silica as outlined below.

Microfabrication

In addition to their filtration capabilities, diatom frustules have potential uses in the production of nanomaterials of constant diameter. Currently, membrane templates have been used to synthesize tubular or cylindrical nanomaterials out of conductive polymers and metals[35]. The synthesis involves the chemical or electrochemical deposition of the molecules within the pores of a membrane template. Depending upon the chemistry of the pore wall, this can lead to the creation of fibrils or tubules. Among other applications, the tubules may be capped and hence used as capsules for drug delivery.

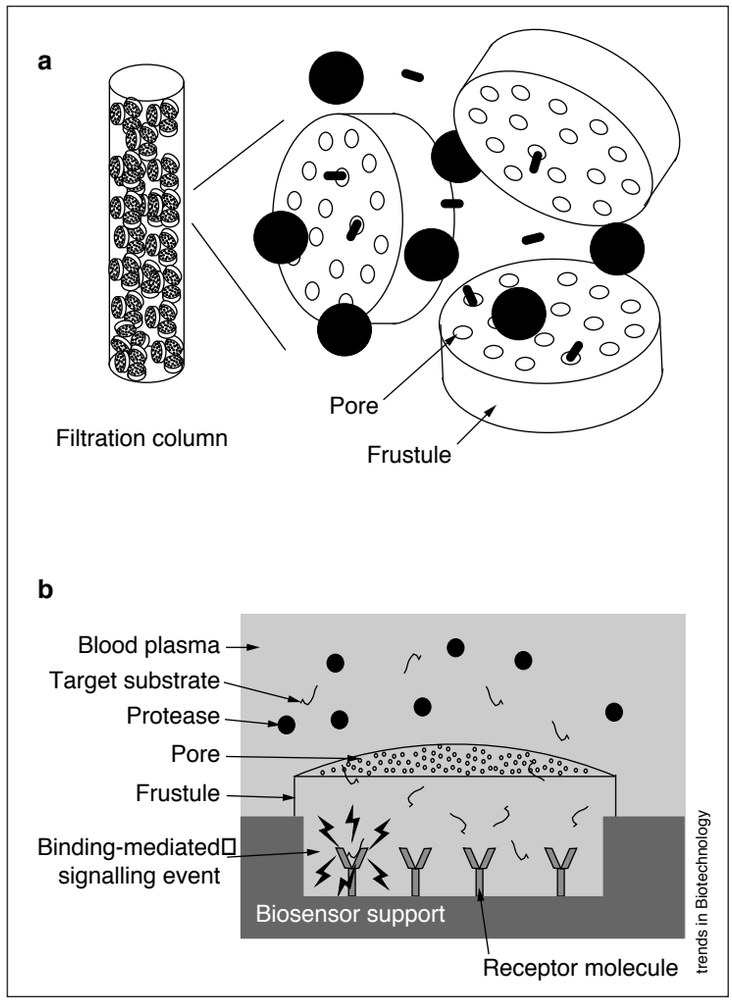

Figure 2

Filtration applications of diatoms. (**a**) A filtration column is packed with diatom frustules. Large molecules will pass through the column relatively quickly, while smaller molecules will be able to enter the frustules via their pores and will thus be eluted at a much lower rate. (**b**) Biosensor filter: in a typical application (e.g. monitoring blood glucose), receptor molecules are contained (either free or fixed to the support, as shown here) within a chamber capped by a diatom frustule. Small molecules may enter via the pores in the frustule and bind to the receptors, eliciting a signal. Larger molecules capable of disrupting the signal (e.g. proteases) are prevented from entering the chamber.

Most of the work in this area has been undertaken using one of two types of synthetic membrane – track-etch polymeric membranes, which are available in a wide range of pore sizes but whose pores are randomly distributed and have low porosity, and porous aluminas, which are available in only a very limited number of pore diameters. Depending upon the lower limit attainable for pore size (which is not currently known), their high, regular porosity combined with the ability to regulate their size tightly could make frustules ideal templates for this procedure (Fig. 3).

