Aqueous Silicates in Biological Sol–Gel Applications: New Perspectives for Old Precursors

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ABSTRACT

Identification of silica sol–gel chemistry with silicon alkoxide hydrolysis and condensation processes is common in modern materials science. However, aqueous silicates exhibit several features indicating that they may be more suitable precursors for specific fields of research and applications related to biology and medicine. In this Account, we illustrate the potentialities of such aqueous precursors for biomimetic studies, bio-hybrid material design, and bioencapsulation routes. We emphasize that the natural relevance, the biocompatibility, and the low ecological impact of silicate chemistry may balance its lack of diversity, flexibility, and processability

I. Introduction

When mentioning "sol–gel chemistry", one often implies the use of hydrolysis and condensation reactions of metal alkoxides to make new materials, mainly oxide phases. However, the synthesis of such alkoxides by Liebig and the discovery of their gel-formation ability by Ebelmen date from the 1840s, the first patent on this process being deposited in the late 1930s.[1] In contrast, the discovery of the gel-forming ability of aqueous salts of metals originates far back in the human history, and silicate-based silica gels were patented in the late 1910s.[2,3]

This supremacy of metal alkoxides over aqueous precursors in today's materials science mainly results from three major aspects. First, alkoxides are available as pure, single molecules whose reactivity toward hydrolysis can be efficiently controlled so that the nature of the species that will effectively condense to form a gel can be selected.[1] In contrast, aqueous metal salt solutions can be prepared in a limited range of pH and concentration and may often contain a mixture of oligomeric species, whose reactivity is more difficult to control.[4] A second aspect is that metal alkoxides are mainly neutral species exhibiting good solubility in certain organic solvents, whereas metal salts tend to form charged species upon dissolution in water, usually leading to their precipitation upon solvent addition. This difference in precursor charge also implies that the degree of ionicity is commonly greater in "aqueous" gels when compared with "alkoxide" gels. Finally, and this may be the key reason for the popularity of silicon alkoxide-based sol–gel chemistry, it is possible to synthesize organosilane precursors bearing a nonhydrolyzable organic function, allowing the design of covalently linked organic–inorganic hybrid materials.[5] Overall, the alkoxide-based sol–gel route is more flexible in terms of reaction conditions, chemical nature, functionality, and processing.

However, when one considers biology-related applications of sol–gel chemistry, aqueous precursors exhibit several interesting features. Indeed, alkoxide hydrolysis leads to the release of parent alcohol molecules that may be detrimental to biological systems.[6] This is a major concern for material design itself but also in terms of ecological impact when large-scale applications are to be developed. In addition, metal salts in aqueous media represent the state where these elements can be found in nature and utilized by living organisms for biomineralization processes.[7] Aqueous solutions may therefore be considered as suitable precursors for biomimetic studies.

In this Account, we would like to illustrate the potentialities of aqueous silicates by presenting recent advances in biology-related studies and applications of silica. In the first part, we show how biomimetic studies performed using aqueous silicate solutions yield a better understanding of some of the processes involved in biosilicification. We then describe the synthesis and properties of silicate-based bio-hybrid particles. Finally, the contribution of "aqueous" routes to recent progress in mineral cell encapsulation is illuminated. Comparison with similar works performed using silicon alkoxide precursors is provided to point out the advantages and limitations of these approaches for future developments.

2. Silicates in Biosilicification Studies

Silica is the major mineral constituting the Earth's crust and is therefore widely found in a soluble form in soils, rivers, lakes, and oceans, where it can be taken up by animals, plants, and marine microorganisms such as diatoms, radiolarians, and sponges (Figure 1).[8] In many instances, evidence is found that silica is incorporated as monosilicic acid, $Si(OH)_4$.[9] Because $Si(OH)_4$ tends to polymerize at concentrations above ca. 1 ppm, it is

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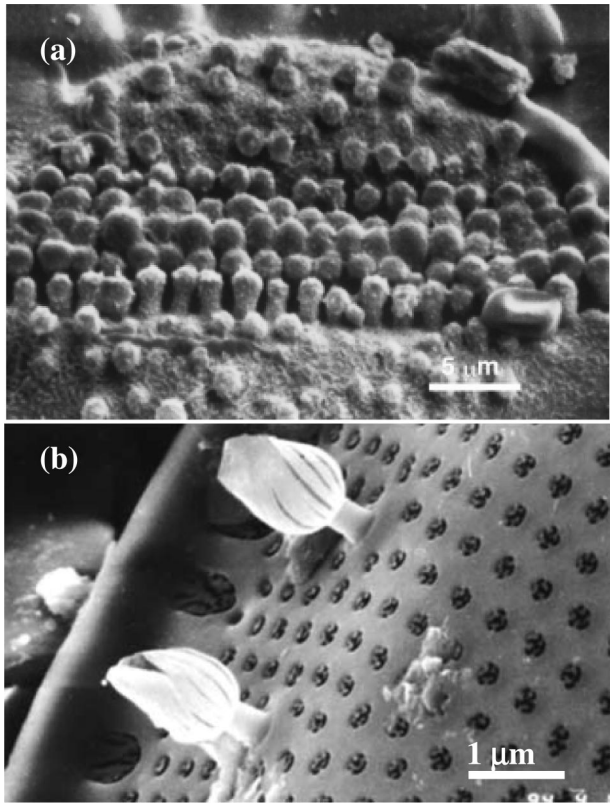

FIGURE 1. Biogenic silica structure. SEM micrographs of (a) chrysoliths of an *Equisitum* sp. horsetail and (b) frustule surface of a *Thalassosiria* sp. diatom.

assumed that it is transported under a stable form to the site of mineralization. Silica formation then proceeds under the control of specific macromolecules that dictate its morphology, up to a tremendous perfection as illustrated by diatom shells.[10,11]

