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Site-selective dye deposition on microstructures of fused silica fabricated using the LIBWE method

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Using laser-induced backside wet etching (LIBWE) technique, microstructures were fabricated onto the surface of fused silica plates, which were pre-coated with self-assembled monolayers (SAMs). Dye molecules and proteins were alternately deposited onto the laser-irradiated or non-irradiated areas by either chemical bonding or physical adsorption.

Formation of functional micropatterns has recently gained substantial interest because of its extensive applications in the fabrication of bio-microarrays[1] and electronic/optoelectronic devices.[2] Considerable efforts have been devoted to the development of simpler, more effective methods to fabricate micropatterns with finer resolutions (on the micron- or even nanometer-scale) and with desirable surface properties. Among the various methods, self-assembling technique and micro-contact printing (μCP) (soft lithography) have been widely exploited.[3] In general, micropatterned self-assembled monolayers (SAMs) are printed on a substrate using an elastomer poly(dimethylsiloxane) (PDMS) stamp. The terminal groups of these SAMs can provide favorable anchoring points (Br, COOH, OH, NH_2, *etc.*) for further chemical reactions,[4] or for the physical adsorption of functional moieties. Moreover, these terminal groups control the surface wettability, biocompatibility, and resistance to a certain types of molecules.

A novel technique, laser-induced backside wet etching (LIBWE), to fabricate fine microstructures on the surface of transparent materials using a mask projection system has been recently developed.[5] This method allows for the control of the depth of the microstructures, from submicron to several tens of microns. In this communication we attempt to extend the LIBWE technique for novel chemical applications. In conjunction with SAMs, microstructures with controllable surface properties can be produced, and accordingly, the site-selective deposition of functional dye molecules or proteins can be achieved.

As shown in procedures I_1–I_3 (Fig. 1), fused silica plates were pre-coated with a SAM of *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane, $(CH_3O)_3Si(CH_2)_3NH(CH_2)_2NH_2$(AEAPS). As a result, the terminal $–NH_2$ group of AEAPS imparted a hydrophilic character on the surface and also provided a point for further modification. During the subsequent LIBWE process,[6] AEAPS SAMs on the laser-irradiated areas were eliminated, along with the underlying bulk fused silica. If the AEAPS SAM on the non-irradiated area survived the LIBWE process, the alternate SAM modified structures on the fused silica plates could be obtained. To visualize these remaining SAMs on the non-irradiated areas, the substrates were sensitized with dansyl chloride.[7] Dansyl chromophores can be anchored to the substrate through $–NH–SO_2–$ bonding, and they are capable of being observed with a fluorescence microscope. Such a fluorescence microscopic image of 10 μm grating microstructures is exhibited in Fig. 2.[8] The green emission (centered at 506 nm) was attributable to the dansyl chromophores excited with UV light. Relatively long exposure times (~4 s) were required due to the weak fluorescence, which is partially attributable to the reported surface coverage of the dansyl monolayer as approximately 60%.[9] The clear-cut microstructures undoubtedly indicated the ruggedness of the NH_2-terminated SAMs anchored to the glass substrate, and that on the non-irradiated areas SAMs can survive the shockwave, high pressure, and high temperature produced during the LIBWE process.[10] Since the immobilization of the dansyl groups to the AEAPS SAM proved to be successful, it is suggested that other functional dye molecules can also be site-selectively immobilized onto the surface of fused silica using similar procedures.

