Review

Potential of silica bodies (phytoliths) for nanotechnology

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Many plant systems accumulate silica in solid form, creating intracellular or extracellular silica bodies (phytoliths) that are essential for growth, mechanical strength, rigidity, predator and fungal defence, stiffness and cooling. Silica is an inorganic amorphous oxide formed by polymerization processes within plants. There has been much research to gain new insights into its biochemistry and to mimic biosilicification. We review the background on plant silica bodies, silica uptake mechanisms and applications, and suggest possible ways of producing plant silica bodies with new functions. Silica bodies offer complementary properties to diatoms for nanotechnology, including large-scale availability from crop wastes, lack of organic impurities (in some), microencapsulation and microcrystalline quartz with possibly unique optical properties.

Introduction

Silica nanoparticles have proven to be important for several biotechnological and biomedical applications, such as biosensor design, drug delivery, cell labelling, cell separation, contrast agents for magnetic resonance and ultrasound medical imaging, and as a targeting and therapeutic platform for drug- or enzyme-released systems [1–5]. Silica bodies are opal phytoliths (*phyto* means 'plant' and *lithos* means 'rock' in Greek) produced by plants when soluble silica from the ground water is absorbed by the roots and carried to different parts of the plant system through the vascular system. Some plants have nonsiliceous phytoliths, such as calcium oxalate (cystoliths [6]).

Most attention has focused on diatom nanotechnology [4] because of their many species, highly regular multiscale structure, fast reproduction and genetic manipulability. As we shall see, silica bodies offer complementary features: huge availability from the unused parts of crop plants [7], high purity of the silica [8], apparent lack of contained organic material in some [8] owing to a precipitation mechanism operating off the cell wall, microencapsulation of organic matter in others, and production of microcrystalline quartz, which might provide unique physical and optical properties.

Precipitation and polymerization of the silica, aided by evaporation and metabolism of water in the plant body, leads to the formation of intra- as well as extracellular silica bodies. Intracellularly, Si accumulates in both the cytoplasm and the vacuoles of the plant cell [8,9]. Plant silica bodies are deposited in roots, stems and leaves. They survive death and decomposition of the plant, are durable in soils and dry environments, and are morphologically distinct and abundant [10]. Silica deposits occur in the form of particles of characteristic shapes, such as dumbbells, saddles, bowls, boats and others (Figure 1), with irregular hollow or porous [11,12] to nearly uniformly solid [8,13] internal textures that are presumably moulded from the intra- or extracellular spaces in which they form. The shapes unfortunately cross genus or species taxonomic boundaries. Nevertheless, because silica bodies are highly durable under a wide range of depositional conditions, they have proven useful in archaeology to identify crop plants [10] and to deduce the presence of forested vegetation and soil horizons [14–16]. Thus, phytolith analysis is a valuable tool for examining the paleoenvironment and gathering evidence of diet and food processing. For example, maize (*Zea mays*) leaves can be differentiated from wild grasses using the shape of silica bodies [17]. Phytolith mapping from different ecological zones is also useful for understanding forest–grassland vegetation dynamics, for analysing the possible causes of loss of biodiversity and for understanding human–land relationships [10,17].

Silica bodies mostly range between 10 and 30 microns and are occasionally up to 200 microns in size [14]. The insoluble silica in a plant's epidermal cell wall is an isotropic (amorphous) deposit with a refractive index ranging from 1.42 to 1.44 [18]. Carbon trapped inside long-lasting silica bodies might be a major component of the carbon cycle on earth [19,20] (although unfortunately DNA has not been found yet [21]). A typical silica body can sequester up to 5% of its weight as carbon [20]. In grass short cells the organic material often appears as a single small 'bubble' (M. Madella, personal communication).

In parallel to the rapid rise of the idea of growing nanotechnology by using diatoms [4], a smaller effort has been underway to examine silica bodies for this endeavour. Our aim here is to provide background on silica bodies and review recent first efforts towards silica body nanotechnology.

