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Bioinspired synthesis of new silica structures†

Siddharth V. Patwardhan,[a] Niloy Mukherjee,[a] Miriam Steinitz-Kannan[b] and Stephen J. Clarson*[a]

[a] *Department of Materials Science and Engineering, University of Cincinnati, Cincinnati, OH 45221-0012, USA. E-mail: stephen.clarson@uc.edu; Fax: 1-513-556-2569*

[b] *Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099, USA*

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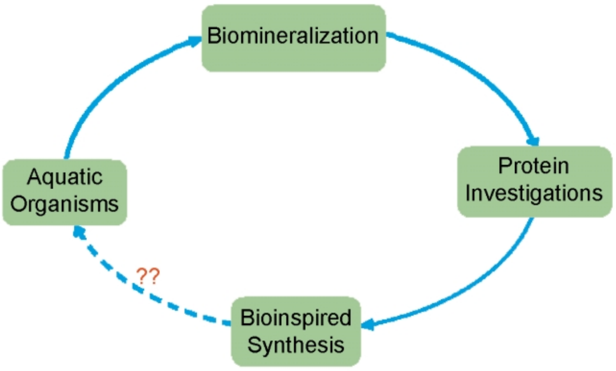
Silicon and oxygen are the two most abundant elements in the Earth's crust[1] but despite the vast scientific literature on crystalline and amorphous silica, new chemistries, structures and applications continue to be discovered for compounds formed from these elements – thus we present here for the first time the formation of new amorphous silica structures that were uniquely synthesized by a bioinspired synthetic system.

The synthesis and chemistry of amorphous and crystalline silica under extreme process conditions have been widely studied over the past several decades. In contrast, living organisms such as diatoms, sponges and grasses are able to form a variety of complex and hierarchical biogenic silica structures under mild physiological conditions (Fig. 1).[2] Aquatic organisms selectively uptake soluble silica from the environment, store the silicic acid and then use it for silica deposition – all this is highly (and presumably genetically) controlled. Biomacromolecules are involved in biosilicification and as they are incorporated into biogenic silica,[3] they may be isolated by selective dissolution of the biosilica and studied. The amino acid compositions and/or the amino acid primary sequences of the proteins that were isolated this way from diatoms (silaffins),[4] grasses[5] and sponges (silicateins)[6a] have been determined. Tacke[7] has proposed that the silaffin and silicatein proteins fulfil multiple roles by acting as catalysts/templates/scaffolds for the formation of silica *in vivo*. The silaffin-1A protein isolated from marine diatom *Cylindrotheca fusiformis* was shown to facilitate the formation of silica spheres from a silica precursor tetramethoxysilane (TMOS) *in vitro*.[4,8]

Studies of biomineralization have highlighted the role of proteins in mediating the formation of biogenic silica. Knowledge of the key features of such biocatalysis can, in turn, be used to design new bioinspired and biomimetic materials (Scheme 1). We have demonstrated elsewhere one such example in which we used a 19-amino acid sequence named R5, derived from the silaffin-1A protein, in order to synthesize bioinspired organic–inorganic optical nano-composites.[9] Furthermore, the hypothesis that the amino acid residues that are cationically charged at neutral pH are key residues in the proteins proposed to be responsible for facilitating biosilicification,[8,10,11] has led us to examine the role of poly-L-lysine (PLL) in silicification. In considering possible silica precursors for use *in vitro*, it was noted that a tetraethoxysilane (TEOS) derived system was reported to be unsuccessful in producing silica using PLL[6b] and hence here we have used TMOS. The polyamine catalyzed polymerization of silicic acid has been reported previously.[12,13] While the incorporation of the polyamines into the silica product was reported, such materials were described as gels and hence the role of the macromolecules as templates/scaffolds was not invoked. The missing link in Scheme 1 is how the information from bioinspired and biomimetic systems can enhance our understanding of related chemistry and processes



Scheme 1 Information flow from biology to bioinspired synthesis. Aquatic organisms form ornate biomineral structures by biomineralization which is facilitated by various biomacromolecules. These biomacromolecules can be studied by selective dissolution of the biomineral phase. The information of the role(s) of these biomacromolecules may enable us to design biomimetic strategies. One interesting question is — what can the information from *in vitro* studies provide that gives new insight into *in vivo* processes?

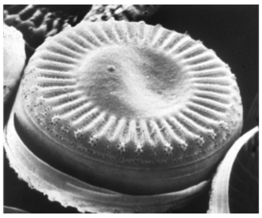


Fig. 1 SEM of centric diatom *Cyclotella meneghiniana*. The sample was collected from Lake San Pablo, Ecuador (typical diameter 10–30 µm).

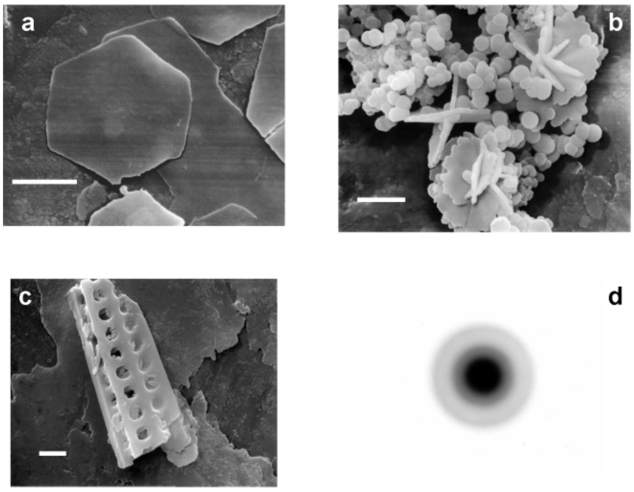


Fig. 2 SEM of silica synthesized using PLL. (a) 50 mg ml^{-1} PLL and 1 M pre-hydrolyzed TMOS (unperturbed), (b) 5 mg ml^{-1} PLL and 0.1 M pre-hydrolyzed TMOS (flowed through a 1/8″ I.D. tube) (c) 5 mg ml^{-1} PLL and 0.1 M pre-hydrolyzed TMOS (stirred for 25 min) and (d) selected area electron diffraction (SAD) pattern of silica (bars = 1 µm).