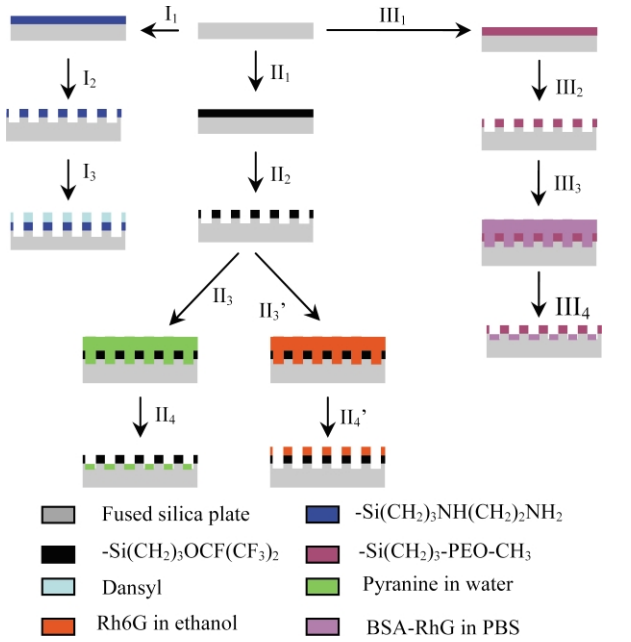

Fig. 1 Schematic illustration of the three procedures to fabricate microstructures, in which dye molecules or protein were site-selectively deposited. I_1 = silanisation with AEAPS, I_2 = LIBWE, I_3 = dye sensitisation with dansyl chloride; II_1 = silanisation with HPFS, II_2 = LIBWE, II_3 = casting using pyranine/H_2O solution, II_3' = casting using Rh6G/ethanol, II_4 and II_4' = solvent evaporation; III_1 = silanisation with PEO-silane, III_2 = LIBWE, III_3 = casting using BSA in PBS solution, III_4 = solvent evaporation.

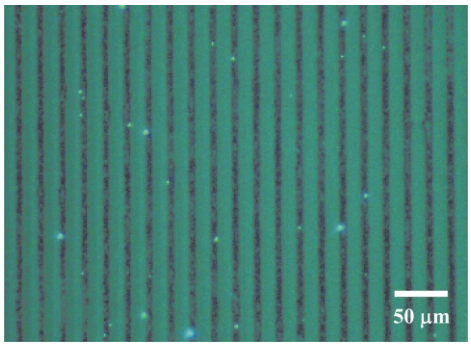

Fig. 2 Fluorescence image of the microstructures on the surface of a fused silica plate sensitized using dansyl chloride and excited by UV-light. The dim areas correspond to the LIBWE-etched channels.

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Based on our observation that the SAMs on the non-irradiated areas can survive the LIBWE process, surface properties can be designed such that the SAMs have higher affinity or resistance to certain types of molecules or solutions. Consequently, dye molecules or even biomolecules can be site-selectively deposited onto the irradiated or non-irradiated areas as required.

As shown in procedures II_1–II_4, II_4' (Fig. 1), the fused silica plates were silanised with 3-(heptafluoroisopropoxy)propyltrimethoxysilane, $(CH_3O)_3Si(CH_2)_3OCF(CF_3)_2$ (HPFS), prior to the LIBWE process. In this case, a reaction time of 16 hours resulted in a closely packed SAM with high surface coverage. The HPFS-modified surface exhibited a contact angle to water of approximately 90°(hydrophobic). After the LIBWE process, the non-irradiated areas remained hydrophobic, whereas the irradiated areas became hydrophilic. XPS analysis of the hydrophilic irradiated area showed, as expected, only a negligible fluorine peak. In this case, the fluorinated and hydrophilic alternate microstructure provides a template for the site-selective deposition of dye molecules upon casting a dilute dye solution.

When an aqueous solution of pyranine (1 μM) was cast, a microscopic fluorescence image of an array of 50 μm × 50 μm square grid was captured, as shown in Fig. 3a. Fluorescence at 512 nm, which originated from the pyranine molecules, was observed on the laser-irradiated area. Since the irradiated areas have higher affinity to the aqueous solution, the pyranine molecules were selectively deposited onto these laser-irradiated areas. Interestingly, switching the dye solution caused a drastic change in the dye deposition selectivity. Upon casting a solution of Rh6G (0.3 μM) in ethanol, fluorescence at 598 nm appeared on the non-irradiated areas, as shown in Fig. 3b. Presumably, the Rh6G solution has a higher affinity to the SAMs than to the hydrophilic fused silica. Furthermore, the fluorescence image remained recognizable, even after the substrate was thoroughly rinsed with ethanol.