Several groups have focused their attention on the extraction of these macromolecules from living organisms.[12,13] In the case of diatoms, proteins termed silaffins, as well as polyamines, could be recovered, and the ability of these extracts to activate silica formation from hydrolyzed silicon alkoxide solutions was reported.[12] However, no data on the reaction between these macromolecules and silicates is available at this time. In parallel, the ability of synthetic or natural polymers bearing amine groups to interact with different silica precursors has been widely studied.[14,15] Different morphologies were obtained depending on the nature of the precursor, but a reliable comparison between these data is made difficult by variations in experimental conditions. In this context, investigations on the silica/collagen system indicate that the nature of the interactions arising between the biopolymer and the silicon species may range between weak hydrogen bond for $Si(OH)_4$ and silicon alkoxide, leading to a limited perturbation of the collagen self-assembly process, and strong electrostatic interactions for silicates and silica nanoparticles, inducing silica precipitation.[16,17]

In the case of silicates, these attractive electrostatic interactions between ammonium groups and poly(silicic acid) oligomers served as the basis for a biomimetic model of silica formation activation by polyamines.[18] In this model, the positively charged organic functions located on the polymer backbone attract the negatively charged inorganic species, bringing them closer to one another and favoring their condensation (Figure 2a). This model was strengthened by the observation that a decrease in ammonium group occurrence on the polymer chain leads to a decrease in the activation process efficiency (Figure 2b).[19] Such an adsorption of silicates on polyamine chains could favor their aggregation and, hence, could lead to the formation of a polymer gel, as demonstrated for bovine serum albumin.[19] As a consequence, silicate condensation occurs within the polymer network, and silica particle growth becomes limited by the voids of this network (Figure 2c). In fact, it was shown that the size of the silica particles formed in the presence of gelatin was inversely proportional to the density of protein chains in the silicate environment.[20,21] Interestingly, it was found that polyamines extracted from diatoms exhibited self-assembly properties that influence silica nanoparticle growth, suggesting that biomimetic studies based on silicates were relevant to understand biosilicification in diatoms.[22]

However, a different situation arises for proteins, termed silicateins, extracted from the silicified sponge *Tethya aurantia*.[13] The activation of silica formation was proposed to involve a protein active site consisting of an asparagine, serine, and histidine triad. This process, which was shown to be efficient for other metal alkoxides,[23] only concerns the hydrolysis step of the sol–gel reaction. As a consequence, it was observed that silicatein does not activate silica formation from silicates. It therefore seems that silicates may not always be suitable when performing such biomimetic studies. In this case, a silicon complex would represent an interesting form of precursor, because it closely mimics the organic hexavalent Si complexes that may be involved in silicon stabilization during intracellular transport.[24] However, with the exception of silicon catecholate,[25] this approach is still largely unexplored.

Another aspect of biomimetic studies performed with silicates deals with the effect of confinement. In fact, in both diatoms and sponges, silica formation has been shown to occur within a specialized vesicle.[8] It was therefore attempted to reproduce similar conditions using multilamellar phospholipids vesicles.[26] In the presence of silicates in acidic conditions (to limit the kinetics of silicate condensation), hybrid vesicles were obtained (Figure 3a). Interestingly, the silica network consists of layers of closely packed nanoparticles whose size appears tailored by the lipid interlayer space. Such an arrangement of nanoparticles is reminiscent of the silica nanostructures observed in sponge silicified architectures.[8] Similar hybrid multilamellar vesicles were described using the self-assembly of surfactants and silicon alkoxides.[27] However, in this case, a fully condensed silica network was observed in the vesicle interlayer space. More recently, the channels of porous membranes were also used as biomimetic confined environments.[28] Silicate gelation within these channels resulted in the formation of hollow tubes with dimensions closely fitting those of the membrane pores

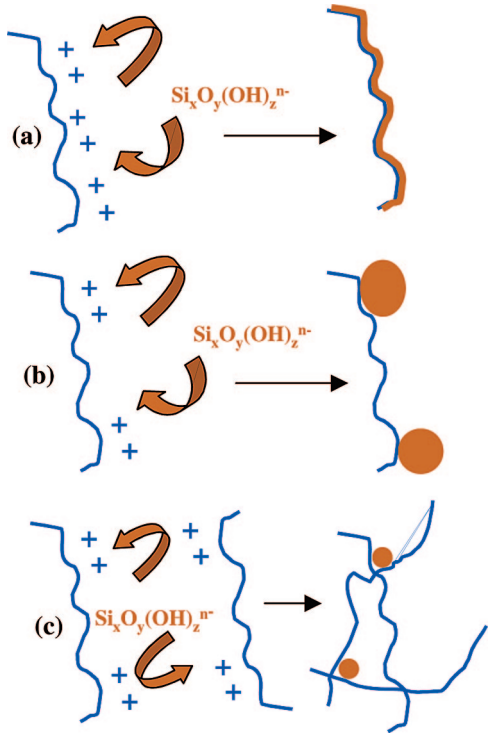

FIGURE 2. Modes of interaction between silicates and polycationic macromolecules: (a) homo-polyamines (e.g., poly(L-lysine)) favor extended silicate condensation to form dense gels, (b) proteins (e.g., lysozyme) favor local silicate condensation to form precipitates, and (c) self-assembling proteins (e.g., gelatin) favor the spatially constrained condensation of silicates to form silica nanoparticles.