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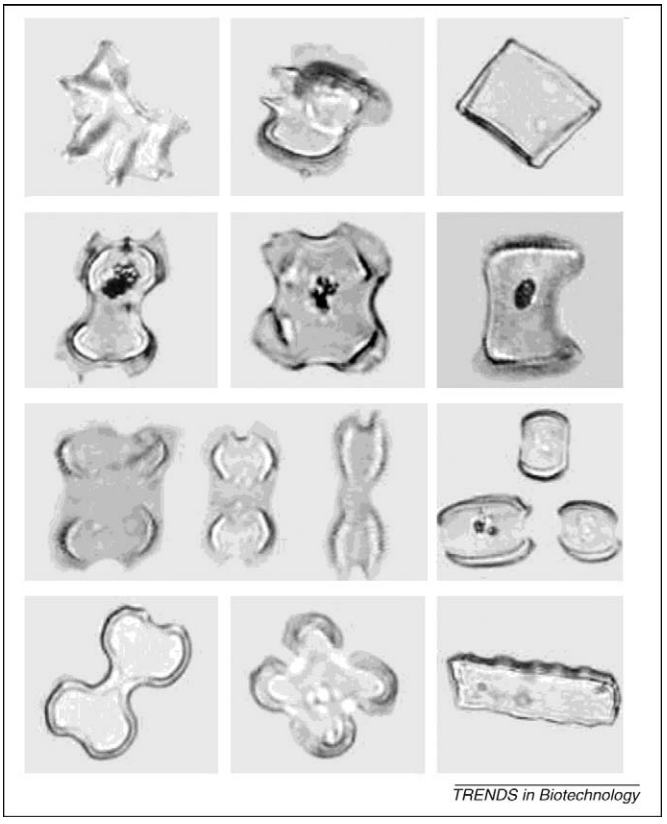

Figure 1. Light micrographs of phytoliths of typical shapes from various families of grasses. Reproduced, with permission, from Ref. [88]. Typical sizes are 10–20 μm.

Functions of phytoliths

Silica bodies in plants serve a variety of purposes, including lending the plant structural rigidity by supporting the shoot [22], giving lodging (falling over) resistance [23] and giving mechanical strength and rigidity to leaves [24]. Their hardness deters obvious predators [10] and, owing to their ability to wear down tooth enamel, they might even provide some (indirect) resistance to mammals, such as *Homo sapiens* [25]. The evolution of horse dentition correlates with increased phytolith content of grasses [26], and silica bodies make some plants distasteful or give their tissues a prickly texture [27]. Silica bodies also conserve water during moisture stress or drought [28], and they have been shown to influence stomata movement on the plant leaf epidermis and reduce the transpiration rate of water in maize [29]. Plant silica bodies promote cell elongation in the growing zone and decrease cell-wall extensibility in the basal zone of stellar tissues in the roots and thereby enhance root elongation of plants [30]. Silica bodies also improve plant tolerance to fungal diseases [31] and metal toxicity [28]. Cells filled with silica bodies allow the plant to capture more light, thus aiding photosynthesis [32], perhaps in the manner of light piping that has been hypothesized for colonial diatoms [4]. At longer wavelengths, in the infrared, silica bodies aid cooling of leaves [33]. In summary, silica helps a plant to survive many abiotic stresses, such as salt, metal toxicity, nutrient imbalance, drought, radiation, high temperature, freezing and ultraviolet [34], and reduce the impact of plant predators.

Silicon uptake mechanism

Plants and other biological organisms make silica in amounts of gigatons per annum, whereas industrial processes only produce mere megatons [7]. Plants extract silica from undersaturated aqueous environments at atmospheric pressure and at temperatures ranging between 4 and 40 °C, which is in contrast to the generally extreme temperatures used in industrial extraction involving silicic acid [7,35]. A better understanding of the bioavailability of phytoliths and the silicon uptake process that takes place inside plants is crucial for the exploitation of their characteristics, for example for the design of nanomaterials with tailored specifications of size, surface area, porosity, morphology and surface functionality.