Currently, many silicon-based microfabrication processes involve the use of a number of lithographic techniques[4,36,37]. At present, it is possible to achieve a resolution in the micrometre range using focused-particle-based lithographies[4]. However, it is not clear that these techniques are suitable for cost-effective, high-volume manufacturing applications. Rather,

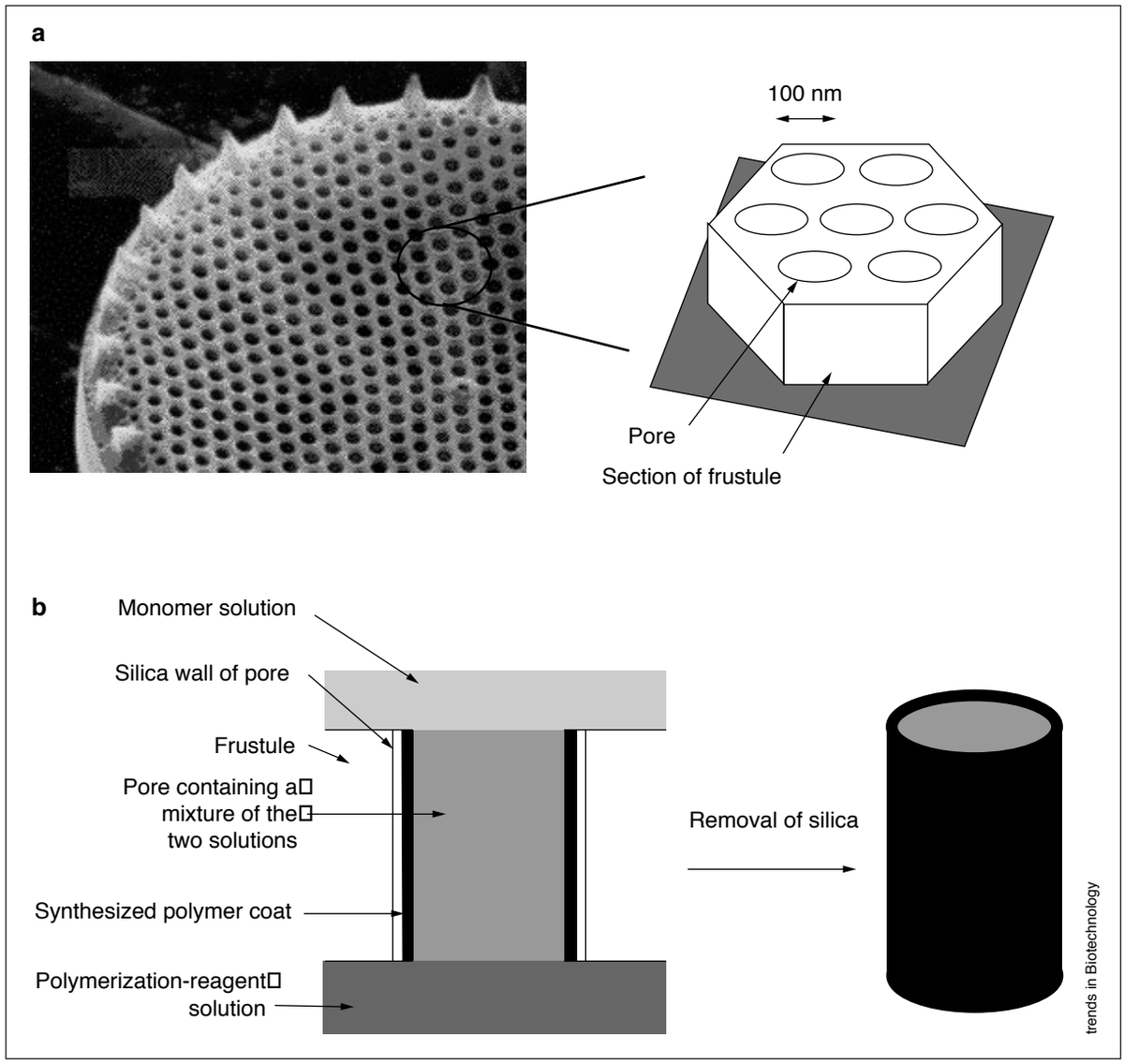

Figure 3

Formation of nanotubes or fibres. (**a**) Certain centric diatom frustules display a high density of regularly ordered pores with diameters from 100 nm. (Micrograph courtesy of D. Kunkel, University of Hawaii, HI, USA.) (**b**) By using the template-synthesis method outlined by C. Martin[35], it is possible to use these pores to form nanometre-sized tubes and fibres. In brief, a solution of monomer is separated from a polymerizing reagent by the frustule. Within the pores, the two solutions mix and, owing to the anionic nature of silica, nucleation of the cationic polymers will preferentially occur on the walls of the pores. Subsequent removal of the silica will lead to the formation of tubules or fibres (depending upon the amount of time the pore is exposed to the solutions).

