BIOLOGICALLY ENABLED MICRO- AND NANOSTENCIL LITHOGRAPHY USING DIATOMS

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ABSTRACT

Diatoms are incredibly diverse, photosynthetic microalgae, which are widely distributed in aquatic environments. The wide array and intricate morphology of the micro- and nanostructured silica exoskeletons encasing the single-celled diatoms offer a tremendous opportunity for biologically-assisted fabrication approaches. Herein, *Coscinodiscus sp.* diatom frustules were aligned on a silicon substrate and subsequently employed as shadow masks during the electron beam deposition of gold. This diatom frustule enabled stencil lithography approach yielded the capacity to precisely pattern gold structures on the silicon substrate with a large range of feature sizes, ranging down to the nanoscale.

INTRODUCTION

Diatoms are microscopic, unicellular photosynthetic algae, which are ubiquitous in aquatic environments, with approximately 100,000 different species ranging from 1 μm to 4 mm in size [1]. The silica exoskeletons of diatoms, termed frustules, feature thousands of distinct morphologies and possess an incredibly wide range of intricate micro- and nanoscale patterns. The unique morphologies of these biologically evolved exoskeletons have been evolutionarily optimized for a variety of functions including photosynthesis, gas and nutrient exchange, as well as offering mechanical protection. Given their highly organized morphology, diatoms represent promising candidates for developing biologically-assisted fabrication approaches [2]. To date, using various molding and templating approaches, the capacity to replicate the intricate morphologies of the diatom frustule has been reported, thus demonstrating the potential of these biological templates to serve as the foundation for next generation, biologically enabled micro- and nanofabrication strategies. For example, the nanoporous morphology of the diatom frustules has been employed to yield nanotextured substrates composed of metal as well as polydimethylsiloxane (PDMS) [3]. Beyond the capacity to generate nanotextured surfaces, however, the use diatoms to create micro- and nanoscale patterns of various materials, such as metals or polymers, on a variety of underlying substrates has not been demonstrated. This capacity to create patterns of materials, which mirror the intricate morphology of the diatom frustule has the potential to facilitate a variety of practical applications and will be specifically addressed in this work. An outstanding limitation of the majority of the prior efforts which employed diatoms in biologically enabled fabrication strategies is the fact that they have focused on either collections of randomly distributed diatoms or single diatoms. However, with the exception of a previously reported method using inkjet printing of diatom frustules, there has been little progress in the development of fabrication strategies which employ uniformly arrayed diatom frustules, a critical step towards the realization of scalable diatom-based micro-/nanomanufacturing and another important focus of this work [4]. In this work, we report the development of a versatile, diatom-enabled fabrication approach with the capacity to generate precisely patterned gold micro- and nanostructures, which mirror the highly-organized, porous frustule morphology on underlying silicon substrates. Specifically, *Coscinodiscus sp.* diatom frustules

were uniformly aligned on silicon substrates and employed as shadow masks in a stencil lithography approach during the electron beam deposition of gold (Au). This novel, biologically-enabled stencil lithography approach yielded the capacity to precisely pattern gold structures on the silicon substrate over large areas with a range of feature sizes, ranging down to the nanoscale.

METHODS

Initial steps in the development of the diatom frustule-based approach included the purification and separation of the frustules. To this end, the diatom *Coscinodiscus sp.* (CCMP 1583) was purchased from the National Center for Marine Algae and Microbiota (NCMA) at the Bigelow Laboratory for Ocean Sciences. Initially, the *Coscinodiscus sp.* diatoms were harvested from 2L of culture media using a sieve, a process which yielded an approximately 10 mL solution of highly concentrated diatoms. This concentrated diatom solution was subsequently gradually poured into a beaker containing 100 mL of concentrated sulfuric acid (98% H_2SO_4)-based solution. Next, this pickling solution was heated in a water bath at 65 $^\circ\text{C}$ for 40 min, thereby ensuring the complete removal of the entirety of the organic contents of diatoms. After cooling this solution, the diatom exoskeletons were filtered with a 40 μm mesh size cell strainer and subsequently continuously flushed for five minutes with deionized (DI) water.

