Merging Biological Self-Assembly with Synthetic Chemical Tailoring: The Potential for 3-D Genetically Engineered Micro/Nano-Devices (3-D GEMS)

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Appreciable global efforts are underway to develop processes for fabricating three-dimensional (3-D) nanostructured assemblies for advanced devices. Widespread commercialization of such devices will require: (i) precise 3-D fabrication of chemically tailored structures on a fine scale and (ii) mass production of such structures on a large scale. These often-conflicting demands can be addressed with a revolutionary new paradigm that couples biological self-assembly with synthetic chemistry: Bioclastic and Shape-preserving Inorganic Conversion (BaSIC). Nature provides numerous examples of microorganisms that assemble biominerals into intricate 3-D structures. Among the most spectacular of these microorganisms are diatoms (unicellular algae). Each of the tens of thousands of diatom species-assembles silica nanoparticles into a microshell with a distinct 3-D shape and pattern of fine (nanoscale) features. The repeated doubling associated with biological reproduction enables enormous numbers of such 3-D microshells to be generated (e.g., only 40 reproduction cycles can yield > 1 trillion 3-D replicas!). Such genetic precision and massive parallelism are highly attractive for device manufacturing. However, the natural chemistries assembled by diatoms (and other microorganisms) are rather limited. With BaSIC processes, biogenic assemblies can be converted into a wide variety of new functional chemistries, while preserving the 3-D morphologies. Ongoing advances in genetic engineering promise to yield microorganisms tailored to assemble nanoparticle structures with device-specific shapes. Large-scale culturing of such genetically tailored microorganisms, coupled with shape-preserving chemical conversion (via BaSIC processes), would then provide low-cost 3-D Genetically Engineered Micro/nano-devices (3-D GEMs).
Introduction

Owing to dramatic improvements in properties, or entirely new properties, exhibited by or predicted for nanostructured materials, appreciable worldwide activity is underway to develop new methods for manufacturing novel devices with nanoscale features. Fabrication routes based on conventional two-dimensional (2-D) layer-by-layer techniques used by the microelectronics industry are not well-suited for the low-cost mass production of nanostructured devices with intricate three-dimensional (3-D) shapes (curved internal and external surfaces) and with complex, non-silicon-based chemistries. However, certain natural microorganisms can generate enormous numbers of nanoparticle-based, 3-D biomineralized structures under ambient conditions.\(^1\),\(^2\) Among the most spectacular examples of such assembly are the microshells (frustules) of diatoms.\(^1\),\(^3\) Diatoms are unicellular algae that form frustules comprised of amorphous silica nanoparticles.\(^4\) Each of the tens of thousands of diatom species forms a frustule with an intricate and unique 3-D shape and with species-specific (genetically controlled) patterns of fine features (10\(^{1}\)–10\(^{2}\) nm pores, channels, nodules, etc.).\(^3\),\(^4\) The open structure and micro to nanoscale porosity of diatom frustules have made these bioclastic structures attractive for wastewater and gas purification, recovery of metals from waste streams, agricultural additives, filtration of beverages, and porous partition columns for chemical analyses.\(^5\)–\(^10\) Continuous reproduction of a given diatom species can lead to enormous numbers of identical 3-D frustules (e.g., 40 sustained reproduction cycles would yield \(2^{40}\), or more than 1 trillion, daughter diatoms with similar frustules).\(^3\),\(^4\),\(^11\) Such massively-parallel and genetically precise (species specific) 3-D nanoparticle assembly has no man-made analog. On the other hand, natural bioclastic structures tend to possess a rather limited range of chemistries. The vast majority of biominalerized structures are comprised of silica or calcium carbonate.\(^2\) These minerals do not possess attractive properties for many device applications.