masking techniques such as photoresist patterning and masked particle lithographies show more potential.

There are four major components of a lithographic system: the source, the aligner, the mask and the resist (Fig. 4a). In a typical lithographic procedure, the sample to be etched is coated in a resist. An aligning tool is then used to position the mask defining the pattern over the sample and the ensemble is exposed to the source (X-ray, ion beam etc.). It is in the design of masks that the diatom frustule has potential, as a cost-effective programmable system for creating a range of different mask morphologies. For example, the constant spacing of striae in pennate diatoms would allow the creation of arrays of channels in which each channel is separated from its neighbour by a constant distance (Fig. 4b). With striae spacings already on the order of μm and the possibility of decreasing this still further using the compustat approach outlined earlier, it should be possible to create thousands of channels on a single silicon chip. The ability to manufacture such arrays cheaply would obviously have implications for a number of biosensor applications[30,31].

Centric diatoms could also be used as lithographic masks, leading to the formation of wheels with spokes of micrometre proportions (Fig. 4c). These spokes could serve as channels, allowing the simultaneous detection of a number of chemicals from a single source of flow. In addition, coating frustules with a hydrofluoric-acid-resistant material followed by removing the silica with hydrofluoric acid would allow replica-plating techniques to be employed. Further refinement of the lithographic process by the incorporation of techniques such as undercutting[38] could lead to the