Silicon uptake has been investigated in more than 500 plant species, and depending on the Si content, plants can be classified as either Si accumulators (rice [*Oryza sativa*], horsetails [*Equisetum arvense*], sugarcane [*Saccharum officinarum* L.], etc.) or Si nonaccumulators (less than 3 mg Si/g dry matter), such as most dicotyledons, including legumes [36,37]. Consequently, Si concentrations in plant tissues vary considerably, from only 0.1% (dry weight basis) in dicotyledons, over 1–3% in grasses, such as oats (*Avena sativa* L.) and rye (*Secale cereale*), up to 10–15% in the Gramineae family, for instance in rice and Cyperaceae (sedges) [38,39]. Si uptake has been reported to be an active process for rice, beans and some species of dicotyledons [40].

Mitani and Ma [41] demonstrated that for rice, tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus* L.), the Si uptake mechanism was transporter-mediated and energy-dependent. They proposed that the actual Si uptake takes place through the radial transport of Si from the external solution to the root cortical cells and, finally, to the xylem. Furthermore, using PCR markers on chromosomes, two transporters (SIT1 and SIT2) that are located at the plasma membranes of root cortical cells and xylem parenchyma cells were shown to facilitate silica transport in rice [42]. The molecular mechanisms for rice [34] involve uptake of silica through lateral root hairs of plants via these transporters in the form of silicic acid and then translocation to the shoot in the same form. In the shoot, and under the cuticle of the plant body, Si is polymerized and deposited as a silica body in conjunction with water loss through transpiration [34].

Extensive systematic leaf tissue analysis using a scanning electron microscope equipped with an energy dispersive X-ray microanalyser [43] revealed that the silica polymerization and deposition process is dependent on the plant cell type. Silica bodies in some higher plants contain protein residues [44,45], and such residues, at least in diatoms, are suspected to have a role in silica precipitation [4]. Silicic acid uptake was also investigated in wheat (*Triticum aestivum*) using solution nuclear magnetic resonance (NMR) techniques [46] with the aim of identifying molecular Si-containing species. However, only monomeric and dimeric silicic acid were detected and there was no evidence of organic components [8], although other plants show some concentrated organic material, perhaps cellulose, near the silica body surface [12]. Thus it would seem that, at least in some plants, silica precipitation

proceeds without nucleation by proteins. Mechanisms might differ between tissues [8].

Examination of the pattern of silica deposition in the abaxial epidermal cells of maize leaves using X-ray microanalysis and fluorescence microscopy by Dorweiler and Doebley [47] helped them to hypothesize that silicification might be linked to loci that are involved in lignifications. Piperno *et al*. [48] suggested that silica deposition is in fact correlated with a genetic locus, *hard rind* (*Hr*), involved in lignin deposition. Perry and Keeling-Tucker [35] and Kauss *et al*. [49] have proposed a molecular model for Si transport in plants that suggests that the kinetics of silica formation and structure regulation are influenced by a proline-rich protein (PRP1) and proteoglycans. The protein enhances cell wall reinforcement, where it provides a barrier to fungal penetration by acting as a catalyst of silica polycondensation [49]. Thus, silica deposition in plants might involve surface catalysis (cf. wall deposition in [12]) without entrapment of the catalysing enzyme within the silica, which is in contrast to the enzyme entrapment seen in diatoms [50]. The extraction and characterization of cell-wall-protein amino acid sequences and structures, the correlation of these proteins with defined functions and the analysis of their roles in the formation of silica will aid our understanding of silica body formation in plants [51].

In spite of the published work on the mechanism of silica uptake and formation of silica bodies in plants, as in diatoms [4], the exact mechanisms that allow silica to pass through cell walls and membranes are not fully understood. With the increasing availability of genetics and genome mapping, it might be possible to identify those genes involved in the silica body silicification process, as is proceeding in diatoms [4].

Structure of phytoliths

There are no accepted methods currently established for investigating patterning in plant silica body sizes. Hansen *et al*. [52] have developed an analytical framework for characterizing size patterning in plant silica body assemblages using a flow-programmed field-flow fractionation technique (a method that would probably also be superior to FACS for diatoms [2]). The solubility, surface properties (such as electrophoretic mobilities and surface charge) and dissolution kinetics of biogenic silica bodies extracted from fresh biomass of larch (*Larix gmelinii*), elm (*Ulmus laevis* Pall.), horsetail (*Equisetum arvense*), fern (*Dicksonia squarrosa*) and four grasses are close to that of synthetic amorphous silica [53].