Figure 1: SEM image demonstrates diatom valves and girdle bands following purification steps and prior to separation of the two components using a combination of filtering/settling.

In order to further separate the diatom frustule components (Figure 1), the thus cleaned frustule valves and girdle bands were again dispersed in DI water and filtered with another 70 μm mesh size cell strainer. Next, leveraging the difference in settling rates of the two components of the diatom frustules (diatom valves and girdle bands), a series of settling processes in DI water were used to separate the diatom valves from the girdle bands [5]. In some

cases, in order to eliminate nanopore layer along the outer surface of the diatom valves, the diatom frustules were further treated with 0.1% HF solution for 1 min, followed by filtering with a 40 μm mesh size cell strainer and flushed thoroughly with DI water.

Figure 2: SEM image demonstrates a single *Coscinodiscus* sp. diatom valve in the 'concave-up' orientation and reveals an intricate, multi-layered pore structure.

Figure 3: Magnification of Figure 2 demonstrates the sub-micron and nanopore structure of the *Coscinodiscus* sp. diatom valve.

Following the cleaning and separation of the diatom frustule components, the assembly of a diatom frustule monolayer was undertaken. Firstly, a 20 mm x 20 mm silicon wafer was immersed in 10% HF solution for 10 min to eliminate the native oxide layer, after which it was flushed for one minute with DI water. Next, approximately 500 μl of the condensed diatom frustule solution, containing ~ 2000 frustules/ml, was pipetted onto the as-prepared silicon wafer, after which the wafer was placed on a heating plate at 130°C to accelerate the water evaporation. In this case, the

diatom-containing solution was heated to the water boiling point in approximately 1 min, yielding the continuous generation of bubbles along the substrate surface and compact monolayers of diatom frustules on the underlying silicon substrate upon evaporation of the water. Next, thin gold films (10 nm to 20nm in thickness) were deposited at a rate of 0.5 $\text{\AA}/\text{s}$ on the diatom monolayers in an electron beam evaporator (Solution, CHA Industries, CA). Following the gold deposition, the diatom frustules were completely removed from the underlying silicon substrate by etching for five minutes using a 1% HF solution. After HF etching and flushing the substrate with DI water, only the patterned gold array persisted on the silicon substrate.

Finally, in order to characterize the deposited gold patterns, a thin gold-platinum layer (approximately 5 nm in thickness) was deposited on dried frustules, after which SEM (Supra 55VP FESEM from Carl Zeiss Microscopy, NY, USA) was employed to image the morphology of diatom frustules.

RESULTS

Of fundamental importance to the approach developed herein were the initial diatom sorting steps, which yield highly monodisperse diatom valves for subsequent alignment upon an underlying substrate. Herein, this was accomplished with a combination of double-filtering to yield highly monodisperse diatom components followed by a series of settling processes to efficiently separate the diatom valves from the girdle bands. The detailed morphology of the unique multi-layered porous structure of the diatom valves is illustrated in Figures 2 and 3.

Figure 4: SEM image demonstrates assembled diatom valve monolayer. Note preferred 'concave-up' orientation of the assembled valves.

Following the isolation of high-purity, cleaned diatom valves, the abovementioned assembly process was employed to yield large-area, close-packed diatom monolayers, illustrated in Figure 4. As noted above, the diatom containing solution was quickly heated to evaporate the DI water, yielding the presence of bubbles along the silicon substrate surface. It is hypothesized that these bubbles yielded an efficient assembly of the diatom frustules in the optimal 'concave-up' orientation due to the perturbation of the frustules, causing them to orient in this more stable 'concave-up' orientation along the underlying silicon substrate. This hypothesis is supported by the fact that condensed diatom frustule solutions

pipetted onto silicon and allowed to dry at room temperature resulted in assemblies in which the ‘concave-up’ and ‘concave-down’ orientations were represented in relatively equal proportions. In addition to resulting in an optimal ‘concave-up’ orientation, the rapid evaporation of the frustule-containing water droplets was found to drive the diatom frustules to form a compact monolayer on the silicon substrates. Using the approach herein, we achieved monolayers of diatoms of up to approximately 4 mm².