The purpose of this article is to demonstrate several approaches that can be used to change the compositions of diatom frustules or other bioclastic structures into a wide variety of non-natural chemistries without loss of the bioassembled 3-D morphology. These processes are referred to collectively as BaSIC (Bioclastic and Shape-preserving Inorganic Conversion).\(^12\)–\(^18\) By merging the attractive self-assembly characteristics of nature with the chemical versatility of synthetic processing, BaSIC processes may be used to mass produce nanostructured microdevices with complex 3-D shapes and tailored chemistries, as is illustrated in Fig. 1. Ongoing advances in the genetic engineering of biominalerizing microorganisms may yield nanoparticle structures with tailored 3-D shapes. Such genetic advances may then be coupled with the BaSIC manufacturing paradigm to yield 3-D Genetically Engineered Micro/nano-devices (3-D GEMs).

Experimental Procedure

To demonstrate the chemical versatility of BaSIC processes, three approaches for altering the compositions of silica-based diatom frustules are described in this article: (i) gas/silica displacement reaction methods,
(ii) conformal coating methods, and (iii) a combination of the displacement reaction and conformal coating methods. The first approach has been used to convert SiO₂ frustules into replicas comprised of MgO (via an oxidation–reduction displacement reaction with Mg(g)) or TiO₂ (via a metathetic displacement reaction with TiF₄(g)). With the second approach, silica frustules have been converted into polymer or ZrO₂ replicas. With the third approach, continuous BaTiO₃ coatings have been applied to MgO-bearing frustule replicas.

**Gas/Silica Displacement Reactions**

Gas/silica displacement reactions were used to convert SiO₂-based diatom frustules into MgO or TiO₂ replicas. Diatom frustules, obtained as diatomaceous earth, were sealed along with a solid reactant precursor inside metal ampoules. Upon heating, the solid precursor to the reactant gas evaporated and then reacted with silica in the frustules to form a new solid phase.

For conversion into MgO, the diatom frustules and solid Mg granules were placed at opposite ends of a steel tube (2.5 cm diameter, 18–20 cm long) that was then welded shut. The center of the tube was crimped and then bent into an inverted “V” shape to avoid contact of the diatom frustules with magnesium liquid during heat treatment (Fig. 2). The molar Mg:SiO₂ ratio sealed within the tube was ≥2.5:1 (i.e., in excess of the stoichiometric 2:1 ratio required for complete conversion of SiO₂ into MgO). The sealed ampoules were heated to 900 °C and held for 1.5–4 h. The tubes were then cut open and the reacted frustules were removed for characterization.

For conversion into TiO₂, the diatom frustules and solid TiF₄ were placed at opposite ends of a titanium tube, which was then welded shut. The molar TiF₄:SiO₂ ratio sealed within the tube was maintained at 2.4:1. The sealed ampoules were heated to 350 °C and held for 2 h. The tubes were then cut open to remove the reacted frustules. These reacted specimens were then heated to 350 °C and held for 2 h, all within a flowing oxygen atmosphere.

**Conformal Coating Methods**

Wet chemical approaches were used to apply conformal coatings of epoxy or zirconia to the diatom frustules. For both types of coatings, the frustules were immersed in a dilute precursor solution dissolved in a volatile solvent. Upon removal of the frustules from the solution, the solvent was allowed to evaporate to leave a thin conformal coating of a precursor to the desired material. The coating was then rigidified, either through cross-linking (for polymer coatings) or calcination (for zirconia coatings). Selective dissolution of the underlying SiO₂ was then used to leave a coating-based replica that preserved the shape and fine features of the starting template.

Polymeric frustule replicas were generated by exposing 1 gram of diatom frustules to 30 mL of a solution comprised of 7 wt% of a two-part, 5 min curing epoxy mixture (Loctite® Quick Set™ Epoxy Gel, Henkel Consumer Adhesives, Avon, OH) dissolved in acetone. After stirring for 15 min, the frustules were filtered from the solution and the acetone was allowed to evaporate from the coated frustules. After curing the coating (hardener + epoxy precursor) at room temperature, the coated frustules were exposed to a 49 wt% HF solution to dissolve the underlying silica. The epoxy replicas were then washed with distilled water and ethanol.

