Nanostructure Synthesis

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Sacrificial Biological Templates for the Formation of Nanostructured Metallic Microshells**

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One of the challenges facing chemists and materials scientists is the fabrication of metallic micro- and macroscale materials that exhibit well-defined and controllable nanometer-scale features. Such materials are generally fabricated by using either lithographic or template-mediated approaches and are potentially useful for micro- and nanoelectronics, plasmon optics, and catalysis. In particular, lithographic techniques have proven useful in the generation of surface-confined nanostructured metallic materials.[1] However, fabrication of similarly structured free-standing and isolable metallic materials has proven more difficult. To create these structures, man-made templates have been used,[2] but they generally lack the structural sophistication necessary to yield the intricate details that lithographic methods routinely provide. Microorganism templates such as viruses,[3] bacteria,[4] diatoms,[5] and fungi,[6] on the other hand, offer unique organism- and species-specific details not provided by man-made templates. In this work, diatoms, a form of unicellular algae, were used as sacrificial templates for the formation of 3D metal-coated diatoms and free-standing nanostructured metallic microshells. Herein, we describe their structures and investigate their potential as substrates for surface-enhanced Raman scattering.

Several reports have described and characterized the nanostructure of diatom cell walls (frustules) and the proteins that direct their formation.[7] Of particular interest is the observation that diatom frustules (typically 1–100 μm in size) appear to be composed of fused or aggregated silica nanoparticles[7b] arranged in a manner that imparts species-specific nanometer-scale features including pores and grooves. These unique topological properties are difficult to generate with conventional materials fabrication techniques, which renders diatoms as attractive templates for the formation of new nanostructured metallic materials having potentially interesting optical and catalytic properties.

To produce metal-coated diatom frustules, pure diatom cultures (*Synedra* spp. and *Thalassiosira* spp.), grown in either

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freshwater (*Synedra*) or seawater (*Thalassiosira*) silicate-rich growth medium, were first subjected to piranha digestion to remove their organic components (Figure 1). The remaining silica frustules were washed with water and then suspended in ethanol and deposited as a non-overlapping monolayer onto a piranha-cleaned glass substrate. Ti metal (5 nm) and Ag metal (30 nm) were evaporated onto the diatom-coated substrate. Following evaporation, the resulting metal-coated diatoms were isolated by brief sonication in EtOH (Figure 1). SEM images of the 3D products reveal that the silver coating is deposited homogeneously across the diatom surfaces and maintains the morphology and intricate nanometer-scale features of the diatom templates (Figure 2).

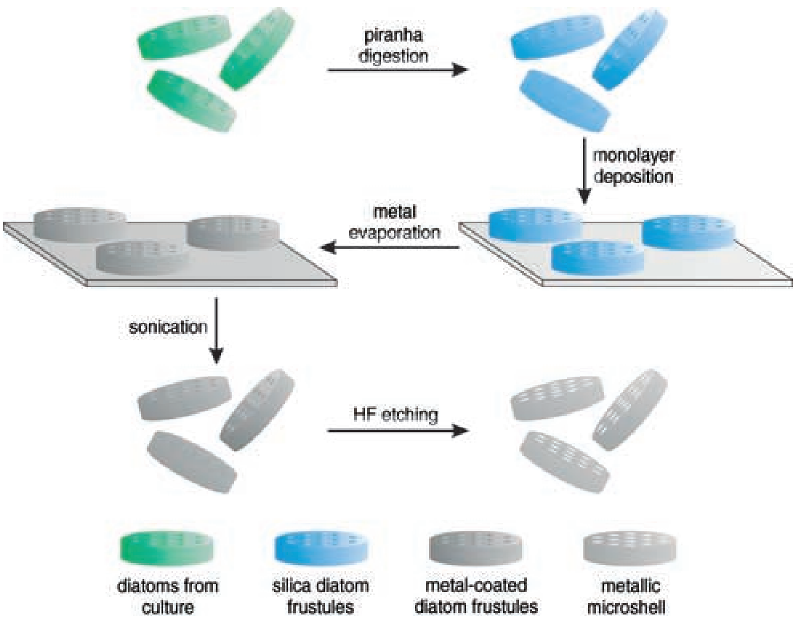

Figure 1. The preparation of metal-coated diatom frustules and the isolation of diatom-templated metallic microshells.