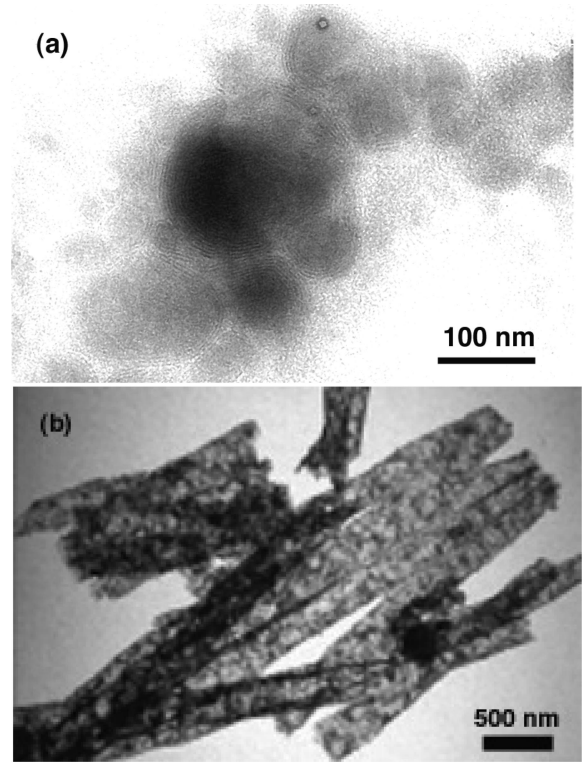

FIGURE 3. Effect of confinement on silica growth. TEM micrographs of (a) hybrid silica/phospholipid multilamellar vesicles and (b) a silica tube formed in a nanoporous membrane.[26,29]

(Figure 3b). The tube shell originates from silicate condensation on the channel's internal surface and consists of densely packed silica nanoparticles, as a result of the negative curvature of this surface. Upon successive impregnations, these tubes were progressively filled-up by silica particles. The size of the primary particles constituting this core silica phase was observed to decrease with pore dimension. It was hypothesized that confinement directly influences the silica formation process by increasing the diffusion coefficient of the growing nanoparticles and favoring their aggregation. Overall, these results indicate that confinement strategies can strongly influence silica growth and especially particle size and packing.

As previously mentioned, the suitability of aqueous silicates for biomimetic studies is debatable. Nevertheless, studies performed over the past few years have allowed the identification of several parameters that control the silica/biopolymer interactions. Their relevance for biosilicification processes in diatoms are clearly identified by comparison with the actual known biochemistry of the living organisms. In addition, recent approaches of confinement effects on silica growth have demonstrated some new aspects of the processes taking place in deposition vesicles found in these organisms. Indeed these data are interesting to understand how nature has learned to cope with silica chemistry. But they may also provide some guidelines for the design of man-made, bio-inspired materials, as shown in the following section.

3. Silicates in Biopolymer–Silica Hybrid Particle Design

The contribution of sol–gel chemistry to medicinal science has been reviewed recently, emphasizing applications in diagnosis, drug delivery systems, and design of artificial organs.[29] The sol–gel process is also widely used to design biomaterials for tissue engineering.[30] In many of these applications, (bio)polymer-based materials have already been widely developed, but several reports suggest that incorporation of a mineral component may improve device properties, especially in terms of chemical and mechanical stability.[31]

As mentioned earlier, a main advantage of aqueous silicates over alkoxides is their low toxicity (due to the absence of labile organic residues) and their low environmental impact.[32] It can therefore be suggested that silicates are particularly suitable for the design of such materials that should function in contact with, or within, the human body.

The possibility to make biopolymer–silicate hybrid materials indeed depends on the nature of the interactions arising between the macromolecules and the inorganic species. In this context, the biomimetic studies presented earlier serve as a useful basis to predict the behavior of such mixed systems. Thus, in neutral pH conditions, silicates bear silanol, Si–OH, and silanolate, $Si–O^-$, groups and are expected to interact strongly with cationic polymers, moderately with neutral chains, and not with polyanionic species (Figure 4). Thus, at pH 7.2, cationic gelatin induces silica precipitation,[20] whereas negatively

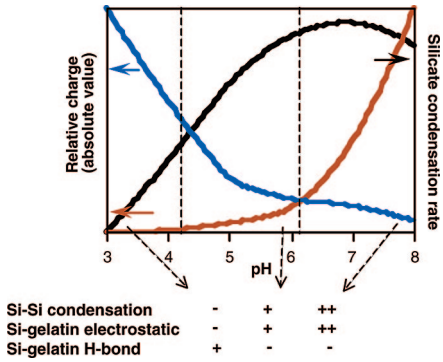

FIGURE 4. Effect of pH on silicate–gelatin interactions. Experimental curves showing the evolution of silicate (orange line) and gelatin (blue line) relative charges with pH together with silicate condensation rate (dark line). Three pH domains, tentatively indicated by dashed vertical lines, can be distinguished, depending on the relative influence of silicate condensation, silicate–gelatin attractive electrostatic interactions, and silicate–gelatin hydrogen bond formation.

charged alginic acid and DNA do not.[33] Decreasing the reaction pH to slightly acidic conditions (pH 4–5) lowers the negative charge and hence the reactivity of silicates, as shown for lysozyme and gelatin (Figure 4).[19,20] However, when more acidic conditions are used, that is, below pH 2–3, silicates are mainly neutral species and can develop hydrogen bonds that may also induce silica precipitation, as shown for gelatin (Figure 4).[2] Overall, the strong reactivity of silicates often leads to the formation of precipitates in the presence of macromolecules, whereas monolith gels can more easily be obtained using silicon alkoxide precursors.[30]

However, such a reactivity is not always a drawback, as illustrated by the design of biopolymer–silica core–shell particles. Biopolymer capsules have already been widely studied for drug delivery or bio-encapsulation devices.[34] It was proposed that the deposition of a silica coating on the capsule surface would lead to an enhanced chemical/mechanical stability.[35] Moreover, whereas polymer hydrogels are often macroporous, the sol–gel process offers the possibility to tailor silica porosity and therefore to modulate the diffusion properties of the particles. The first attempt to use silicates for capsule coating was described on alginate macrospheres. Alginic acid is a polysaccharide bearing carboxylic acid functions and is therefore negatively charged at pH 7, preventing direct deposition of silicates on its surface. Thus, alginate capsules were first coated with poly(L-lysine) (PLL). This outer layer renders the particle surface positively charged and allows the deposition of silica from diluted silicate solutions at neutral pH (Figure 5a).[36] The resulting coating consists of a dense network of silica. Alginate–PLL–silica hybrid capsules were shown to have enhanced mechanical stability when compared with alginate–PLL–alginate beads. A similar approach was later developed for the design of alginate–PLL–silica microcapsules (Figure 5c).[37] However, it was difficult to elaborate hydrogel spheres with submicrometric dimensions and alginate/silica nanocapsules could only be obtained using a spray-drying approach, with loss of the core–shell structure.