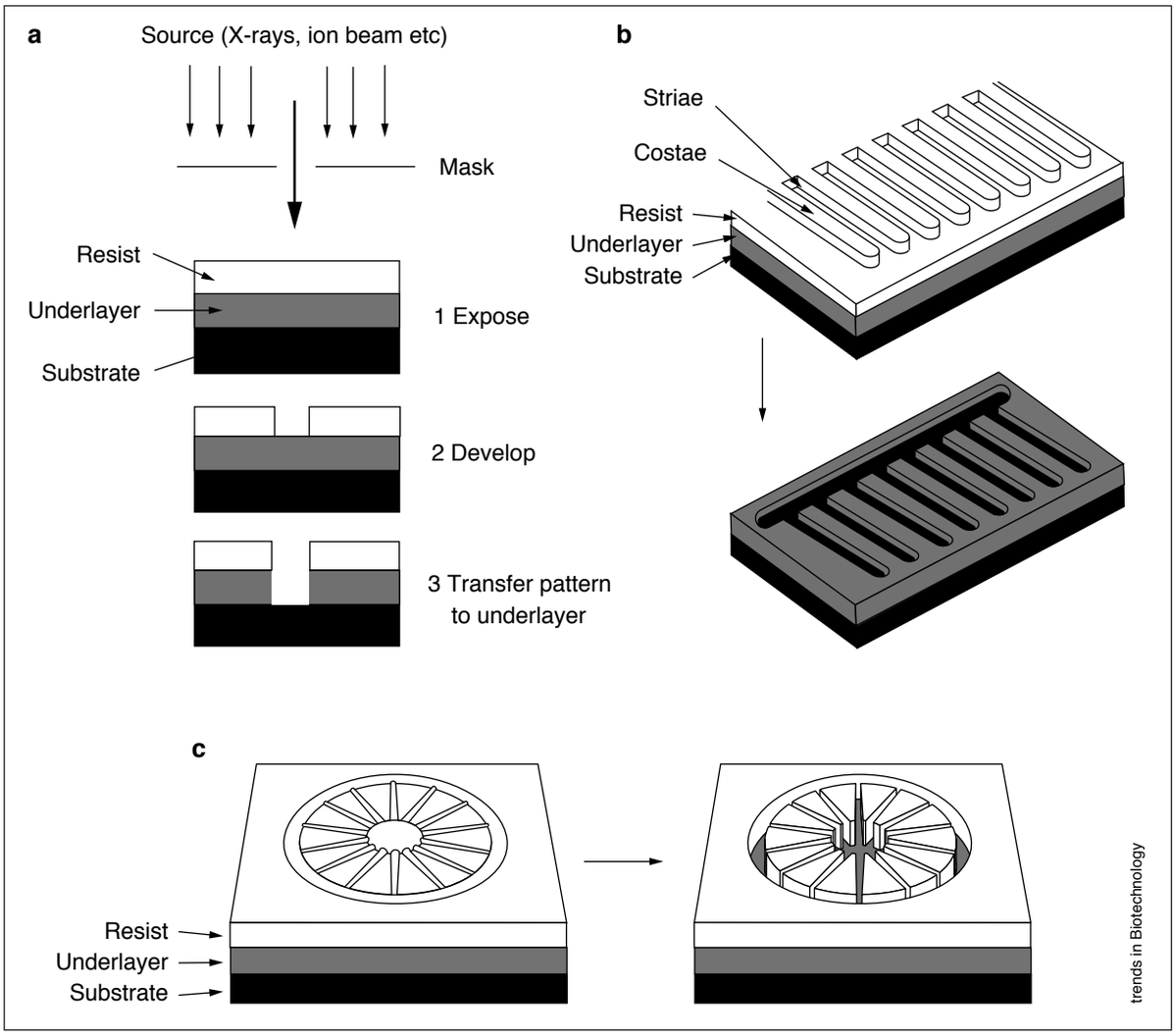

Figure 4

Lithographic applications of diatoms. (**a**) Masked-pattern lithography: the resist is exposed to the source in areas exposed by the mask (1); developing (removing) the exposed resist reveals the underlying layer (2); the pattern is then transferred to the underlayer via etching (3). (**b**) Using the costae of a pennate diatom as a mask in the lithographic process leads to the formation of parallel arrays of uniform channels. (**c**) The use of a centric diatom in combination with a negative resist (in which the exposed surface is resistant to the etching process) leads to the formation of radiating channels.

cheap manufacture of components such as wheels and gears on the micrometre scale.

Applications of non-siliceous frustules

Thus far, we have only addressed the uses of silica-based frustules. Over two decades ago, Azam and co-workers showed that germanium may also be incorporated into frustules through the same pathway as silica[27,28]. There thus remains the intriguing question of which other elements could be used, and to what extent, instead of silica in frustule formation. Silicate is known to be transported into the cell via an ionophore in the form of either $Si(OH)_4$ or $H_3SiO_4^-$ (Refs 39,40). Furthermore, the successful incorporation of $Ge(OH)_4$ into the frustule[27,28] suggests that those elements capable of forming a tetrahydroxide, such as lead and tin, could potentially be used to generate diatom frustules.

In addition to forming frustules with unique material properties, it should be possible to create frustules composed of more than one type of material. At this stage, it should be noted that the design of frustules composed of more than one material is limited by the fact that the diatom grows from the centre out (or in the case of pennate diatoms, from the central raphe). Hence, if it were possible to switch between two types of material during the growth of the frustule, each material will be laid down as a band. The ability to switch materials will depend upon the time of formation of the frustule: the initial frustule pattern is known to take 3–4 min to form and a subsequent 1–2 h to thicken[9,10].

Although it is possible to slow this process down either by cooling the diatom or by limiting the light or nutrient source, repeating flushing and filtering of the chemical environment has the potential for cellular damage and may affect morphogenesis. An alternative would be to engineer a switch between the deposition of two or more types of component using, for example, temperature-sensitive pathways. In this approach, a protein involved in the transport of material to the periphery of the SDV would be identified and isolated. Mutational studies would then be undertaken,

