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Assembling and Patterning of Diatom Frustules onto PDMS Substrates Using Photoassisted Chemical Bonding

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A photoassisted chemical bonding method was used to accurately position diatom frustules into complex microscale patterns on a PDMS surface. The micropores and fine features of the diatom frustules were perfectly preserved because neither pressure exerted on diatoms nor etchant contacted in the whole process. This method has the potential to enable diatom device applications utilizing the functionalized frustules especially for biosensors, biodetection chips, and microfluidic chips.

Diatoms are unicellular photosynthetic algae that possess silica frustules with delicate micro- or nanoscale structures and two-dimensional pore arrays.[1,2] Clean diatom frustules feature large specific surface areas (up to 500 $m^2 g^{-1}$) and photoluminescence.[3] In addition, the biosilica shell can be converted to silicon[4] or titanate,[5] or be used as a template to obtain replication products.[6,7] With its unique properties, diatoms have potential applications in sensors for gas detection,[8] electroluminescent thin film devices,[9] and biosensors.[10–13] One technical difficulty for the above device applications is to accurately assemble the diatoms with microscale SiO_2 structures into defined patterns onto a substrate.

Recently, a multilayer deposition method aided by inkjet printing has been reported to assemble diatom frustules into rectangular arrays onto a glass substrate.[14] Poly(dimethylsiloxane) (PDMS) is another widely used substrate material which can be activated and sealed to SiO_2 substrate.[15,16] Since the diatom frustules are composed of SiO_2, they have potential to be bonded with PDMS substrate.

In this letter, we describe the assembly of diatom frustules into designed patterns onto a PDMS surface. The fabrication process is based on the selective adsorption of the diatom frustules onto a partially activated PDMS surface. The diatom biosilica and PDMS are bonded together after ultraviolet radiation with an intensity of 175 mJ cm^{-2} and simply laid for 48 h.

Figure 1 shows a schematic diagram of the whole process (specific procedures, parameters, and precautions of the patterning method refer to Supporting Information[21]). The PDMS substrate was immersed in *n*-hexane, acetone, and anhydrous alcohol in sequence and followed by ultrasonic cleaning for 15 min to increase surface silanol groups (Si–OH). The PDMS is attached onto a glass substrate to avoid being warped (Step 1 in Figure 1). Then, a photoresist film with a thickness of 2 μm was spin-coated (ca. 2000 rpm) on PDMS substrate (Step 1). After photolithography to form the photoresist patterns (Step 2, photoetching machine: URE-2000, IOE, wavelength: 365 nm), diatom frustules were put on the PDMS surface to fill the patterned pits (Step 3). The diatom frustules used here were purified by H_2SO_4/H_2O_2 and anhydrous alcohol in sequence to

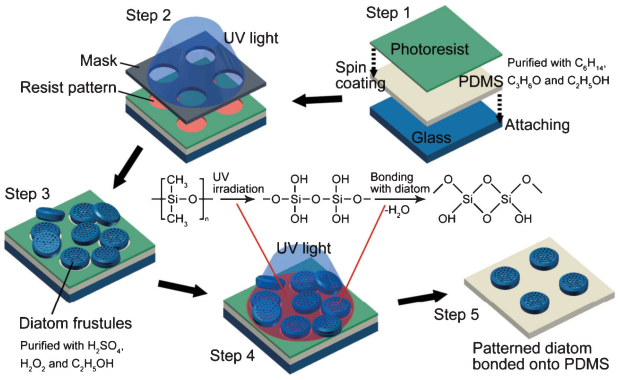

Figure 1. Schematic diagram of the patterning process.

increase surface area and surface silanol groups in advance.[17] Through 3 h of ultraviolet irradiation (UV curing machine: KW-4AC, CHEMAT, wavelength: 254 nm, UV power: 12 μW cm^{-2}), the $–OSi(CH_3)_2O–$ groups of PDMS surface were changed into $–O_4Si(OH)_{4-n}–$, and more Si–OH groups generated.[18,19] The active Si–OH groups on the contact surface of PDMS and diatom may form a –Si–O–Si– crosslinking structure via condensation reaction (Step 4). After 48 h, the photoresist was removed by glue-dispenser, and the patterned frustules were firmly bonded onto the PDMS surface (Step 5).

Three types of diatom frustules were used for experiments. *Coscinodiscus* diatomites were provided by Linjiang Sailite Diatomite Co., Ltd. Cultivated *Navicula* and *Nitzschia* diatoms were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences. Using the fabrication process shown in Figure 1, rectangular arrays of individual *Coscinodiscus* frustules and *Navicula* frustules clusters were formed. Figure 2 shows scanning electron microscopy (SEM) images of the patterned diatom frustules on a PDMS substrate (SEM Machine: CS3400, Camscan; FESEM machine: Product type: Apollo300, Camscan). Since the diameter of arrayed photoresist patterns is 50 μm, which is similar with the averaged diameter of *Coscinodiscus* frustules (40 μm), diatom frustules are adhered to a given pit individually (Figure 2a). Figure 2b indicates that no photoresist or PDMS remained on the diatom surface or covered the micropores. With a rectangularly arrayed circular mask of 80 μm in diameter, clusters of *Navicula* frustules with a length of ca. 15 μm and a width of 12 μm were precisely assembled in circular shaped microarrays as illustrated in Figures 2c and 2d (discussion of diatom size and pattern resolution refer to Supporting Information). Figure 2e shows the fine features of the patterned *Navicula* biosilica at the submicron scale.