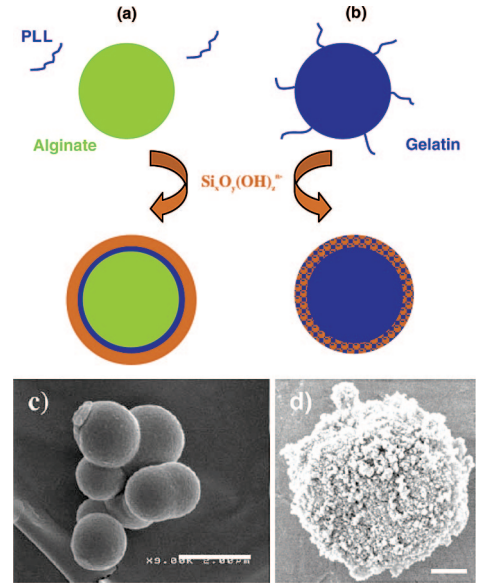

FIGURE 5. Comparison of alginate–silica and gelatin–silica core–shell microparticles. Elaboration of (a) silica-coated alginate capsules involving the deposition of PLL and (b) silica-coated gelatin capsules are shown. SEM micrographs of resulting microspheres for (c) alginate and (d) gelatin capsules are also shown (scale bar = 2 μm).[37,39]

A similar approach was followed to design gelatin–silica hybrid capsules. In this case, the biopolymer bears a positive charge, so direct deposition of silicates is possible (Figure 5b). Macro-, micro-, and nanocapsules of gelatin and corresponding hybrid particles exhibiting core–shell structures could be elaborated (Figure 5d).[38,39] The presence of the silica coating appeared to stabilize significantly the hydrogel network, especially toward dissolution in water.

Preliminary investigations on the cytocompatibility and cellular up-take of alginate- and gelatin-based nanocomposites were also performed using fibroblast cells.[37,39] In both cases, the hybrid nanoparticles could be internalized without causing rapid cell death and were found in cell compartments where their degradation occurs. In fact, it seems that only the biopolymer component of these particles was digested intracellularly, leaving fractured or hollow silica particles. Data on long-term cell viability are not available at this time. In fact, nothing is known about the systemic fate of silica, and such data would be of great help to evaluate the suitability of silica-based materials for biomedical applications.

To our knowledge, no equivalent alkoxide-based nanocomposites have been described so far. In the case of alginate, other groups have reported the synthesis of silica-coated macrocapsules using organosilanes.[35,40] For instance, Sakai et al. used aminopropyltrimethoxysilane (APTS) together with tetramethoxysilane (TMOS) to coat alginate beads.[41] The hydrolyzable methoxy functions of both precursors allowed the formation of a hybrid silica coating, whereas the ammonium groups from APTS favor anchoring on the alginate surface. When compared with the silicate approach, this method relies on a similar mechanism by using a cationic bridge between the

polymer and silica. However, it is a simpler one-step process that takes advantage of organosilane properties to introduce covalency, and therefore chemical stability, in the material.

Overall, silicate reactivity toward biomacromolecules is controlled by its ability to form electrostatic interactions and hydrogen bonds in a wide range of pH and concentration conditions. As a consequence, silicates tend to form hybrid precipitates rather than gels. This limits their application for bulk material design but favors their formation at specific interfaces, as shown for core–shell nanoparticles. Another key aspect of silicate chemistry is its expected good biocompatibility. If the demonstration of hybrid nanoparticle internalization by cells suggests that silicate-based materials may exhibit suitable cytocompatibility, even more convincing data are provided by the cell encapsulation experiments that are discussed in the next section.

4. Silicates in Bioencapsulation

Among the wide diversity of applications of sol–gel hybrid materials, bioencapsulation approaches may be some of the most fascinating. The possibility to immobilize biological systems, such as enzymes or cells, within silica gels opens the route for the design of biosensors or bioreactors.[42–44]

The success of these encapsulation approaches relies on silica formation routes that are compatible with the preservation of biological activities. Traditional silicon alkoxide-based sol–gel chemistry makes use of an alcohol as a cosolvent to water to ensure the solubility of the starting precursor. Indeed, it is well-known that alcohol can induce enzyme denaturation and cell death. This problem was solved in the early 1980s by Avnir et al. who showed that alcohol was not necessary to form silica gels.[45] In fact, upon hydrolysis, alkoxides release alcohol molecules in the solution that allow their rapid solubilization. Optimization of this approach using sonication was described by Ellerby et al.,[46] offering a suitable procedure for enzyme encapsulation that is now widely used in this field.

If the presence of alcohol in the starting mixture can be avoided, the problem of alcohol release in the medium during hydrolysis still remains. Several approaches have been described to address this point. These include the use of a high hydrolysis ratio,[47] the evaporation/distillation of the released alcohol before enzyme addition,[48] and the synthesis of chemically modified alkoxides bearing nontoxic alcohol groups such as glycerol.[49] Carturan et al. also developed the so-called Biosil process based on the use of alkoxide vapors flowed over the biological system so that the released alcohol molecules are rapidly withdrawn by the vector gas flux.[40]

In fact, the same group pioneered the field of sol–gel cell encapsulation using tetraethoxysilane (TEOS) solution to immobilize yeast cells.[50] The success of this approach was due to two factors. First, yeasts are able to withstand a large quantity of ethanol in their environment since this alcohol is a byproduct of their fermentation activity. In addition, the silica gel was deposited as a thin film, allowing a rapid evaporation of the alcohol from the surface layer. Such an ability of thin films to maintain whole cell viability was more recently used for the encapsulation of genetically engineered luminescent *Escherichia coli* bacteria,[51] *Bacillus sphaericus* spores,[52] and several kinds of living organisms in association with phospholipids.[53] This ability of silica gels to present a high surface-to-volume ratio for rapid alcohol evaporation and thus to present good cytocompatibility is probably also responsible for the successful encapsulation of islets of Langerhans in alkoxide-based microspheres described by Pope et al.[54]

However, when bulk gels are designed, the problem of alcohol release appears more difficult to solve. For instance, it was shown that the amount of methanol released during the formation of TMOS-based gels following traditional routes used for enzyme encapsulation was harmful for *E. coli* bacteria.[6] Several groups have therefore investigated the possibility to use aqueous precursors, that is, silicates and colloidal silica.