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† Electronic supplementary information (ESI) available: Methods, EDS for specimens in Fig. 2c and Fig. 2b; additional SEM and TEM of silica. See http://www.rsc.org/suppdata/cc/b3/b302056h/

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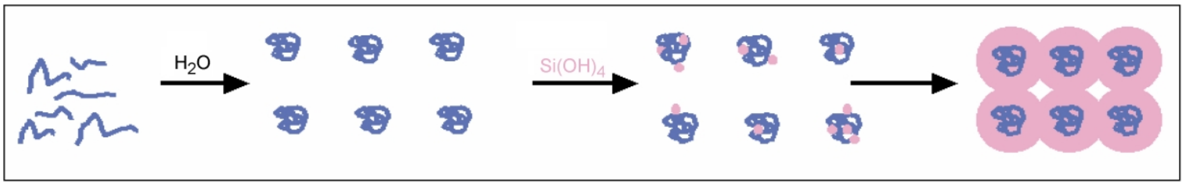

Scheme 2 Schematic representation of the role of macromolecules that act as scaffolds for the formation of mineral structures. Macromolecule chains (in blue) self-assemble and/or form domains in aqueous solution with characteristic spatial arrangements. These may act as scaffolds for the growth of the silica or polysilicic acid (in pink), thus governing the final structure.

in living organisms. In this context, using a PLL based bioinspired synthetic system, we present here new amorphous silica structures.

The results shown in Fig. 2 represent the silica synthesized using PLL as described in the experimental methods.† In the absence of PLL this system formed a gel. Despite the vast scientific literature on crystalline and amorphous silica, we have observed new amorphous silica structures, although the synthesis of crystalline lamellar silica has been reported previously.[14] Without any perturbation (stirring or flowing) of the system, we typically observed the formation of hexagons (Fig. 2a) and spheres. Flowing the reaction mixture through a tube while it was reacting, typically yielded petal-like (Fig. 2b) and fiber-like (see ESI†) silica structures in addition to spheres and hexagons. These structures were seen to be made up of fused spherical particles similar to those that we have previously reported for a related system which gave fiber-like silica structures.[11] When the reaction mixture was stirred, three dimensional silica structures with periodic voids (ladder shaped) were seen (Fig. 2c) in addition to spheres, hexagons and petals. These structures that have a regular spacing of holes resemble the ornate structures that have been seen in diatoms.[15] The representative selected area electron diffraction (SAD) pattern obtained (Fig. 2d) shows the silica produced to be amorphous. From Energy Dispersive Spectroscopy (EDS) and Fourier Transform Infra Red Spectroscopy (FTIR) data of the silica, it was found that the PLL was incorporated into all of the silica structures; thus it appears to fulfil the role of catalyst/template/structure directing agent *in vitro*. It is interesting to note that despite the *in vitro* studies of various proteins or synthetic macromolecules in this context,[4,6,10] only the PLL based system was able to produce the aforesaid silica structures for the conditions studied to date.

The conformation and orientation of the cationically charged macromolecule (PLL in this case), either before or during the silica formation, may be key to the formation of oriented silica structures (Scheme 2). It has been demonstrated both here and elsewhere that the presence of a field (shear force[11,16] or an electric field[16]) enhances the ability of mineralizing systems to form novel oriented structures. It is known that polyelectrolytes may self assemble in solution and thus provide nucleating and templating sites for silicification.[10,17] The close resemblance of the silica structures formed using PLL *in vitro* with those seen in living organisms is consistent with the importance of cationically charged amino acids residues (in this case lysine) in the proteins catalyzing biosilicification. These findings may help in the strategic biomimetic synthesis of tailor made silica structures[18] and lead us to a better understanding of the role of macromolecules in biosilicification (Scheme 1).

As to potential commercial applications of these methods, the industries producing speciality silica globally have sales close to two billion dollars per annum.[19] This quantity clearly illustrates the importance of the industry and processes dealing with silica. One example of an application of precipitated silica is its use as a reinforcing agent in a variety of organic–inorganic hybrid materials. The properties of such materials to date are characteristically isotropic due to the use of spherical silica particles. Novel routes to preparing oriented silica structures, such as described here, present the fascinating possibility of preparing analogous new organic–inorganic hybrid materials where the properties are highly anisotropic. We are currently studying the mechanical properties of bioinspired nano-composite materials.

(Bio)macromolecules typically facilitate (bio)mineralization *via* ionic bridges and hydrogen bonding with the growing minerals. These interactions facilitate the self organization of the (bio)macromolecules (referred to as the *organic matrix* [3]) that create appropriate scaffolds for structure direction of the growing (bio)minerals. In addition, catalytic residues/sites become available for facilitating the growth of the mineral phase. This mechanism can be further exploited not only for silica synthesis but also for the synthesis and nano-patterning of various other systems based on aluminium, germanium, boron, tin, silver, gold, iron, calcium and so on. It is clear that future research in the field of biomineralization and biomimetic materials synthesis based on these concepts will be highly fruitful.

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