ZrO₂ frustule replicas were generated with a sol-gel coating process. The diatom frustules were first exposed to a boiling ammonium hydroxide solution (pH = 10) for 4 h. The frustules were then immersed in an anhydrous ethanol-based solution containing zirconium n-propoxide (Aldrich Chemical Co., Milwaukee, WI) along with ammonium hydroxide and deionized water. The molar Zr(OC₃H₇)₄:NH₄OH:H₂O:EtOH ratio of this solution was 1.0:0.67:3.36:480. Diatom frustules (0.4 g) were introduced into 20 mL of this solution. The frustules were stirred in the solution, as the solution was refluxed, for a total of 6 h at 76°C, with intermediate stages of evaporation of the solution after 3, 4, and 6 h. The coated frustules were then heated in air to 650°C...
for 3 h. The resulting ZrO$_2$-coated frustules were then immersed for 2 h in an aqueous 30 wt% NaOH solution at 85°C to selectively remove the underlying silica.

**Combined Use of the Displacement Reaction and Coating Methods**

The displacement reaction and conformal coating methods were combined to synthesize composite frustule replicas. MgO-converted frustule replicas (formed by the oxidation–reduction displacement reaction discussed above) were coated with a thin BaTiO$_3$ layer. MgO-bearing replicas (0.4 g) were immersed in 20 mL of an ethanol-based solution containing barium titanium ethylhexano-isopropoxide (Alfa Aesar, Ward Hill, MA) along with ammonium hydroxide and de-ionized water. The molar BaTi(OOC$_8$H$_{15}$)(OC$_7$H$_7$)$_3$: NH$_4$OH:H$_2$O:EtOH ratio of the solution was 1.0:0.15:0.77:110. The frustule replicas were stirred in this solution, as the solution was refluxed, for a total of 5 h at 70°C, with intermediate stages of evaporation of the solution after 3, 4, and 5 h. The coated specimens were then heated in air at 700°C for 1.5 h.

**Results and Discussion**

**Diatom Frustule Templates**

The diatomaceous earth used in the present work was largely comprised of capsule-shaped *Aulacoseira* diatom frustules. The morphologies of these frustules are shown in Fig. 3. These cylindrical frustules were decorated with rows of fine (few hundred nm diameter) pores that ran parallel to the length of the frustule (Fig. 3a). One end face of each frustule possessed a circular hole and a protruding outer rim (Fig. 3b), whereas the other end possessed finger-like extensions. Larger, paired assemblies were often observed to form by the intercalation of the finger-like extensions from one cylinder with those of another (the narrow channels in Fig. 3a are gaps between intercalating finger-like extensions). Secondary electron images revealing the hollow internal structure of the *Aulacoseira* frustules are shown in Fig. 4. These images were obtained by gradual ion beam milling of the same frustule from one end toward the other end (note: during the milling process between the images in 4b and 4c, the frustule rolled to the right, so that part of the frustule circumference was not milled away).
Chemical Conversion via Gas/Silica Displacement Reactions

Two types of displacement reactions were used to replace the silica in the diatom frustules:

i) oxidation/reduction (with elemental gas reactants) and ii) metathetic (with halide gas reactants).

The following oxidation-reduction displacement reaction was used at 900°C to convert SiO$_2$ *Aulacoseira* frustules into MgO-based replicas:

$$2\text{Mg}(g) + \text{SiO}_2(s) \rightarrow 2\text{MgO}(s) + \text{Si}(s) \quad (1)$$

From available data for the Gibbs energies of formation of SiO$_2$(s) (as cristobalite) and MgO(s), the partial pressure of Mg(g) required for this reaction to proceed spontaneously at 900°C was calculated to be only $1.1 \times 10^{-7}$ atm ($8.0 \times 10^{-5}$ Torr). For a metastable amorphous silica reactant, the required magnesium partial pressure would be even lower. By sealing the diatoms and magnesium within steel ampoules (as shown in Fig. 2), the magnesium vapor pressure generated over molten magnesium could be effectively utilized for this reaction. At 900°C, the equilibrium vapor pressure of Mg(g) over Mg(l) is 0.16 atm (120 Torr). Because this value is six orders of magnitude greater than the magnesium partial pressure needed to conduct this reaction, direct contact of the diatoms with molten magnesium was not required for rapid reaction at 900°C. Direct contact of diatoms with molten magnesium was, in fact, prevented by crimping and bending the center of the steel ampoule, in order to avoid the need for extracting reacted diatoms from a solidified Mg-rich matrix. Steel was selected as the ampoule material, as magnesium exhibits negligible chemical interaction with iron or carbon.