First attempts in that direction were performed for enzyme encapsulation, using either commercial silica sols,[55] or sodium silicate solutions acidified with an ion-exchange resin to get rid of sodium ions.[56] Silicate precursors were also found suitable to design pin-printed protein microarrays.[57] In contrast, it was shown that enzyme adsorption on silica particle surfaces may lead to their denaturation.[58] Silica colloids and silicates were also independently used for cell immobilization.[43,59–61] However, the most successful approach so far involves a mixture of silicates and colloidal silica. In this case, the silica network originating from silicate condensation acts as a cement to ensure the cohesion of the colloidal assembly (Figure 6).[6]

Although such mixed gels were found more suitable than TMOS-based hosts for short-term encapsulation of *E. coli* bacteria, a rapid decay of cell viability was observed over a few days.[6] In order to enhance the survival rate of entrapped cells, several organic additives, gelatin, poly(vinyl alcohol) (PVA) and glycerol, were incorporated in the silica gel.[62] Only the latter led to a significant increase in cell viability, with 50% of the initial bacteria *E. coli* population being metabolically active after 1 month.[63] Investigations of the gel porous structure revealed that gelatin or PVA do not modify the network porosity, whereas glycerol addition leads to a strong decrease in surface area. This suggests that glycerol is located in the mesopores of the silica gel and may therefore be in close contact with the encapsulated cells. Further experiments showed that ethylene glycol does not favor bacteria survival, despite its close chemical similarity with glycerol.[64] In parallel, ethylene glycol addition did not modify the silica gel porous structure, suggesting that it behaves similarly to gelatin and PVA. Overall, these data indicate that the localization of the additive is a determining factor

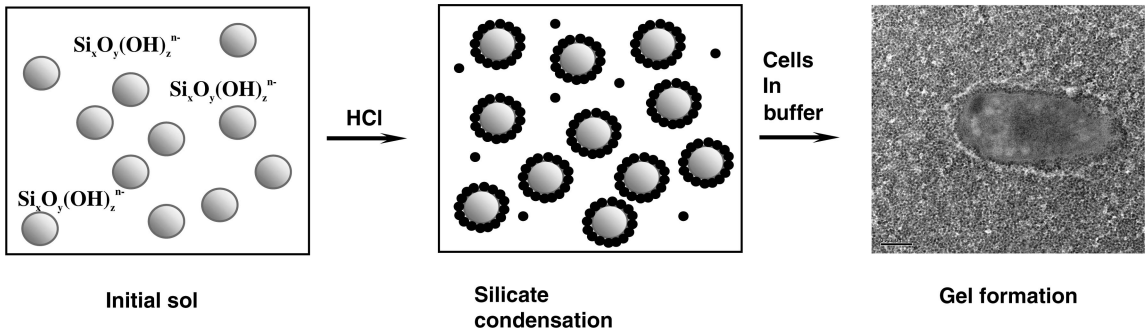

FIGURE 6. Encapsulation of bacteria in silicate/colloidal silica mixed matrices. The initial precursor solution is neutralized by HCl addition, inducing silicate condensation in the solution and on the surface of the colloids. Upon cell addition, gel formation proceeds, leading to their encapsulation in the silica network.[62]

for its ability to stabilize encapsulated cells. This hypothesis was strengthened by recent cryo-scanning electron microscopy (SEM) experiments performed on TEOS/glycerol gels demonstrating that the organic additive is located around the bacteria, avoiding direct contact between the silica gel pore surface and the cell.[65] The suitability of glycerol to maintain the long-term viability of encapsulated *E. coli* bacteria was confirmed in silicate-based matrices for *E. coli*.[66]

Considering the actual knowledge in the field of whole cell encapsulation, it can be proposed that "alkoxide" and "aqueous" routes may be suitable for different applications, related to their processing. Silicon alkoxides appear convenient precursors for the design of thin films that may be suitable for the development of biosensors.[51,53] Silicates and colloidal silica are more adapted to bulk gel elaboration, allowing the conception of bioreactors. In this context, it was shown that *Serratia marcescens* bacteria encapsulated in SiO_2/glycerol hosts were capable of producing prodigiosin, a promising pharmaceutical agent.[67] Moreover, this production could be enhanced by addition of interbacterial communication molecules to the gel. Indeed, in both kinds of systems, a key factor is the possibility to maintain the long-term viability of entrapped cells, because no possibility for cell division inside the gel network has been reported so far.

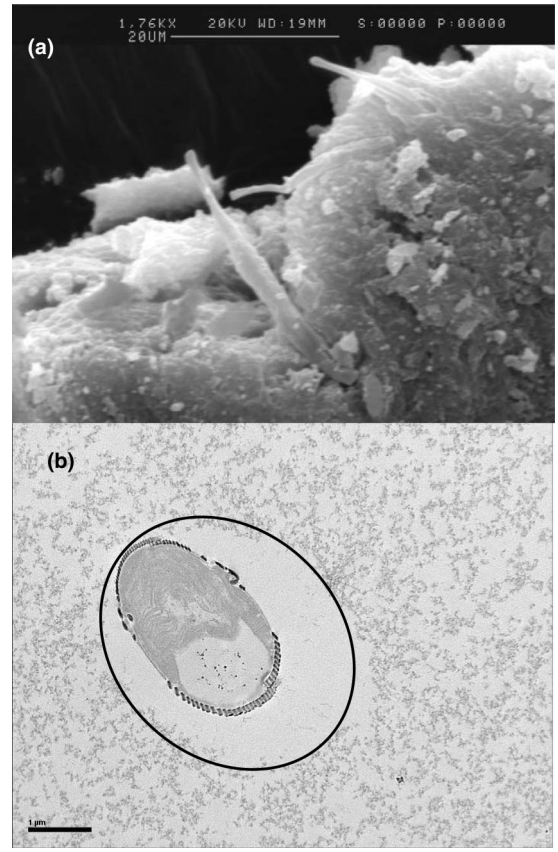

FIGURE 7. Encapsulation of diatoms in silica gels: (a) SEM and (b) TEM micrographs of *Cylindrotheca fusiformis* diatoms in a silicate matrix. The oval line shows the cavity observed in the vicinity of the cell, suggesting silica dissolution by the diatoms.[68]

