Sol-gel synthesis on self-replicating single-cell scaffolds: applying complex chemistries to nature's 3-D nanostructured templates

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A sol-gel process was used, for the first time, to apply a multi-component, nanocrystalline, functional ceramic compound ($BaTiO_3$) to a three-dimensional, self-replicating scaffold derived from a single-celled micro-organism (a diatom).

Intense global activity to produce innovative micro-to-nanoscale devices has led to appreciable interest in self-assembly. Numerous examples of self-assembly can be found in nature.[1] Certain micro-organisms are adept at assembling three-dimensional (3-D) biomineralized micro-structures with precise shapes and fine (submicron) features. An exceptional variety of silica-based structures are generated by aquatic micro-algae known as diatoms.[2a,b] Diatoms are single-celled organisms that assemble microshells (frustules) comprised of silica nanoparticles.[2c] Each diatom frustule possesses a 3-D shape decorated with a regular pattern of fine features (10^2 nm pores, channels, protuberances) that are species specific.[2a–d] The frustule morphology for a given diatom species is replicated with high fidelity upon biological reproduction.[2d] Consequently, enormous numbers of identically-shaped frustules can be generated by sustained reproduction of a single parent diatom (*e.g.*, more than 1 trillion daughter diatoms with similar frustules could be produced from a parent diatom after only 40 reproduction cycles).[2d,e] Such massively parallel and genetically precise 3-D nanoparticle assembly has no man-made analog. With $\approx 10^5$ extant diatom species, a rich variety of frustule morphologies exists for potential applications.[2a–d,f] However, the utility of these natural assemblies is severely limited by the silica-based chemistry. Extensive work over the last half of the past century has yielded a variety of synthetic, solution-based routes (sol-gel, polymer precursor, hydrothermal, *etc.*) to ceramic compounds with complex chemistries. The purpose of this paper is to demonstrate, for the first time, how a solution-based synthetic process may be used to apply a multi-component, functional ceramic compound ($BaTiO_3$) to a 3-D scaffold derived from a self-replicating, single-celled organism (a diatom).

For this demonstration, a sol-gel process was used to apply a thin barium titanate coating to a diatom-derived scaffold. $BaTiO_3$ is a widely-used compound with attractive dielectric, ferroelectric, piezoelectric, chemical, and electro-optical properties for capacitors, actuators, thermistors, sensors, waveguides, and numerous other current or potential devices.[3] The sol-gel process has been used to apply $BaTiO_3$ coatings to a variety of substrates.[4] In the present case, the silica-based composition of diatom frustules is not chemically compatible with barium titanate. The $BaO–TiO_2–SiO_2$ ternary phase diagram reveals that the compound $BaTiSiO_5$ can form as a stable intermediate phase upon firing of a $BaTiO_3$ coating on SiO_2.[5a] In order to avoid this undesired silicate compound, a silica scaffold may be coated with, or converted into, a chemically compatible material. One such compatible material is magnesia, MgO. The $BaO–TiO_2–MgO$ phase diagram reveals that MgO and $BaTiO_3$ share a tie line (*i.e.*, no new compounds can form between these oxides at ambient pressure[5b]). Indeed, composites of $BaTiO_3$ nanoparticles dispersed in a MgO matrix have been synthesized by hot pressing powder mixtures at 1350 °C for 1 h.[5c] MgO is also a desired substrate for electro-optical $BaTiO_3$ coatings, due to its optical transparency, modest refractive index, and thermal expansion match to $BaTiO_3$.[3a,b]

The bioscaffolds used in this work were the frustules of *Aulacoseira* diatoms (obtained as diatomaceous earth). A secondary electron image of an *Aulacoseira* frustule is shown in Fig. 1(a). These frustules were cylindrical in shape and were lined with rows of fine (10^2 nm dia.) pores. One end face of each frustule possessed a circular hole with a protruding outer rim, whereas the other end was closed and exhibited fingerlike extensions. Prior to the