Secondary electron images of the same *Aulacoseira* frustules before and after reaction with Mg(g) at 900°C are shown in Fig. 5a and b, respectively. Energy-dispersive X-ray (EDX) analysis obtained from reacted frustules is shown in Fig. 5c. The reacted frustules had been converted into MgO, with little Si detected in the EDX pattern. The overall morphology of the starting diatom frustules was well-preserved after conversion into MgO. Indeed, 10 different features (along with the fine pores) present in the starting frustules in Fig. 5a were preserved in the reacted frustules in Fig. 5b. The elemental Si product of reaction (1) was largely removed from the frustules via the formation of a Si-bearing liquid that poured out of the MgO-converted frustules (as seen in Fig. 5b). The Si reacted with excess Mg gas in the sealed ampoules to form a Mg-rich, Mg-Si liquid, as expected from the Mg–Si phase diagram.

Silica-based *Aulacoseira* frustules were converted into TiO$_2$ replicas at 350°C through the use of the net metathetic displacement reaction:

$$\text{TiF}_4(g) + \text{SiO}_2(s) \rightarrow \text{TiO}_2(s) + \text{SiF}_4(g) \quad (2)$$

In this case, the Si-bearing product is a halide gas that can diffuse away to yield a TiO$_2$ frustule replica. The standard Gibbs energy change for this reaction (with SiO$_2$ as cristobalite and TiO$_2$ as rutile) at 350°C is $-82.7$ kJ/mol. The calculated TiF$_4$(g):SiF$_4$(g) molar ratio required for this reaction to proceed at 350°C (for pure cristobalite and rutile phases) was only $1.2 \times 10^{-7}$. In this work, the diatom frustules were exposed to TiF$_4$(g) that was generated by heating TiF$_4$(s) to 350°C within sealed titanium ampoules. Given that more TiF$_4$(s) was sealed within the ampoules than SiO$_2$(s) frustules (on a molar basis), a strong thermodynamic driving force existed for reaction. TiF$_4$ gas pressures >1 atm were achieved by heating TiF$_4$(s) powder sealed within the ampoules to a temperature above the sublimation point for this halide compound (285°C).
The net reaction (2) was conducted in two steps: (i) exposure of SiO2 frustules to TiF4(g) within sealed Ti ampoules at 350°C, and then (ii) treatment of the reacted frustules in flowing oxygen, also at 350°C. The second step was conducted to remove fluorine (present as TiOF2 or dissolved in titania) from the reaction product. A secondary electron image, and an X-ray diffraction (XRD) pattern, of converted Aulacoseira frustules are shown in Fig. 6. The XRD pattern revealed that the frustules had been fully converted into anatase titania. The anatase frustules retained the morphology and fine features (pores, channels between intercalating finger-like extensions) of the starting Aulacoseira frustules.

**Chemical Conversion via Conformal Coating Methods**

A second BaSIC approach involves the use of bioclastic structures as 3-D templates onto which thin conformal coatings of desired composition are deposited. If the coating is continuous and rigid, then the underlying bioclastic template may be selectively removed (e.g., by selective dissolution) to yield a coating-based replica with a tailored composition. The aim of such a coating-based BaSIC approach is to form a positive replica that retains the fine features of the starting bioclastic template (as opposed to a negative structure that assumes the morphology of continuous pore networks, such as has been accomplished by the Replamine process using coral templates). To form a positive replica, the conformal coating must be applied as a sufficiently thin layer that the fine features are not filled or covered. This was accomplished in the present work with wet chemical approaches that utilized dilute precursor solutions with volatile solvents.