Figure 5: SEM images demonstrate gold nanopatterns following dissolution of the diatom frustules reflecting the nanohole pattern of the diatom valves.

The diatom monolayers overlying the silicon substrates were subsequently employed as shadow masks in a stencil lithography process during which gold thin films were deposited using electron beam evaporation. Figure 5 illustrates the appearance of the silicon substrate following diatom dissolution. In this case, the gold nanopatterns demonstrate feature sizes on the order of 20-40 nm, mirroring both the size and pattern of the nanopores of the diatom frustule valves. Of note, using thin gold films on the order of 10-20 nm, as were employed herein, the distinct nanoscale features reflecting the sizes of the nanopores were readily reproduced. In addition to the gold nanopatterns, the diatom valves, which were modified by removal of the nanopore layer prior to assembly, were employed to yield gold patterns with larger, sub-micron feature sizes. In this case, following valve assembly and gold deposition, patterns with feature sizes on the order of 200 nm were achieved, illustrated in Figure 6, reflecting both the size and periodicity of the second layer of the diatom valve holes.

DISCUSSION

The diatom-enabled approach reported herein, which was specifically employed to fabricate precisely patterned gold structures reflecting the micro- and nanoscale features of the diatom frustule, may find utility in a range of photonics applications. For example, the reported diatom-enabled stencil

lithography approach, given its capacity to pattern a landscape of rationally designed gold micro- and nanostructured features, has the potential to be uniquely suited towards the scalable fabrication of SERS substrates. Beyond photonics applications, the capability to fabricate a precisely patterned landscape of micro- and nanoscale features of various materials using a diatom enabled stencil lithography technique potentially enables a wide array of applications, from photovoltaic devices to a host of biomedical technologies.

Figure 6: SEM images demonstrate sub-micron scale gold patterns reflecting the sub-micron pore layer of the diatom valve following initial dissolution of the nanopore layer prior to gold deposition.

Finally, it is instructive to consider the potential advantages of the biologically enabled approach reported in this work in comparison to alternative approaches to large area nanopatterning. While enabling the requisite precision to create nanopatterned surfaces, conventional nanolithography techniques such as electron-beam lithography or focused ion beam approaches are fundamentally serial in nature and lack the scalability required for many practical applications. Beyond conventional nanolithography approaches, a host of nanoimprint lithography-based techniques have been developed for diverse applications. However, while imprint lithography approaches yield the requisite precision and their parallel, scalable nature achieves the capacity for increasingly large-area nanopatterning, there remains an inherent need for master molds, which are typically fabricated using costly, serial nanolithographic approaches such as electron-beam lithography. As an alternative, a host of ‘bottom-up’, self-assembly-based methods have been developed. These ‘bottom-up’ methods offer a highly scalable approach to nanomanufacturing but lack a similar degree of control over and variety of the patterned nanoscale features when compared to the aforementioned ‘top-down’ approaches. In comparison to the diverse array of alternative approaches noted above, the diatom enabled stencil lithography approach reported herein mitigates several existing limitations of

these techniques. The proposed diatom-based approach is envisioned to be yield the requisite scalability to enable a diverse array of applications given the capacity to assemble these naturally occurring templates into large-area arrays. In addition to the scalability of diatom enabled lithography given its biological templates, leveraging the staggering variety of nanoscale features, which exist in these naturally occurring organisms yields a large degree of freedom in the achievable nanopatterns.

In summary, this paper reports a novel micro- and nanostencil lithography approach using diatom frustules. Beyond the materials employed herein, this biologically enabled approach may be readily tailored to pattern a variety of materials on various substrates and has the potential to represent a next generation micro- and nanomanufacturing technology which may be optimized towards a diverse array of applications.

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