5. Perspectives

We have recently reported the long-term viability of diatom cells within silicate gels (Figure 7).[68] Encapsulated cells could dissolve the silica network in their surroundings, suggesting that diatoms may not only form Si–O–Si bonds but also cleave these siloxane bridges. To our sense, these experiments illustrate the main features of silicate-based processes. These aqueous precursors are the silicon source available for most living organisms and are therefore relevant for biomimetic studies. Such studies not only concern the molecular mechanisms of silicification but may also contribute to the field of cell–mineral interfaces in geological environments.[64] In addition, silicate chemistry is environmentally friendly and biocompatible and can be combined with many biological systems from molecules to single cells (i.e., *in vitro*) and, hopefully in the near future, to whole organisms (i.e., *in vivo*).

For all the reasons already mentioned above, it is very likely that silicon alkoxides will remain the dominant silica precursor for the design of hybrid materials. However, increasing concern for ecological issues in the context of sustainable development has already triggered considerable efforts to develop "greener" chemical processes. Whether there will be a need for a "green" sol–gel chemistry is difficult to ascertain at this time, but it is our belief (and hope) that actual studies on aqueous silicate-based material design will significantly contribute to future developments in this field.

References

(1) Brinker, C. J.; Scherrer, G. *Sol-Gel Science, The Physics and Chemistry of Sol-Gel Processing*; Academic: Boston, MA, 1990.

(2) Iler, R. K. *The Chemistry of Silica*; Wiley-Interscience: New York, 1979.

(3) Feldman, M.; Desrochers, P. Research Universities and Local Economic Development: Lessons from the History of the Johns Hopkins University. *Ind. Innovation* **2003**, *10*, 5–24.

(4) Jolivet, J. P. *Metal Oxide Chemistry and Synthesis*; Wiley: Chichester, U.K., 2000.

(5) Sanchez, C.; Ribot, F. Design of hybrid organic-inorganic materials synthesized via sol-gel chemistry. *New. J. Chem.* **1994**, *18*, 1007–1047.

(6) Coiffier, A.; Coradin, T.; Roux, C.; Bouvet, O. M. M.; Livage, J. Sol-gel encapsulation of bacteria: a comparison between alkoxide and aqueous routes. *J. Mater. Chem.* **2001**, *11*, 2039–2044.

(7) Lowenstam, H. A.; Weiner, S. *On Biomineralization*; Oxford University Press: New York, 1989.

(8) *Silicon and Siliceous Structures in Biological Systems*; Simpson, T. L., Volcani, B. E., Eds.; Springer-Verlag: New York, 1981.

(9) Coradin, T.; Desclés, J.; Luo, G. -Z.; Lopez, P. J. Silicon in the Photosynthetic Lineages: Molecular Mechanisms for Uptake and Deposition. In *Floriculture, Ornamental and Plant Biotechnology*; Teixeira da Silva, J. A., Ed.; Global Science Books: London, 2006; Vol. *IV, Chapter 24*.

(10) Perry, C. C.; Keeling-Tucker, T. Biosilicification: the role of the organic matrix in structure control. *J. Biol. Inorg. Chem.* **2000**, *5*, 537–550.

(11) Coradin, T.; Lopez, P. J. Biogenic silica patterning: simple chemistry or subtle biology? *ChemBioChem* **2003**, *4*, 251–255.

(12) Kröger, N.; Deutzmann, R.; Sumper, M. Polycationic peptides from diatom biosilica that direct silica nanosphere formation. *Science* **1999**, *286*, 1129–1132.

(13) Cha, J. N.; Shimizu, K.; Zhou, Y.; Christiansen, S. C.; Chmelka, B. F.; Stucky, G. D.; Morse, D. E. Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicone in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 361–365.

(14) Patwardhan, S. V.; Clarson, S. J.; Perry, C. C. On the role(s) of additives in bioinspired silicification. *Chem. Commun.* **2005**, 1113–1121.

(15) Lopez, P. J.; Gautier, C.; Livage, J.; Coradin, T. Mimicking biogenic silica nanostructures formation. *Curr. Nanosci.* **2005**, *1*, 73–83.

(16) Eglin, D.; Mosser, G.; Giraud-Guille, M. -M.; Livage, J.; Coradin, T. Type I Collagen, A Versatile Liquid Crystal Biological Template for Silica Structuration from Nano- to Microscopic Scales. *Soft Matter* **2005**, *1*, 29–32.

(17) Eglin, D.; Shafran, K. L.; Livage, J.; Coradin, T.; Perry, C. C. Comparative study of the influence of several silica precursors on collagen self-assembly and of collagen on 'Si' speciation and condensation. *J. Mater. Chem.* **2006**, *16*, 4220–4230.

(18) Coradin, T.; Durupthy, O.; Livage, J. Interactions of amino-containing peptides with sodium silicate and colloidal silica: a biomimetic approach of silicification. *Langmuir* **2002**, *18*, 2331–2336.

(19) Coradin, T.; Coupé, A.; Livage, J. Interactions of bovine serum albumin and lysozyme with sodium silicate solutions. *Colloids Surf., B* **2003**, *29*, 189–196.

(20) Coradin, T.; Bah, S.; Livage, J.Gelatine/silicate interactions: from nanoparticles to composite gels *Colloids Surf., B* **2004**, *35*, 53–58.