To isolate metallic microshell replicas of the diatoms, the metal-coated diatoms were subjected to HF (1%) etching solutions for 12–15 h which removed the silica shell and the 5-nm Ti layer (Figure 1 and Supporting Information). The resulting metallic materials (Figure 3) are structurally identical to their respective diatom templates in that they retain all of the nanostructural detail present on the diatom surfaces. Moreover, the 30-nm thick silver microshell is robust and remains intact throughout the etching process. This fabrication process is important for a number of reasons. First, thousands of structurally unique diatoms can be chosen as templates,[8] which allows one to choose particular species with topological patterns that best suit their interests. Second, the process is straightforward, high-yielding, easily reproducible, and can be performed with a variety of metals and metal compositions, depending on the desired application. Finally, the resulting 3D free-standing products exhibit nanostructural detail, including regularly patterned sub-200-nm pore sizes (Figure 3b,d). Thus, the diatoms act as sacrificial templates for the formation of 3D nanostructured metallic microshells.

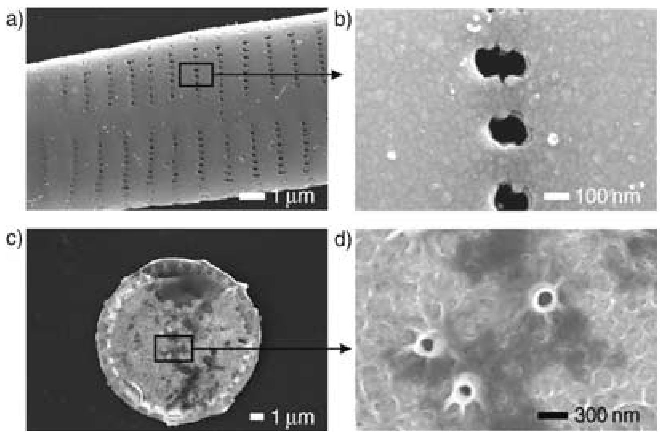

Figure 2. SEM images of the a) and b) *Synedra*, and c) and d) *Thalassiosira* frustules coated with silver (30 nm). The nanometer-scale features of the diatoms are preserved during the coating process.

Both the silver-coated diatoms and silver microshell diatom replicas (Figure 2b,d and Figure 3b,d) display pronounced sub-100-nm topological features. Similar features were not observed upon the evaporation of 30 nm Ag onto a glass microscope cover slip, showing that the fused silica particles that constitute the underlying structure of the diatom frustules are responsible for the observed roughness of the resulting metallic materials. As roughened metal surfaces and silver colloids have proven effective as surface-enhanced Raman scattering (SERS) substrates, we hypothesized that the diatom-templated metallic materials could potentially be used as substrates for the optical detection of analytes by using SERS.[9]

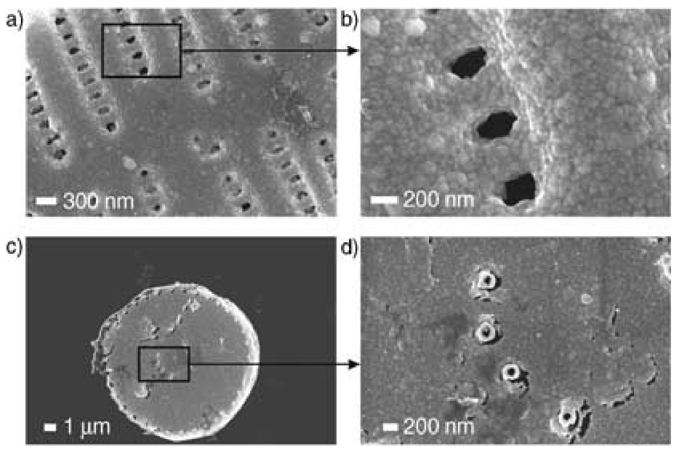

Figure 3. SEM images of silver metallic microshells after HF (1%) etching. The microshells exhibit the nanostructural detail present on the diatom templates.

As a first step in this direction, we began by investigating the SERS activity of the silver-coated diatom frustules. After soaking for four hours in aqueous solutions of rhodamine 6G (R6G) of varying concentration (1 mM, 1 μM, 100 nM), the diatom-templated materials were dispersed onto a piranha-cleaned glass slide and the dye solution was left to evaporate. SERS spectra were collected with a He/Ne laser (633 nm) excitation source with a power of 30 mW. For both *Synedra* and *Thalassiosira*, only the highest R6G concentration (1 mM) resulted in detectable SERS signal (Figure 4). Assuming 100 % coverage of the dye on the silver-coated diatom substrate, the enhancement factor was calculated to be approximately 4×10^5 for the *Synedra* and 2×10^5 for the *Thalassiosira*.[10]