Many techniques are available for studying the structure and chemical composition of plant silica bodies [51,54], including transmission electron microscopy (TEM), scanning electron microscopy (SEM, Figure 2), atomic force microscopy (AFM), energy dispersive X-ray analysis (EDXA), X-ray diffraction techniques and biochemical separation procedures.

Conventional biochemical separation procedures for the extraction of silica bodies, such as wet oxidation, dry ashing and acid wash burning, frequently led to cross-contamination and possibly to alterations in phytolith assemblies [55]. To measure Si concentrations, colourimetric methods, atomic absorption and inductively coupled plasma analysis are the methods of choice [7], whereas SEM and EDXA are most commonly used for investigating the silica particles in detail. Although X-ray diffraction techniques can be used for determining the organization of silica bodies at the nano level, TEM has proven useful for investigating particles with regard to size, interactions and relative orientation, as well as for following structural changes observed during the development of silica bodies [12].

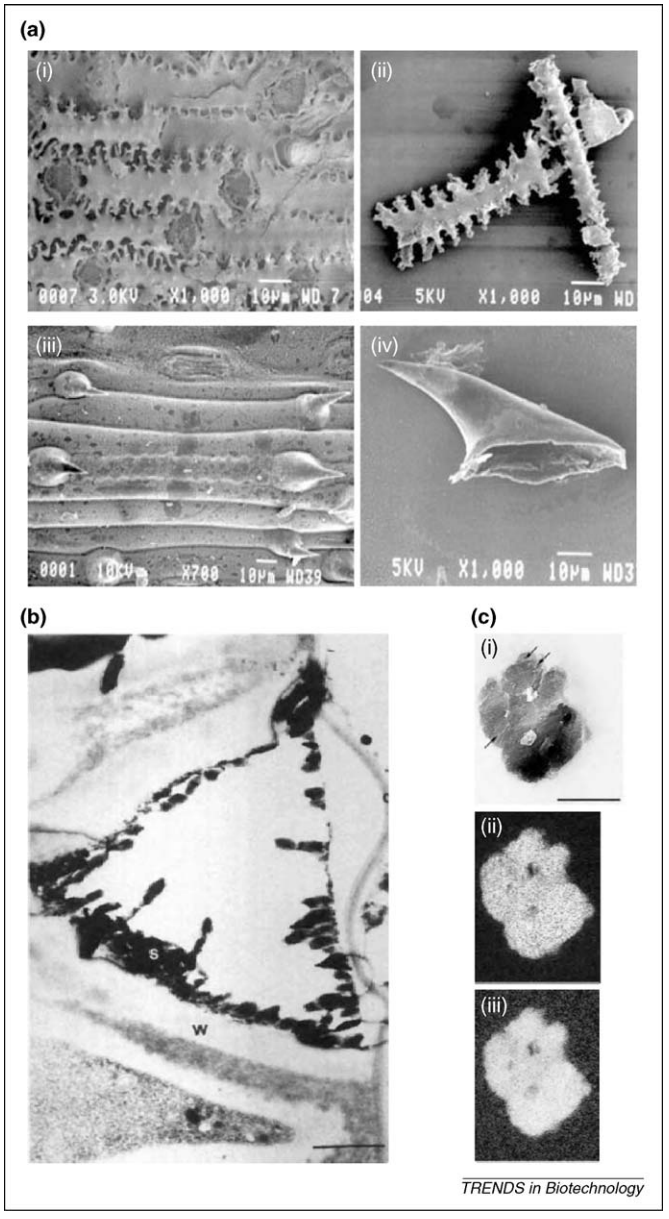

Figure 2. Electron micrographs of a variety of silica bodies. (a) Scanning electron micrographs of different silica bodies in the Einkorn wheat *Triticum monococcum*. (i) Inflorescence of dendriform, *in situ*. (ii) Inflorescence of dendriform and rod with clavate processes. (iii) Laminae of adaxial leaf section, showing structures that silicify. (iv) Lamina of hair cell. Panel (a) reproduced with permission of Terry Ball (http://home.byu.net/tbb/). **(b)** Transmission electron micrograph of a phytolith growing inwards from the cell wall (w) in the monocot *Deschampsia caespitosa*. Scale bar represents 1 μm. Reproduced, with permission, from Ref. [11]. **(c)** Transmission electron loss microscopy of vacuolar silica granules in palisade cells of the palm *Syagrus coronata*. (i) Zero loss image. (ii) L edge silicon image. (iii) L edge oxygen image. (C and N images showed no structure.) The arrows in (i) indicate some porosity. Scale bar represents 200 nm. Panel (c) reproduced, with permission, from Ref. [8].