(21) Coradin, T.; Marchal, A.; Abdoul-Aribi, N.; Livage, J. Gelatine thin films as biomimetic surfaces for silica particles formation. *Colloids Surf., B* **2005**, *44*, 191–196.

(22) Sumper, M.; Brunner, E. Learning from diatoms: Nature's tools for the production of nanostructured silica. *Adv. Funct. Mater.* **2006**, *16*, 17–26.

(23) Summerel, J. L.; Yang, W.; Kisailus, D.; Weaver, J. C.; Choi, J. H.; Morse, D. E. Biocatalytically templated synthesis of titanium dioxide. *Chem. Mater.* **2003**, *15*, 4804–4809.

(24) Kinrade, S. D.; Gillson, A.-M. E.; Knight, C. T. G. Silicon-29 NMR evidence of a transient hexavalent silicon complex in the diatom *Navicula pelliculosa*. *J. Chem. Soc., Dalton Trans.* **2002**, 307–309.

(25) Harrison, C. C.; Loton, N. Novel routes to designer silicas: studies of the decomposition of $(M^{+})_2[Si(C_6H_4O_2)_3]$. xH_2O. *J. Chem. Soc., Faraday Trans* **1995**, *91*, 4287–4297.

(26) El Rassy, H.; Belamie, E.; Livage, J.; Coradin, T. Onion phases as biomimetic confined media for silica nanoparticles growth. *Langmuir* **2005**, *21*, 8584–8586.

(27) Tanev, P. T.; Pinnavaia, T. J. Biomimetic templating of porous lamellar silicas by vesicular surfactant assemblies. *Science* **1996**, *271*, 1267–1269.

(28) Gautier, C.; Lopez, P. J.; Hemadi, M.; Livage, J.; Coradin, T. Biomimetic growth of silica tubes in confined media. *Langmuir* **2006**, *22*, 9092–9095.

(29) Coradin, T.; Boissiére, M.; Livage, J. Sol-gel chemistry in medicinal science. *Curr. Med. Chem.* **2006**, *13*, 99–108.

(30) Vallet-Regi, M.; Arcos, D. Nanostructured hybrid materials for bone tissue regeneration. *Curr. Nanosci.* **2006**, *2*, 179–189.

(31) Coradin, T.; Allouche, J.; Boissière, M.; Livage, J. Sol-gel biopolymer/silica nanocomposites in biotechnology. *Curr. Nanosci.* **2006**, *2*, 219–230.

(32) Van Dokkum, H. P.; Hulskotte, J. H. J.; Kramer, K. J. M.; Wilmot, J. Emission, fate and effects of soluble silicates (waterglass) in the aquatic environment. *Environ. Sci. Technol.* **2004**, *38*, 515–521.

(33) Coradin, T.; Coupé, A.; Livage, J. Influence of DNA, alginate, lysozyme and bovine serum albumin on sodium silicate condensation. *Mater. Res. Soc. Symp. Proc.* **2002**, *724*, N.7.20.1–5.

(34) Wang, W.; Liu, X.; Xie, Y.; Zhang, H.; Yu, W.; Xiong, Y.; Xie, W.; Ma, X. Microencapsulation using natural polysaccharides for drug delivery and cell implantation. *J. Mater. Chem.* **2006**, *16*, 3252–3267.

(35) Coradin, T.; Nassif, N.; Livage, J. Silica/alginate composites for microencapsulation. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 429–434.

(36) Coradin, T.; Mercey, E.; Lisnard, L.; Livage, J. Design of silica-coated microcapsules for bioencapsulation. *Chem. Commun* **2001**, 2496–2497.

(37) Boissière, M.; Meadows, P. J.; Brayner, R.; Hélary, C.; Livage, J.; Coradin, T. Turning biopolymer particles into hybrid capsules: the example of silica/alginate nanocomposites. *J. Mater. Chem.* **2006**, *16*, 1178–1182.

(38) Coradin, T.; Livage, J. Synthesis, characterization and diffusion properties of biomimetic silica-coated gelatine beads. *Mater. Sci. Eng. C* **2005**, *25*, 201–205.

(39) Allouche, J.; Boissière, M.; Hélary, C.; Livage, J.; Coradin, T. Biomimetic core-shell gelatine/silica nanoparticles: a new example of biopolymer-based nanocomposites. *J. Mater. Chem.* **2006**, *16*, 3121–3126.

(40) Carturan, G.; Dal Toso, R.; Boninsegna, S.; Dal Monte, R. Encapsulation of functional cells by sol-gel silica: actual progress and perspectives for cell therapy. *J. Mater. Chem.* **2004**, *14*, 2087–2098.

(41) Sakai, S.; Ono, T.; Ijima, H.; Kawakami, K. Synthesis and transport characterization of alginate/aminopropyl-silicate/alginate microcapsule: application to bioartificial pancreas. *Biomaterials* **2001**, *22*, 2827–2834.

(42) Jin, W.; Brennan, J. D. Properties and applications of proteins encapsulated within sol-gel derived materials. *Anal. Chim. Acta* **2002**, *461*, 1–36.

(43) Böttcher, H.; Soltmann, U.; Mertig, M.; Pompe, W. Biocers: ceramics with incorporated microorganisms for biocatalytic, biosorptive and functional materials development. *J. Mater. Chem.* **2004**, *14*, 2176–2188.

(44) Avnir, D.; Coradin, T.; Lev, O.; Livage, J. Recent bio-applications of sol-gel materials. *J. Mater. Chem.* **2006**, *16*, 1013–1030.

(45) Avnir, D.; Kaufman, V. R. Alcohol is an unnecessary additive in the silicon alkoxide sol-gel process. *J. Non-Cryst. Solids* **1987**, *192*, 180–182.

(46) Ellerby, L. M.; Nishida, C. R.; Nishida, F.; Yamanaka, S. A.; Dunn, B.; Valentine, J. S.; Zink, J. I. Encapsulation of proteins in transparent porous silicate glasses prepared by the sol-gel method. *Science* **1992**, *255*, 1113–1115.