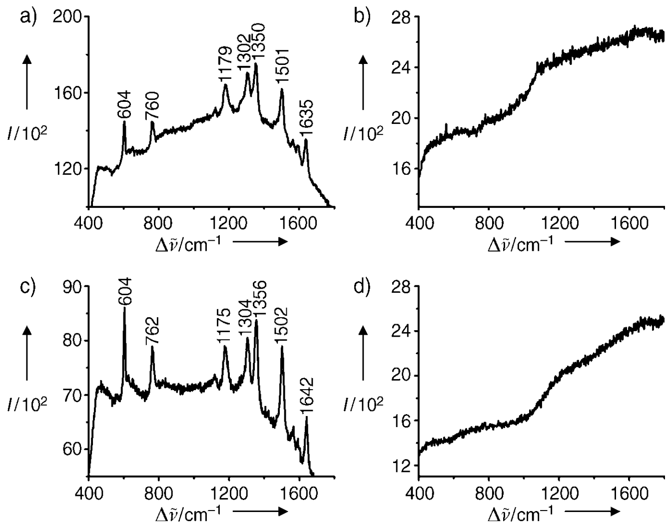

Figure 4. SERS spectra of a) 1-mM R6G and b) 1-μM R6G dye concentrations adsorbed on the silver-coated *Synedra* diatom frustules; c) and d) SERS spectra for 1-mM and 1-μM R6G, respectively, on silver-coated *Thalassiosira* frustules.

The SERS signal intensity for the 1-mM solution increased dramatically in identical SERS experiments that were performed on the isolated silver microshells, and significant signal was recorded for both the 1-μM and 100-nM dye solutions (Figure 5). In this case, the enhancement factor was calculated to be 1×10^6 for the *Synedra* and 7×10^5 for *Thalassiosira*. The striking increase in SERS intensity upon template removal can be partially attributed to an increase in the exposed metal surface area onto which the Raman dye can associate, but this does not entirely explain the observed change. It is possible that HF treatment could also impact the SERS signal, as others have reported that mineral acids and anions can result in chemical enhancement of a SERS substrate.[11]

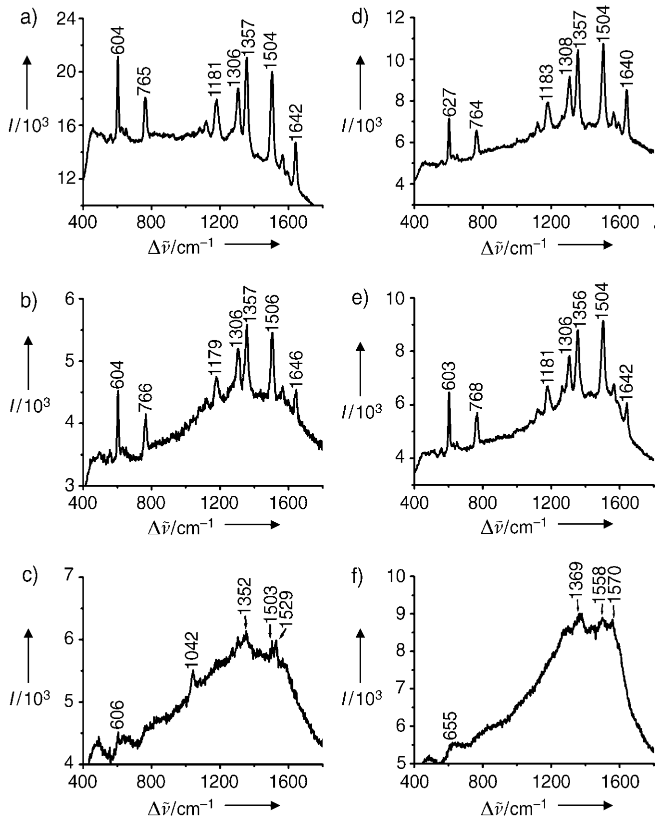

Figure 5. SERS spectra of a) 1-mM R6G, b) 1-μM R6G, and c) 100-nM R6G dye concentrations adsorbed on the silver microshell replicas of the *Synedra* frustules; d), e), and f) SERS spectra for 1-mM, 1-μM, and 100-nM R6G, respectively, on the silver microshell replicas of the *Thalassiosira* frustules.

In conclusion, we report a straightforward fabrication method that employs diatoms as templates for the formation of 3D microscale metallic materials that exhibit elaborately detailed and patterned nanometer-scale features. The procedure is general and can be used with a variety of metals and any diatom species to produce a library of structurally unique materials. An important aspect of this work involves the selection of microorganism templates based on specific structural features that may benefit targeted applications. In this regard, we have shown that the nanostructured surfaces of diatom-templated metallic materials, which are inherent to the chosen diatom template, render them useful as SERS substrates.

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