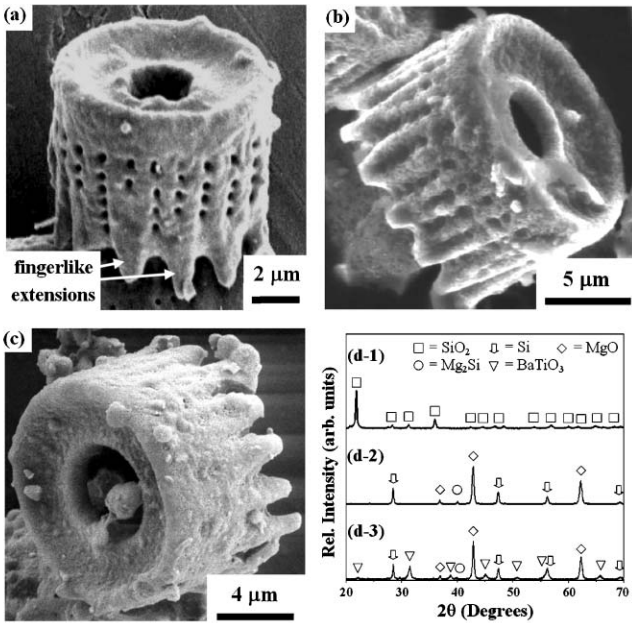

Fig. 1 Secondary electron images of (a) an *Aulacoseira* diatom frustule, (b) a MgO-bearing *Aulacoseira* frustule after reaction with Mg(g) for 1.5 h at 900 °C, (c) a MgO-bearing *Aulacoseira* frustule coated with a $BaTiO_3$ sol-gel precursor and fired at 700 °C for 1.5 h. (d) XRD analyses of (1) SiO_2-based *Aulacoseira* frustules, (2) MgO-bearing frustules after reaction with Mg(g) for 1.5 h at 900 °C, and (3) MgO-bearing frustules coated with sol-gel-derived $BaTiO_3$ after firing at 700 °C for 1.5 h.

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application of sol-gel-derived barium titanate, these silica-based frustules were converted into magnesia-bearing scaffolds by the net gas/silica oxidation–reduction reaction:

$$2Mg(g) + SiO_2(s) \Rightarrow 2MgO(s) + \{Si\}$$

where {Si} refers to the silicon reduction product present in an elemental or alloyed state (as Mg–Si liquid or solid Mg_2Si). Recent work has shown that this displacement reaction can yield MgO-based structures that retain the 3-D shapes and fine features of the starting frustules.[6] The diatom frustules and magnesium reactants (SiO_2 : Mg molar ratio = 1 : 3.5) were placed within, and at opposite ends, of a steel tube (2.5 cm dia., 20 cm long), that was then crimped and welded shut. The specimen-bearing tube was then thrust into a horizontal tube furnace preheated to 900 °C, and held at this temperature for 1.5 h. X-Ray diffraction (XRD) analyses of the frustules before and after this treatment are shown in Figs. 1(d-1) and 1(d-2), respectively. After the 900 °C exposure to Mg(g), the diffraction peaks for cristobalite silica in the starting frustules vanished and were replaced by those for MgO and Si, along with a weak peak for the compound Mg_2Si. A secondary electron image of such a converted and shape-preserved frustule is shown in Fig. 1(b). A continuous layer of barium titanate was then applied to these magnesia-bearing scaffolds by a sol-gel process. The barium titanate precursor consisted of a solution of barium titanium ethylhexano-isopropoxide (Alfa Aesar Chemical Co., Ward Hill, MA) dissolved in absolute ethanol, along with smaller amounts of concentrated (29%) ammonium hydroxide, and deionised water. The molar ratio of $BaTi(OOC_8H_{15})(OC_3H_7)_5$: NH_4OH : H_2O : EtOH in the solution was 1.0 : 0.15 : 0.77 : 110. After introducing 0.4 g of the magnesia-bearing frustules into 20 ml of this solution, the mixture was stirred and refluxed at 70 °C for 3 h. 5 ml of the solution was then allowed to evaporate at 56 °C. The refluxing treatment was then repeated for 1 more hour, after which 5 more ml of the solution were removed by evaporation. After refluxing for a final hour, the remaining volatile solution was allowed to evaporate at 56 °C. The coated frustules were then placed within a magnesia crucible and heated at 3 °C per min in ambient air to 700 °C for 1.5 h. XRD analysis (Fig. 1(d-3)) of the coated and fired specimens revealed distinct peaks for $BaTiO_3$. The relative intensities of the MgO and Si diffraction peaks in Fig. 1(d-3) were similar to those for uncoated specimens (Fig. 1(d-2)). Other titanate (than $BaTiO_3$) or silicate phases were not detected. These latter observations indicated that the barium titanate coating underwent negligible reaction with the underlying scaffold. Measurement of peak broadening in the XRD pattern and use of the Scherrer equation yielded an average $BaTiO_3$ crystallite size of 20 nm.[7] A secondary electron image of a barium titanate-coated frustule is shown in Fig. 1(c). The absence of gaps or cracks on the coated frustule surface, and the retention of the frustule morphology (*i.e.*, the protruding rim and circular hole on one end, and the fingerlike extensions on the other end), were consistent with the presence of a continuous, thin coating over the entire frustule surface. A transmission electron image of a cross-section of a coated specimen is shown in Fig. 2(a). A continuous, 150 nm thick coating was detected on the frustule surface. Electron diffraction analyses of this coating (Fig. 2(b)) and the underlying scaffold (Fig. 2(c)) were consistent with a layer of tetragonal $BaTiO_3$ on a composite mixture of MgO, Si, and Mg_2Si, respectively. A higher magnification image of the barium titanate coating is shown in Fig. 2(d). The $BaTiO_3$ grains were 10 to 40 nm in size, which was consistent with the Scherrer analysis.