(47) Conroy, J. F. T.; Power, M. E.; Martin, J.; Earp, B.; Hostica, B.; Daitch, C. E.; Norris, P. M. Cells in sol-gels I: A cytocompatible route for the production of macroporous silica gels. *J. Sol-Gel Sci. Technol.* **2000**, *18*, 269–283.

(48) Ferrer, M. L.; del Monte, F.; Levy, D. A novel and simple alcohol-free sol-gel route for encapsulation of labile proteins. *Chem. Mater.* **2002**, *14*, 3619–3621.

(49) Gill, I.; Ballesteros, A. Encapsulation of biologicals within silicate, siloxane, and hybrid sol-gel polymers: an efficient and generic approach. *J. Am. Chem. Soc.* **1998**, *120*, 8587–8598.

(50) Carturan, G.; Campostrini, R.; Diré, S.; Scardi, V.; de Alteris, E. Inorganic gels for immobilization of biocatalysts: inclusion of invertase-active whole cells of yeast (*Saccharomyces cerevisiae*) into thin layers of SiO_2 gel deposited on glass sheets. *J. Mol. Catal.* **1989**, *57*, L13–L16.

(51) Premkumar, J. R.; Lev, O.; Rosen, R.; Belkin, S. Encapsulation of luminous recombinant *E. coli* in sol-gel silicate films. *Adv. Mater.* **2001**, *13*, 1773–1775.

(52) Matys, S.; Raff, J.; Soltmann, U.; Selenska-Pobell, S.; Böttcher, H.; Pompe, W. Calcium dipicolinate induced germination of *Bacillus* spores embedded in thin silica layers: Novel perspectives for the usage of biocers. *Chem. Mater.* **2004**, *16*, 5549–5551.
(53) Baca, H. K.; Ashley, C.; Carnes, E.; Lopez, D.; Flemming, J.; Dunphy, D.; Singh, S.; Chen, Z.; Liu, N.; Fan, H.; Lopez, G. P.; Brozik, S. M.; Werner-Washburne, M.; Brinker, C. J. Cell-directed assembly of lipid-silica nanostructures providing extended cell viability. *Science* **2006**, *313*, 337–341.
(54) Pope, E. J. A.; Braun, K.; Peterson, C. M. Bioartificial organs I: Silica gel encapsulated pancreatic islets for the treatment of diabetes mellitus. *J. Sol-Gel Sci. Technol.* **1997**, *8*, 635–639.
(55) Liu, D. M.; Chen, J. W. Encapsulation of protein molecules in transparent porous silica matrices via an aqueous colloidal sol-gel process. *Acta Mater.* **1999**, *47*, 4535–4544.
(56) Bhatia, R. B.; Brinker, C. J.; Gupta, A. K.; Singh, A. K. Aqueous sol-gel process for protein encapsulation. *Chem. Mater.* **2000**, *12*, 2434–2441.
(57) Rupcich, N.; Goldstein, A.; Brennan, J. D. Optimization of sol-gel formulations and surface treatments for the development of pin-printed protein microarrays. *Chem. Mater.* **2003**, *15*, 1803–1811.
(58) Bower, C. K.; Sananikone, S.; Bothwell, M. K.; McGuire, J. Activity losses among T4 lysozyme charge variants after adsorption to colloidal silica. *Biotechnol. Bioeng.* **1999**, *64*, 373–376.
(59) Finnie, K. S.; Bartlett, J. R.; Woolfrey, J. L. Encapsulation of sulfate-reducing bacteria in a silica host. *J. Mater. Chem.* **2000**, *10*, 1099–1101.
(60) Chen, J.; Xu, Y.; Xin, J.; Li, S.; Xia, C.; Cui, J. Efficient immobilization of whole cells of *Methylomonas* sp. strain GYJ3 by sol-gel entrapment. *J. Mol. Catal. B* **2004**, *30*, 167–172.
(61) Yu, D.; Volponi, J.; Chhabra, S.; Brinker, C. J.; Mulchandani, A.; Singh, A. K. Aqueous sol-gel encapsulation of genetically engineered Moraxella sp. cells for the detection of organophosphates. *Biosens. Bioelectron.* **2005**, *20*, 1433–1437.
(62) Nassif, N.; Roux, C.; Coradin, T.; Rager, M. N.; Bouvet, O. M. M.; Livage, J. A sol-gel matrix to preserve the viability of encapsulated bacteria. *J. Mater. Chem.* **2003**, *13*, 203–208.
(63) Nassif, N.; Bouvet, O.; Rager, M. N.; Roux, C.; Coradin, T.; Livage, J. Living bacteria in silica gels. *Nat. Mater.* **2002**, *1*, 42–44.
(64) Livage, J.; Coradin, T. Living cells in oxide glasses. In *Medical Mineralogy and Geochemistry*; Sahai, N., Schoonen, M., Eds.; Reviews in Mineralogy and Geochemistry Series, Vol. *63*; Mineralogical Society of America: Washington, DC, 2006.
(65) Ferrer, M. L.Garcia-; Carvajal, Z. Y.; Yuste, L.; Rojo, F.; del Monte, F. Bacteria viability in sol-gel materials revisited: Cryo-SEM as a suitable tool to study the structural integrity of encapsulated bacteria. *Chem. Mater.* **2006**, *18*, 1458–1463.
(66) Desimone, M. F.De; Marzi, M. C.; Copello, G. J.; Fernandez, M. M.; Malchiodi, E. L.; Diaz, L. E. Efficient preservation in a silicon oxide matrix of *Escherichia coli*, producer of recombinant proteins. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 747–752.
(67) Nassif, N.; Roux, C.; Coradin, T.; Bouvet, O. M. M.; Livage, J. Bacteria quorum sensing in silica matrices. *J. Mater Chem.* **2004**, *14*, 2264–2268.
(68) Gautier, C.; Livage, J.; Coradin, T.; Lopez, P. J. Sol-gel encapsulation extends diatoms viability and reveals their silica dissolution capability. *Chem. Commun.* **2006**, 4611–4613.

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