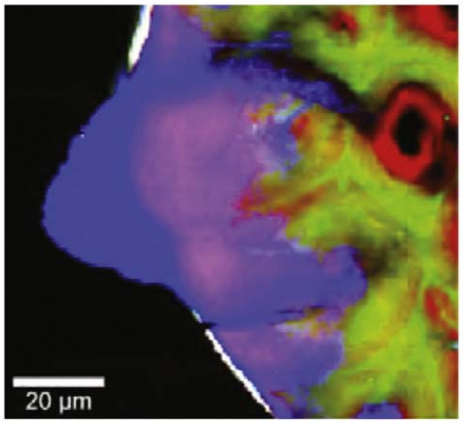

Figure 3. Confocal Raman microscopy showing chemical composition of a cross section of the horsetail *Equisetum hyemale*: blue = silica, green = pectin, red = cellulose, white = cuticular layer. Reproduced, with permission, from Ref. [58].

Using solid state Si NMR spectroscopy, Perry *et al.* [56] were able to determine that plant silica bodies consist of mostly extensively hydrated silica gels with Si-O-Si bond angles of less than 145°, indicating an amorphous nature. The oxygen bridge bonds among silicon atoms give SiO_2 many of its unique properties. SEM in combination with EDXA was used to locate the composition of minerals and other constituents within the plant silica body, for example that of carbon sequestered inside the plant silica body [20], and to determine the hydration levels for individual structural phytolith types within a mixed sample [57]. The internal structural information, such as bond strength and hydroxylation in the silica body, was studied using infrared spectroscopy, and solid state NMR spectroscopy has been used to look at the local environment around silicon atoms [7]. Holzhüter *et al.* [54] have used small-angle and wide-angle X-ray diffraction techniques to detect the nanometre-level structure of plant silica bodies in the primitive horsetail plant *Equisetum arvense* and have found no evidence for crystallinity, that is, further evidence that silica bodies are made of amorphous silica, similar to diatoms [4].

Using two-dimensional spectral maps of high resolution (<1 μm) confocal Raman microscopy, the direct visualization of differences in chemical composition of cross sections of *Equisetum hyemale* was accomplished [58] (Figure 3). AFM has been used to analyse diatom cell wall structures, and an unexpected nanostructured granular surface was revealed [59] that consisted of fused spherical silica nanoparticles, perhaps corresponding to a 'granular structure at the nanometre scale' in silica bodies [12]. This level of structure might represent sintering of colloidal silica [4]. In general, contrary to diatoms, except for these possible sintered spheres of around 50 nm diameter [4], no regular hierarchical structures have been observed in phytoliths.

Applications of silica bodies to nanotechnology

There are two general possible approaches for silica bionanotechnology: (i) the direct use of naturally produced silica structures; and (ii) the biomimicking of selected components, such as proteins or metabolites involved in silica processing in organisms, with the aim of producing artificial structures [4,60,61]. Perry and Lu [62] have identified $K_2[Si(C_6H_4O_2)_3]\ 2H_2O$ as a silicon complex that is very similar (biomimetic) to the silicic acid complex inside plants at approximately neutral pH and ambient temperature conditions. However, we will focus here on the direct use of phytoliths.

Several research efforts have focused on producing specific nanoparticles in plants. We have synthesized organic silica materials in intercellular spaces of epidermal cells of tall fescue (*Festuca arundinacea* Schreb.) leaves through molecular recognition between Si-OH and polysaccharide-OH or glycoprotein-OH using tetraethoxysilane rather than monosilicic acid [63]. Further, Bansal *et al*. [64] have utilized the fungus *Fusarium oxysporum* to transform the naturally occurring amorphous biosilica in rice husks into quasispherical shaped, highly crystalline silica nanoparticles (quartz) of 2 to 6 nm diameter. The authors claim that this method of producing biosilica nanomaterials constitutes an energy-conserving and economically viable 'green' approach for quartz production compared with the respective industrial processes performed at high temperatures [65,66]. The transformation of amorphous silica to quartz has also been suggested to take place in the horsetail *Equisetum telmateia* [67] and might also occur in the electric organs of fish and in human brains [68].