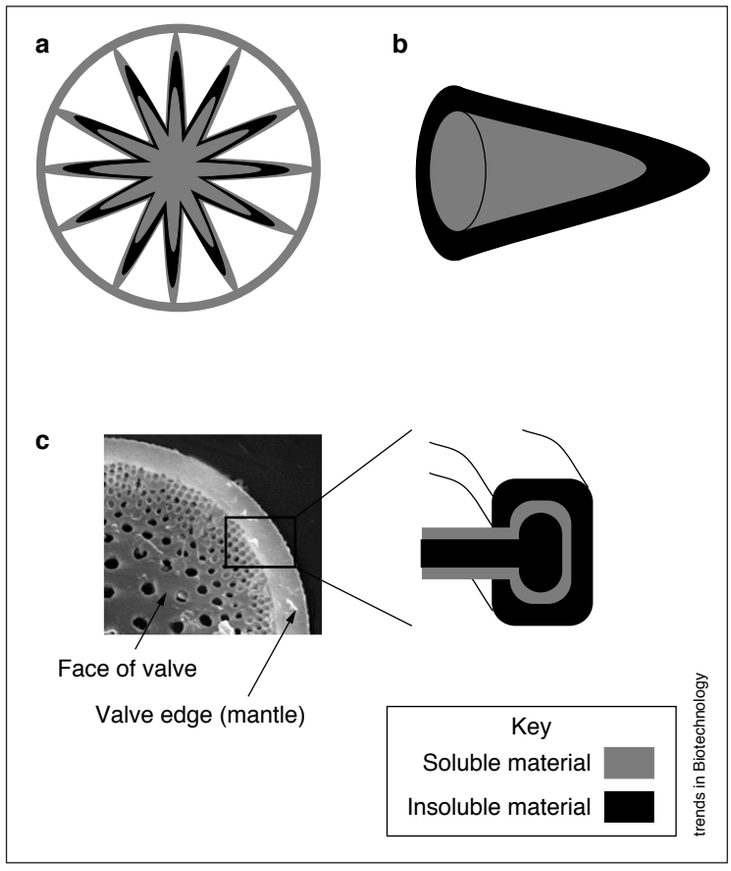

Figure 5

Growth of diatoms using two types of material. (**a**) During the growth of a centric-diatom's frustule, a switch between the deposition of two types of material leads to the formation of a mosaic frustule. Dissolution of one of the materials leads to the production of cogs. The use of two switching regimes leads to the formation of hollow spokes (**b**), thus reducing the overall weight of the cog. A switch in material deposition during the growth of the girdle of the diatom frustule could result in the formation of an axle (**c**).

leading to the alteration of the protein such that a novel material is preferentially transported by this pathway. Such a mutation could be selected for on the basis of temperature sensitivity (a common approach used in isolating mutant forms of proteins in many organisms). The introduction of this new protein into a diatom containing the original protein would then allow a switch in the type of material deposited simply by altering the growth temperature.

The incorporation of non-siliceous material into the structure of frustules and its subsequent removal could allow the production of moving parts such as cogs and hinges (Fig. 5). This may provide an even cheaper method of producing such structures than the lithographic procedure outlined earlier.

Conclusions

Diatoms have potential as factories for the production of a wide range of materials that may be of great benefit to nanotechnology and microfabrication. Although a compustat approach may prove useful in the generation of a number of useful morphologies, the true potential of diatoms will become clearer when we have unravelled the pathways by which silica is transported within the diatom and deposited.

