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Biologically enabled micro- and nanostencil lithography using diatoms

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ABSTRACT

The development of a biologically enabled micro- and nanostencil lithography approach using diatoms is demonstrated. Diatom frustules are initially purified, sorted, and aligned into compact monolayers on underlying silicon substrates. Subsequently, the diatom monolayers are employed as shadow masks during the electron beam deposition of gold (Au) thin films, a process which enables the capacity to mirror the intricate micro- and nanoporous frustule architecture on the underlying silicon substrates. Following Au deposition and diatom frustule dissolution, both sub-micron and nanoscale gold patterns on silicon are realized using this approach. This unique method yields the highly structured patterning of gold and other materials on a variety of substrates, with feature sizes ranging from the sub-micron to the nanoscale, enabling a host of diverse applications.

1. 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, not only have thousands of distinct morphologies but also possess a wide range of intricate micro- and nanoscale patterns. The unique morphologies of these biologically evolved exoskeletons are optimized for a variety of functions including mechanical protection, gas and nutrient exchange, as well as offering mechanical protection [2,3]. Given their highly organized morphology, diatoms represent promising candidates for developing biologically assisted fabrication approaches. A further consideration regarding the potential of these organisms in nanofabrication is the capacity to exert a degree of control over the morphology of the diatom exoskeleton using genetic engineering approaches. Since the initial sequencing of the diatom genome, the door towards rationally designing three-dimensional nanostructures by manipulating the diatom genome has been opened and offers a tremendous potential for biologically-enabled technology [4,5]. To date, the capacity to replicate the diatom morphology using various molding and templating approaches has been reported, thereby establishing the potential of these biological templates to enable novel micro- and nanofabrication strategies [6,7]. In these instances, the nanoporous morphology of the diatom frustules has been employed to yield nanotextured substrates composed of metal as well as polydimethylsiloxane (PDMS) [8–10]. Beyond the capacity to generate nanotextured surfaces, however, the use of diatoms to create micro- and nanoscale patterns of various materials,

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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 these biological templates has the potential to facilitate a variety of practical applications and will be specifically addressed herein [11]. Furthermore, the majority of the prior efforts which employed diatoms in biologically enabled fabrication strategies have focused on either collections of randomly distributed diatoms or single diatoms [8–10]. 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 [12]. In this letter, 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.

2. Experimental

Purification and separation of diatom frustule: The diatom *Coscinodiscus sp.* (CCMP 1583) was purchased from the National Center for Marine Algae and Microbiota (NCMA, formerly CCMP) at the Bigelow Laboratory for Ocean Sciences. The *Coscinodiscus sp.* diatoms were harvested from 2L of culture media using a sieve, yielding approximately of 10 mL of concentrated diatoms. The concentrated diatom solution was gradually poured into a beaker containing 100 mL of concentrated sulfuric acid (98% H_2SO_4)-based pickling solution. Subsequently, this solution was heated in a water bath at 65 °C for 40 min to ensure the complete removal of all the organic contents of diatoms. After the solution was cooled, the diatom exoskeletons were filtered with a cell strainer (40 μm in mesh size, 352 340, BD Falcon) and subsequently continuously flushed with deionized water for 5 min. The cleaned frustule valves and girdle bands were then again dispersed in deionized water and filtered with another cell strainer (70 μm in mesh size, 352 350, BD Falcon) to remove any frustule components exceeding 70 μm in size. Next, a series of settling processes were used to separate the diatom valves from the girdle bands [11]. 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, then filtered with a cell strainer (40 μm) and flushed thoroughly with deionized water.

Assembly and lithography of diatom frustules monolayer: A 20 mm × 20 mm silicon wafer was immersed in 10% HF solution for 10 min to eliminate the silica film, after which it was flushed with deionized water for 1 min and then blown dry with compressed air flow. Approximately 500 μl of a condensed diatom frustule solution (∼2000 frustules/ml) was pipetted onto the as-prepared silicon wafer to form a small pool, after which the wafer was placed on a heating plate at 130 °C to accelerate the water evaporation. The solution was heated to the water boiling point in approximately 1 min, yielding the continuous generation of bubbles along the substrate surface.

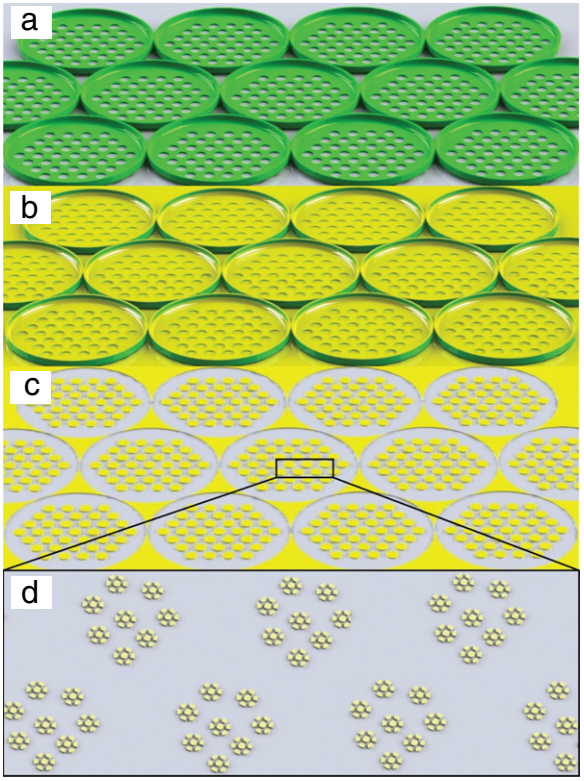

Fig. 1. Illustration of diatom assembly and stencil lithography approach. (a) Assembled monolayer of diatom frustules on the silicon wafer; (b) Deposit gold layer with electron beam evaporation; (c) Removal of diatom frustules with HF solution, yielding deposited gold layer; (d) Magnification of Fig. 1(c) demonstrating a detailed view of the final gold pattern.

Next, thin gold films (10–20 nm in thickness) were deposited at a rate of 0.05 nm/s in an electron beam evaporation deposition machine (Solution, CHA Industries, CA). Subsequently, the diatoms were completely removed by etching using a 1% HF solution for 5 min. After HF etching and flushing the substrate with deionized water, only the patterned gold array persisted on the silicon substrate. Fig. 1 demonstrates the process flow during which gold thin films were patterned upon the diatom monolayers, followed by dissolution of the diatoms, yielding a replication of the intricate porous pattern of the diatom valves.

Characterization of diatom frustules and patterned gold arrays: A thin gold–platinum layer (approximately 5 nm in thickness) was deposited on dried frustules on the silicon wafers, after which SEM (Supra 55VP FESEM from Carl Zeiss Microscopy, NY, USA) was used to investigate the