Amorphous nanosilica can be obtained from burnt and hot organic acid pretreated rice hulls and straw or from the epidermis of vegetables [69]. Plant-stem-derived nanosilica is capable of reducing amounts of certain classes of lipoproteins produced by the polyhydrosis virus in silkworm larvae [70]. Insects develop resistance to chemical insecticides by using a variety of cuticular lipids to protect their water barrier and avoid death by desiccation [71]. Surface charged, modified hydrophobic nanosilicas bypass this defence by being absorbed into the cuticular lipids of the insects through physiosorption, causing the desiccation and death of the insects. Hence 3–5 nm nanosilica particles could be used as an effective nanobiopesticide for controlling a range of agricultural insect pests and animal ectoparasites [71].

Rice hull ash (RHA) silica can be depolymerized in aqueous alcohol with $[NR_4]_8OH$ (R = Me, CH_2CH_2OH) under ambient conditions with the selective formation of octasilicate anions [72], $[NR_4]_8[OSiO_{1.5}]_8$. Octasilicate anions offer access to novel polyfunctional silsesquioxane platforms in which each functional group occupies a single octant in Cartesian space. These platforms offer potential as precursors to dendrimers and hyperbranched polymers and as nanobuilding blocks for the formation of nanocomposites [72].

Parr and Sullivan [20] identified the occlusion of appreciable quantities of carbon inside plant silica bodies and, having demonstrated the stability of this carbon fraction against decomposition in soil for millennia, proposed that plant species that yield high amounts of phytolith-occluded carbon could be employed to enhance terrestrial carbon sequestration. We add to the speculated mechanism of physical entrapment [20] the possible existence of silica-binding proteins [4] in silica bodies that also entrap other organic material. Hence greenhouse gas emis-

sions might be reduced by capturing enormous amounts of carbon dioxide inside silica nano powders and sinters stored harmlessly just about anywhere in the environment. Deeper understanding of the sequestration mechanism of carbon within plant silica bodies could lead to the design of biomimetic approaches for the dynamic manipulation of bionanodevices and for the specific control of nanotransporters that could be used for the targeted delivery of proteins or nucleic acids.

Confocal microscopy and X-ray microradiography analysis of dumbbell-shaped silica bodies of rice and maize plants revealed strong birefringence (double refraction) along with strong polarization-dependent second harmonic generation optical properties [73,74]. These studies provide opportunities for synthesizing biogenic photonic crystals from plant silica bodies, which, with the transition to microcrystalline quartz [64], could have some optical properties complementing those of diatoms [4]. It might be possible to exert control over the nucleation and growth, crystal polymorphism, morphology, size, location and orientation of silica bodies in plants to tune the wavelengths, band gaps and refractive index of photonic crystals. The colours (browns, greys and blacks) and transparency of silica bodies can be altered by fire [75].

A detailed study of leaf temperature of silicified leaves of creeping bentgrass (*Agrostis palustris*) by thermal infrared imaging showed that silica bodies in epidermal cells reduce the heat load of leaves and were remarkably effective in cooling leaves by virtue of their highly efficient thermal emission in the mid-infrared range (wavelength 8–12 μm) [33]. Hence, biogenic plant silica bodies could be used in thermal protection mechanisms.

Zeolites, with a three-dimensional honeycomb structure, have the ability to selectively absorb and release liquids and gases in applications such as petroleum processing. Crystalline perfection in zeolite is complex and essential to improve the structure for better functionality. Highly reactive silica in plants can promote zeolite crystallization. The large amount of silica found in the epidermal surface of *Equisetum arvense* (Figure 3) facilitates zeolite nucleation, providing homogeneously and densely distributed zeolite nuclei [76]. Thus a micro-/mesoporous material that retains all of the morphological features of the plant silica bodies was obtained. This study opens up routes for the zeolitization of silica-containing plants and the preparation of materials with controlled porosity and specific macromorphological features [76].