References

1 Whole section (1991) *Science* 254, 1300–1342
2 Drexler, K. E., Peterson, C. and Pergamit, G. (1991) *Unbounding the Future: The Nanotechnology Revolution*, Morrow
3 Krummenacker, M. and Lewis, J. (1995) *Prospects in Nanotechnology: Toward Molecular Manufacturing*, Wiley
4 Chieh, Y. S., Krusius, J. P. and Chapman, P. (1994) *J. Electrochem. Soc.* 141, 1585–1589
5 Gordon, R. and Aguda, B. D. (1988) in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Part 1/4: Cardiology and Imaging* (Vol. 10), pp. 273–274, Institute of Electrical and Electronics Engineers, New York, NY, USA
6 Werner, D. (1977) *The Biology of Diatoms*, University of California Press
7 Round, F. E., Crawford, R. M. and Mann, D. G. (1990) *The Diatoms: Biology and Morphology of the Genera*, Cambridge University Press
8 Lewin, J. C. and Guillard, R. R. L. (1963) *Annu. Rev. Microbiol.* 17, 373–414
9 Gordon, R. and Drum, R. W. (1994) *Int. J. Cytol.* 150, 243–372
10 Gordon, R. and Drum, R. W. (1994) *Int. J. Cytol.* 150, 421–422
11 Allen, L. H. and Matijevic, E. (1969) *J. Colloid Interface Sci.* 31, 287–296
12 Righetto, L., Polissi, A., Comi, D., Marcandalli, B., Bellobono, I. R. and Bidoglio, G. (1987) *Ann. Chim.* 77, 437–455
13 Preisig, H. R. (1994) *Protoplasma* 181, 29–42
14 Conley, D. J. and Kilham, S. S. (1989) *Limnol. Oceanogr.* 34, 205–213
15 Pickett-Heaps, J. D., Schmid, A. M. M. and Edgar, L. A. (1990) *Prog. Phycol. Res.* 7, 1–168
16 Schmid, A. M. M. (1986) in *Proceedings of the Eighth International Symposium on Living and Fossil Diatoms*, (Ricard, M., ed.), pp. 293–314, O. Koeltz, Koenigstein, Germany
17 Schultze, M. J. S. (1863) *Quart. J. Microscop. Sci.* 3, 120–134
18 Oliver, S., Kuperman, A., Coombs, N., Lough, A. and Ozin, G. A. (1995) *Nature* 378, 47–50
19 Gordon, R. (1996) *Nova Hedwigia* 112, 213–216
20 Drum, R. W., Gordon, R., Bender, R. and Goel, N. S. (1971) *J. Phycol.* 7 (Suppl.), 13–14
21 Dunahay, T. G., Jarvis, E. F., Davis, S. S. and Roessler, P. G. (1996) *Appl. Biochem. Biotechnol.* 57–58, 223–231
22 Hasle, G. R. and Fryxell, G. A. (1970) *Trans. Am. Microscop. Soc.* 89, 469–474
23 Schuler, P. F., Ghosh, M. M. and Gopalan, P. (1991) *Water Res.* 25, 995–1005
24 Ongerth, J. E. and Hutton, P. E. (1997) *J. Am. Water Works Assoc.* 89, 39–46
25 Kilham, P. (1990) in *Large Lakes* (Tilzer, M. M., ed.), pp. 414–427, Springer-Verlag
26 Gordon, R. and Brodland, G. W. (1990) *Diatom Res.* 5, 409–413
27 Chiappino, M. L., Azam, F. and Volcani, B. E. (1977) *Protoplasma* 93, 191–204
28 Azam, F., Hemmingsen, B. B. and Volcani, B. E. (1973) *Arch. Mikrobiol.* 92, 11–20
29 Collings, A. F. and Caruso, F. (1997) *Rep. Prog. Phys.* 60, 1397–1440
30 Meyer, H. *et al.* (1995) *Anal. Chem.* 67, 1164–1170
31 Dempsey, E. *et al.* (1997) *Anal. Chim. Acta* 346, 341–349
32 Desai, T. A., Chu, W. H., Tu, J. K., Beattie, G. M., Hayek, A. and Ferrari, M. (1998) *Biotech. Bioeng.* 57, 118–120
33 Colton, C. K. (1995) *Cell Transplant.* 4, 415–436
34 Anderegg, U., Vorberg, S., Herrmann, K. and Haustein, U. F. (1997) *Eur. J. Dermatol.* 7, 27–31
35 Martin, C. R. (1994) *Science* 266, 1961–1966
36 Seeger, D. E., La Tulipe, D. C., Jr, Kunz, R. R., Garza, C. M. and Hanratty, M. A. (1997) *IBM J. Res. Devel.* 41, 105–118
37 Silverman, J. P. (1997) *J. Vac. Sci. Technol. B* 15, 2117–2124
38 Carr, D. W. and Craighead, H. G. (1997) *J. Vac. Sci. Technol. B* 15, 2760–2763
39 Bhattacharyya, P. and Volcani, B. E. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 6386–6390
40 Bhattacharyya, P. and Volcani, B. E. (1983) *Biochem. Biophys. Res. Commun.* 114, 365–372