To form polymeric replicas, Aulacoseira frustules were immersed in a dilute solution (7 wt%) of an epoxy precursor dissolved in acetone. After removal from the solution, evaporation of the acetone, room-temperature curing of the thin epoxy coating, and then selective dissolution of the silica in a HF solution, polymeric replicas of the type shown in Fig. 7a were produced. Complete removal of silica from the polymeric coating was confirmed by EDX analyses (i.e., by the absence of peaks for silicon) and thermogravimetric analyses in flowing oxygen at 550°C (i.e., the polymer could be completely burned away with no residual weight from undissolved silica). By controlling the concentration of the epoxy precursor in the coating solution, a continuous polymeric coating was generated that did not completely fill or cover the fine pores or narrow channels present in the starting frustule. A cross-section of the wall of a polymeric replica is shown in Fig. 7b. The wall of the replica was hollow, and consisted of thin internal and external layers that were separated by cylindrical struts. These struts were generated upon coating of the walls of pore channels that ran through the thickness of the Aulacoseira frustules.

The successful synthesis of polymeric frustule replicas was then followed by the development of wet chemical BaSIC methods for generating zirconia and other ceramic replicas. To form a ZrO2 coating, the frustules were immersed in an ethanol-based solution containing a dilute concentration (1.45 wt%) of a zirconium alkoxide. Upon removal from solution and evaporation of the ethanol, the coated frustules were fired in air at a heating rate of 30°C/min up to 1000°C in a high-temperature XRD system (X’Pert PRO System, PANanalytical Corp., Almalo, The Netherlands). The high-temperature XRD patterns obtained from...
200°C to 1000°C are shown in Fig. 8a. The onset of zirconia formation was observed to occur between 400°C and 450°C. Based on this information, heat treatments of the coated frustules were then conducted at \( \geq 550°C \) for 3 h to allow for complete zirconia formation. A \( \text{ZrO}_2 \) frustule formed by firing of the coating at 650°C for 3 h, and then selective dissolution of the underlying silica in a hot 30 wt% NaOH solution for 2 h, is shown in Fig. 8b. This \( \text{ZrO}_2 \) replica retained the overall morphology and features of the starting \textit{Aulacoseira} frustules. An EDX pattern obtained from such replicas (Fig. 8c) revealed the presence and absence of Zr and Si peaks, respectively, which indicated that the underlying silica template had been completely dissolved away (note: the Al peak in the EDX pattern was obtained from the underlying aluminum substrate and the Au peak was obtained from a coating applied to the specimen to avoid charging).

**Chemical Conversion via Combined Use of Displacement Reaction and Coating Methods**

The composition of a native bioclastic structure may not be chemically compatible with a desired coating material. If a thermal treatment is required to convert an as-deposited coating into a desired material/phase, then an undesired chemical reaction may occur between the coating material and a chemically incompatible bioclastic template. Consider, for example, the formation of a barium titanate (\( \text{BaTiO}_3 \)) coating on a \( \text{SiO}_2 \)-based diatom frustule. As shown in Fig. 9a, barium titanate and silica are not chemically compatible (i.e., several intermediate \( \text{BaO–TiO}_2–\text{SiO}_2 \)-bearing compounds may form).\(^{25}\) Indeed, initial attempts to generate a \( \text{BaTiO}_3 \) coating on \( \text{SiO}_2 \) frustules via firing of a sol–gel coating resulted in the formation of \( \text{BaTiSiO}_5 \). Examination of the \( \text{BaO–MgO–TiO}_2 \) ternary phase diagram (Fig. 9b) reveals that \( \text{BaTiO}_3 \) is chemically compatible with MgO.\(^{26}\) That is, MgO-converted frustule replicas should act as chemically inert templates onto which sol–gel-derived \( \text{BaTiO}_3 \) coatings may be generated.