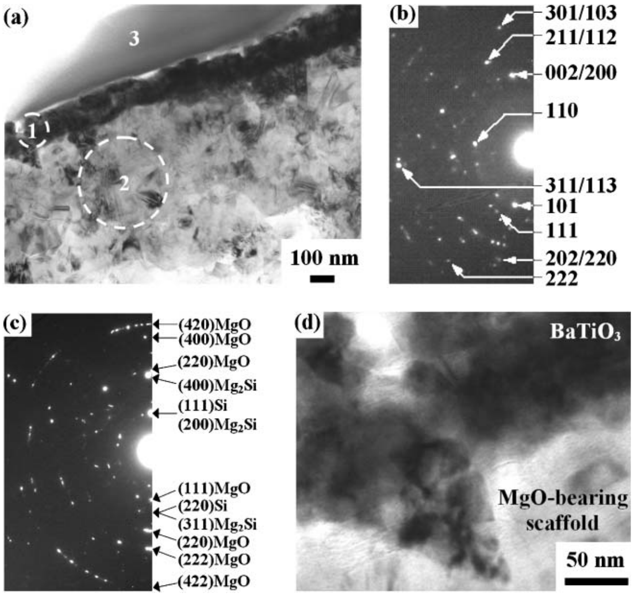

Fig. 2 (a) Transmission electron (TE) micrograph of an ion-milled cross-section of a $BaTiO_3$-coated, MgO-converted *Aulacoseira* diatom frustule. Electron diffraction patterns obtained from (b) region 1, consistent with $BaTiO_3$ and (c) region 2, consistent with a mixture of MgO, Mg_2Si and Si (region 3 is an epoxy matrix applied during specimen preparation). (d) Higher magnification TE micrograph of the interface between the $BaTiO_3$ coating and the MgO-bearing scaffold near region 1 in (a).

This work demonstrates how the attractive characteristics of both synthetic chemical processing and biological self-assembly can be merged; that is, we show how complex and highly-tailorable chemistries accessible with solution processing can be applied to intricate, genetically-precise 3-D micro/nanostructures that can be generated in a massively-parallel fashion by single-celled organisms. Although this work has focused on a sol-gel-derived compound and a diatom-derived scaffold, other solution-based routes, chemistries, and bioclastic structures (*e.g.*, from coccolithophorids, silicoflagellates, radiolarians, bacteria, sponges, *etc.*[1]) may be used to produce micro/nanostructures with a nearly endless variety of functional chemistries and shapes. Unlike other approaches for fabricating 3-D ceramic micro/nanostructures, this solution-based bioscaffold coating process does not require expensive equipment or facilities for patterning or for chemical processing, and is highly scalable. Furthermore, future work on the genetic modification of single-celled, biomineralizing organisms may enable the mass production of self-replicating structures with engineered shapes. The recent mapping of the genome of the diatom *Thalassiosira pseudonana* is a first step in this direction.[8] The present process could then be used to produce **G**enetically **E**ngineered **M**icro/nanodevice**s** (**GEMs**) with tailored shapes and compositions for numerous applications.

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