In another application, as shown in procedures III_1–III_4,[11] the fused silica plates were pre-coated with the SAM of methoxy(polyethyleneoxy)propyltrimethoxysilane, $(CH_3O)_3Si(CH_2)_3O(CH_2CH_2O)_{4-6}CH_3$ (PEO-silane). Subsequently, bovine serum albumin tetramethylrhodamine conjugate (BSA-RhG) in a phosphate buffer solution (PBS, pH = 7.4) was cast onto the microstructure. As shown in the resulting microscopic fluorescence image (Fig. 3c), the apparent array of the red squares indicated that the BSA molecules were selectively adsorbed onto the irradiated areas. On the non-irradiated areas, protein adsorption was prevented by the PEO chains.[12] Using this methodology, protein microarrays can be readily fabricated with the protein embedded in a deep cavity or channel, and can prove to be valuable in the fabrication of biosensors or microreactors.

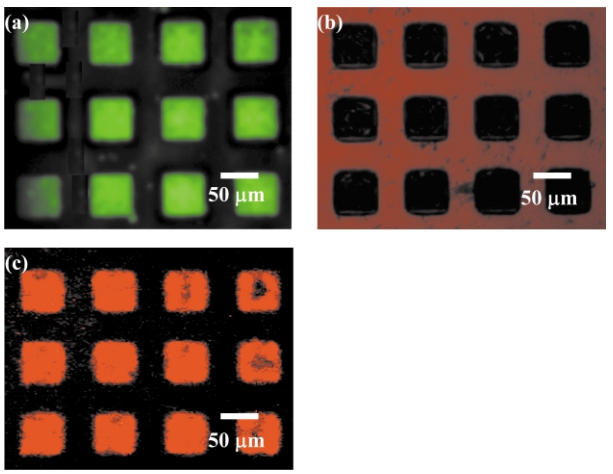

Fig. 3 Fluorescence images of the microstructures on the surface of fused silica plates onto which various dye solutions were cast. The inside of the squares correspond to the LIBWE-etched cavities: (a) an aqueous solution of pyranine was cast, excited by UV-light, (b) Rh6G ethanol solution was cast, excited by green light, (c) BSA-RhG in PBS was cast, excited by green light.

In summary, it is shown that SAMs on non-laser-irradiated areas of fused silica plates can survive the shockwaves, high temperatures, or high pressure during the LIBWE process. The SAMs that survive can act as a template for dye molecule or protein deposition through covalent bonding or physical adsorption. With careful tuning of the terminal group of the SAMs, dye molecules or proteins can be selectively deposited onto the laser-irradiated or non-irradiated areas. In comparison to other micro-structuring approaches, this method offers an array of channels or cavities with controllable depths (from submicron to several tens of microns), and can serve as a promising tool in the fabrication of microarrays, micro-reactors, and biosensors on fused silica or other transparent materials.

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6 Details of the LIBWE process can be found in ref. 5. In this communication, a KrF excimer laser was employed as the light source. The repetition rate of laser irradiation was set at 2 Hz. In a typical process, the pulse number was set as 200 and the laser fluence was 1.0 J cm^{-2}. The etching medium was a saturated solution of pyrene in acetone. Under these conditions, the depth of the etched channels or cavities was estimated to be approximately 2 μm.

7 The sensitization of the $-NH_2$ terminated SAM can be realized by immersing the AEAPS SAM into a solution of dansyl chloride (10 mg) and triethylamine (0.1 mL) in dehydrated acetonitrile (10 mL) for 16 h in a glovebox at a relative humidity below 10%, followed with successive rinses of acetonitrile, acetone, and ethanol.

8 Fluorescence images of microstructures were obtained using an inverted fluorescence microscope (ECLIPSE TE2000-U, Nikon) equipped with a high-pressure Hg-lamp. The pictures were recorded using a CCD camera (Coolpix4500, Nikon).

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