To demonstrate that layered composite materials may be synthesized via combined use of the displacement reaction and conformal coating BaSIC methods, a sol–gel-derived \( \text{BaTiO}_3 \) coating was applied to MgO-converted diatom frustules. The converted frustules were immersed in an ethanol-based solution that contained barium titanium ethylhexano-isopropoxide. High-temperature XRD analyses (analogous to the analyses in Fig. 8a) indicated that the onset of \( \text{BaTiO}_3 \) formation occurred between 550°C and 575°C. Hence, the coated MgO-bearing frustules were fired at 700°C for 1.5 h to allow for complete \( \text{BaTiO}_3 \) formation. An XRD pattern obtained from such specimens is shown in Fig. 10a. The only \( \text{BaO–TiO}_2 \)-bearing phase detected was the desired \( \text{BaTiO}_3 \) compound. As expected, the dominant diffraction peaks were obtained from the MgO phase in the underlying template. Secondary silicon-bearing phases (Si, Mg,Si) detected in the template formed during the MgO conversion reaction (1). A secondary electron image of a \( \text{BaTiO}_3 \)-coated frustule is shown in Fig. 10b. The overall morphology of the starting diatom frustules was preserved in the \( \text{BaTiO}_3/\text{MgO} \)-bearing composite frustule.

**Genetic Engineering and Large-Scale Culturing of Diatoms**

In a BaSIC manufacturing paradigm, the sustained reproduction of microorganisms would be used to gen-
erate large numbers of identical 3-D bioclastic structures that, in turn, would be chemically tailored to achieve desired properties. The technology for large-scale culturing of microorganisms that form bioclastic structures currently exists. Large-scale diatom culturing (with \( \geq 30,000 \) L culturing tanks) is currently being conducted for commercial aquaculture operations (for shrimp, clams, oysters, scallops, etc.) and for the synthesis of biotechnological compounds.27,28

The reaction-based and coating-based BaSIC methods can be used to convert diatom frustules and other bioclastic structures into a wide variety of functional materials for devices (sensors, filters, reactors, capsules, actuators, etc.) for environmental, transportation, biomedical, agricultural, chemical/petrochemical, manu-

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**Fig. 8.** (a) High-temperature X-ray diffraction patterns obtained upon heating of a sol-gel-coated Aulacoseira diatom frustule in air at 30°C/min (CoK\( \alpha \) radiation); (b) a secondary electron image and (c) an energy-dispersive X-ray pattern obtained from a zirconia frustule replica that had been heated to 650°C in air for 3 h and then exposed to a hot sodium hydroxide solution to selectively dissolve the underlying silica.

**Fig. 9.** (a) \( \text{BaTiO}_3-\text{SiO}_2 \) binary phase diagram\(^{25} \) and (b) \( \text{BaO-} \text{TiO}_2-\text{MgO} \) ternary phase diagram\(^{26} \) (both diagrams reprinted with permission from the American Ceramic Society).
facturing, telecommunications, aerospace, homeland security, and other applications. The multifarious shapes available among natural bioclastic structures may be selected for specific device applications. Indeed, the growth of several diatom species with varied frustule shapes is underway at Scripps Institution of Oceanography and the Georgia Institute of Technology (see Fig. 11). The genetic engineering of diatoms and other biomineralizing organisms may lead to an even greater variety of tailored (non-natural) shapes. The mapping of a diatom genome, and the development of transformation-based approaches for expressing genes in diatoms, are initial steps in this direction.29,30

Conclusions

Several reaction-based and coating-based methods for altering the silica-based composition of diatom microshells (frustules), while preserving the starting frustule shapes, have been demonstrated. Gas/solid displacement (oxidation–reduction or metathetic) reactions were used to convert diatom microshells into magnesia-based and titania-based replicas. Wet chemical conformal coating methods were used to generate polymeric and zirconia frustule replicas. A combination of the displacement reaction and coating methods was used to generate composite replicas comprised of a continuous barium titanate coating on a magnesia-bearing frustule template. These BaSIC approaches may be used to convert the microshells of diatoms (or other biomineralized structures) into a wide variety of other functional chemistries. This synergistic combination of biological assembly with synthetic chemical functionalization opens the door to large numbers of 3-D micro/nanostructures with chemistries and properties that can be tailored for a host of device applications. If future advances in the genetic engineering of biomineralizing microorganisms lead to precise control over the morphologies of bioclastic structures, then the coupling of BaSIC processes with such genetic tailoring promises to make the manufacturing of low-cost 3-D GEMs a reality.

References