We also patterned a small pinnate diatom *Nitzschia* (ca. 10 μm in length and 6 μm in width) to form more complex

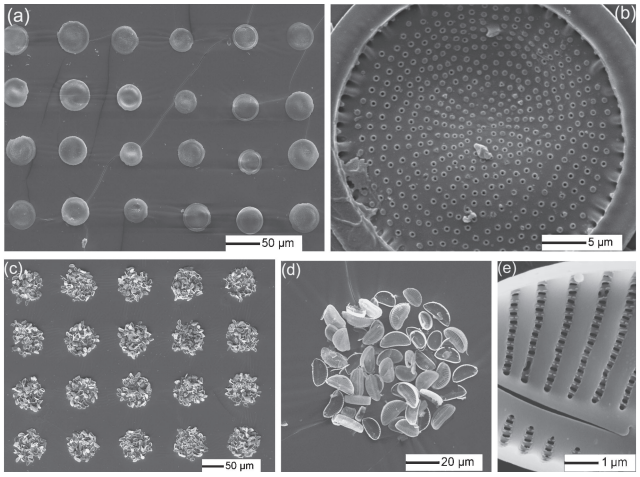

Figure 2. SEM images of diatom frustules in rectangular arrays: (a) 4 × 6 array of single *Coscinodiscus* frustules; (b) pore array of a single *Coscinodiscus* frustules bonded on PDMS; (c) 4 × 5 array of *Navicula* frustules dots, each containing dozens of diatom frustules; (d) single layer of *Navicula* frustules on a single circular dot; (e) fine feature and pore array of *Navicula* frustules bonded on PDMS (FESEM).

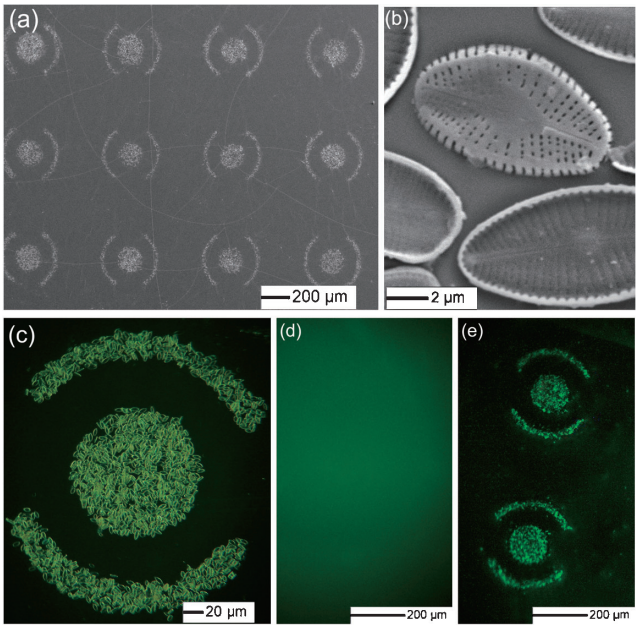

Figure 3. Eye patterns consist of hundreds of *Nitzschia* diatom frustules. (a) 3 × 4 array of eye patterns after peel test; (b) fine features of *Nitzschia* frustules bonded on PDMS; (c) PL image of the patterned frustules; (d) fluorescence-labeled protein solution added onto diatom substrate; (e) eye patterns imaged by fluorescence microscopy after the protein solution evaporated.

patterns using the same method. As shown in Figure 3a, after a three times peel test with sticky tape (product type: 3M 9690, cohesive force $>7.5\,\mathrm{N\,cm^{-2}}$), hundreds of the *Nitzschia* frustules still closely assembled as a monolayer that conformed to the designed shape of the mask. The test result indicates that the bonding force of a single valve of diatom *Nitzschia* may reach 100 μN (test and discussion of bonding force refer to Supporting Information). Figure 3b shows the fine features of the patterned *Nitzschia* biosilica. Figure 3c shows a photoluminescence (PL) image of the patterned frustules under the excitation of high-power mercury lamp, which indicates the frustule kept its original optical characteristics after the patterning and bonding process (same excitation peak of 490 nm as the original frustules, PL images of other diatom patterns, refer to Supporting Information). In order to test the adsorption capacity of the patterned frustules, FITC-labeled protein solution was added onto the surface of PDMS and then dried by heating. As Figures 3d and 3e show, most of the labeled protein self-assembled to the diatom area (details of adsorption experiment refer to Supporting Information), which indicates that the patterned frustules kept their large surface area after the bonding process. After vigorous washing with deionized water, the fluorescence still did not completely vanish. This experiment indicates that the patterned frustules have potential in biodetection to fulfill high-density probe binding (combine with the chemical modify method in reference[20]) or target molecular enrichment.

In conclusion, diatom frustules assembled into designed patterns and bonded onto a PDMS surface through ultraviolet irradiation. The crossbonding characteristic of SiO_2 and PDMS was utilized to accurately position the diatom frustules into complex microscale patterns. The micro- or nanoscale pores and fine features of the diatom frustules were perfectly preserved because neither pressure exerted on diatom nor etchant contacted in the whole process. Moreover, the PL characteristics and large surface areas of diatom frustules were perfectly preserved after the patterning and bonding process. The bonding force between a single diatom valve and PDMS may reach 100 μN via peel test. The UV bonding technique can also be used to handle other SiO_2–PDMS bonding problems especially for powders or micro- or nanoscale particles composed of SiO_2. Furthermore, the method is useful in PDMS microfluidic substrate. After UV irradiation, the diatom can be fixed at the microchannel, and the two PDMS substrates can seal together simultaneously. This method has the potential to enable diatom device applications utilizing the functionalized frustules especially for biosensors, biodetection chips, and microfluidic chips.

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21 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