Future uses of phytoliths for nanotechnology

As we have seen, silica transporters have been demonstrated in plants [23], but silica-precipitating molecules have yet to be unequivocally proven to exist and isolated. If they were found, large-scale purification of silica-precipitating proteins might best utilize crop plants, rather than cultivated diatoms or sponges, because of their abundance. This would facilitate the design of novel silica-based materials with desired architectures [39], and a large range of applications in coatings, catalysis and separation science would ensue. Although the higher order mesoscale-pattern-formation mechanisms seen in diatoms [4] are apparently not present in phytolith biosynthesis, a significant variety of shapes is still available because plant cells, vacuoles and intracellular spaces seem to act as moulds for silica precipitation.

Direct interaction of soluble anionic silicates from the plant silica body with the positively charged groups on the peptidoglycane of the bacterial envelopes on the peptide chain could be used to entrap bacteria inside plant silica bodies. Encapsulated bacteria [77] retain their bioactivity and remain accessible to external reagents by diffusion through the porous silica. For example, *Azolla* contains symbiotic nitrogen-fixing cyanobacteria [78], a relationship that could possibly be duplicated in crop plants via silica body encapsulation. Due to its specific morphological properties, nanosilica is an effective carrier of organic compounds for developing new drugs in medicine and biotechnology [79], and it has been used for encapsulating enzymes that in turn can be used for *in vivo* drug release and drug targeting enzyme therapeutics [80]. Perhaps silica bodies could perform the encapsulation.

Deposition of silica in confined nanoenvironments, analogous to the formation of silica bodies, was mimicked in part by using phospholipids [81] or pores in polycarbonate membranes [82] as nanoreactors. Similar reproduction of silica-body-like structures for making artificial organs, bone repair scaffolds, drug vectors, holographic systems and other biofunctional nanomaterials has been attempted by associating biological polymers and silica [83–85]. Commercial synthesis of silica gel from tetraethoxysilane by hydrolysis and condensation reactions is often affected by poor water solubility caused by the involvement of alcohol as a cosolvent. The presence of alcohol affects the rapid formation of biofunctional silica gels by influencing proteins, enzymes and antibodies. This has been overcome via a biofriendly sol-gel synthesis [86] in which a biopolymer with good biocompatibility was used to assist the gelation of glycol-modified tetraethoxysilane in an aqueous system without the addition of any organic solvents. Plant silica bodies, being amorphous in nature, are highly versatile, thermally stable and compatible with both biological systems and the silicon-processing technology used in the electronics industry. Phytoliths are excellent platforms for nanoscale structures due to their attractive chemical, optical, biological and electric properties, their water solubility and their ability to encapsulate both hydrophobic and hydrophilic organic substances. It is expected that such a silica gel matrix could offer several advantages over conventional silica materials as the immobilization platform for bioactive molecules such as proteins, cells, drugs and enzymes [86].

Conclusions

Silica body nanotechnology lags well behind diatom nanotechnology. It has not yet settled on one or more model organisms with sequenced genomes. It has barely begun to characterize silica transporters or proteins or other macromolecules that might be involved in nucleating and thereby localizing silica deposition. Despite the high variability of phytolith size and shape, certain types are characteristic of certain cereals and plants, suggesting a role of the genome in their morphogenesis and thus the possibility of genetic manipulation. This is likely to be indirect, as silica bodies

seem to form via the coating or filling of cells, cell compartments and intercellular spaces. There are hints that the silica in phytoliths can be purer than diatom silica and yet encapsulate organic matter, perhaps of our choosing. The mechanism of silica precipitation and deposition in plants is likely to be different from that of diatoms, although only deeper investigation will resolve this issue. No research has yet been done on the engineering aspects of how silica bodies collectively provide rigidity or optical piping and radiant cooling to plants. Significant advantages of silica bodies include their sheer total biomass compared with that of cultivated diatoms or sponges and the fact that most silica bodies are in the parts of plants that are not consumed. Thus crop plants could serve quadruple purposes for food, fibre, biofuel and growing our nanotechnology.

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