Immobilization of Biomolecules in Nanobottles

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ABSTRACT

A new bioimmobilization technology was developed using nanometer-sized, glass nanobottles made by using polymer nanostructures as template, to capture and contain biomolecules in their free state on surfaces. The polymer nanostructures were formed by film symmetry break-up during microwave irradiation under liquid environment.

Keywords: silica spheres, hollow capsules, solvothermal, MCM-41

1 INTRODUCTION

The immobilization of different biological constructs (e.g., DNAs, mRNAs, proteins, cells and tissues) is critical to many areas of application including commercial bioprocesses for chemical, pharmaceutical and food productions as well as environmental remediation, biosensors for process, environment, safety and health monitoring and diagnosis, and recent biochip technologies that promises rapid drug discovery and advanced diagnostic tools [1-4]. The lack of general technique invariably means that a variety of methods must be tested for each new biological system that has to immobilized and for every new application being sought, making it difficult if not impossible to establish an optimal protocol. This is particularly true for proteins because of their greater chemical and structural complexity and diversity. Proteins also readily lost their bioactivity by denaturation, dehydration and oxidation. One possible solution is to contain and store the suspended biomolecules in their free state using nanobottles. The high cost of biomolecules and recent advances in detection technology motivate the creation of smaller and finer pattern features. High signal fidelity is obtained by increasing the probe density per sites and optimizing the spatial presentation of probe molecules. It is therefore also our aim to create well-defined spatial patterns of immobilized, bioactive molecules of precise quantity in a fast and reliable manner using nanobottles.

A serendipitous discovery made in our laboratory formed the basis of the fabrication of SiO_2 or glass nanobottles. The discovery revealed that polymer films undergo rapid symmetry break-up during microwave irradiation in liquid environment forming regular nano- and microscale features of uniform size. The phenomenon was observed in both thick and thin films and for thermoplastics, block co-polymers, resins and even photoresists. Figure 1a shows a 6 nm thick PMMA film breaks-up into uniform sized hemispherical droplets of 48 nm in diameter and 19 nm in height. The presence of dust particles that reflect microwaves induced ripple-like pattern of droplets as shown in Fig. 1b. Unlike dewetting, the new phenomenon also occurred in thick (ca. 0.5-10 μm) polymer photoresists (cf. Fig. 1c) that are optimized against dewetting. It is possible to guide the symmetry break-up to create self-organizing patterns and hierarchal structures.

2 EXPERIMENTAL

The preparation of SiO_2 nanobottles starts with the deposition of a thin layer of poly(methyl methacrylate) on a clean Si(100) wafer by spin-coating from a PMMA solution (Polysciences, MW_{ave} =25,000). The deposited polymer was outgassed overnight at room temperature and the film thickness measured by ellipsometry (L116C, Gaertner). The exact film thickness could be obtained by adjusting the concentration of the polymer solution and the rotational speed of the spin-coater. A SiO_2 sol was prepared by hydrolysis of tetraethyl orthosilicate (TEOS, 98 %, Aldrich) in an alcohol-water solution followed by acid peptization under reflux at 328 K for 6 h. A 0.25 mole/L SiO_2 in ethanol was prepared and spin-coated on the sample. The polymer core was removed by air calcination. The nanobottles were imaged by scanning electron microscope (SEM, JEOL 6300F) and tapping mode atomic force microscope (Nanoscope IIIα, Digital Instruments). The surface composition was analyzed by X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5600) and Fourier transformed infrared spectroscopy (FTIR, Perkin-Elmer Spectrum GX).

Well defined micropatterns of nanobottles were fabricated using traditional photolithography technique. The fabricated samples were used to immobilized Fluorescein isothiocyanate conjugated bovine serum
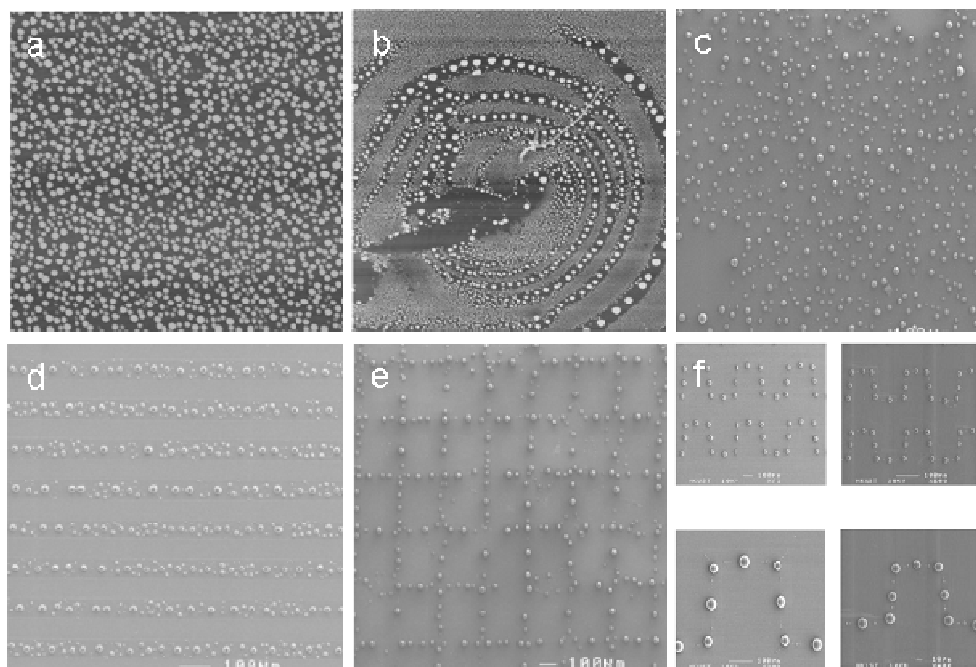
Figure 1: Film symmetry break-up of ultrathin film of (a) PMMA (2 x 2 μm^2) and (b) SBS (20 x 20 μm^2) and (c) one micron thick photoresist HPR1075.(c)-(e) Self-organizing pattern created by guiding the symmetry break-up of polymer photoresist (HPR1075). The mark in each pictures represent 100 μm.

albumin (BSA-FITC, Sigma). The the patterned sample was placed in 1 mg.ml^{-1} aqueous BSA-FITC suspension. A light sonication was used to first remove the air trapped inside the nanobottles. The incubated sample was washed six times with phosphate buffered saline (PBS) solution before rinsing with deionized, distilled water. The sample was then examined under a fluorescent microscope (BX41, Olympus) at an excitation wavelength of 460-490 nm. Pictures were taken at a 10x magnification at an exposure time of 2000 s with the light shutter fully opened.

3 RESULTS AND DISCUSSION

Polymer films undergo large-scale symmetry break-up when irradiated with microwave under hydrothermal condition forming well-defined nanoscale structure. Figure 2a depicts the surface topology of a thin layer (ca. 6 ± 0.2 nm) of poly(methyl methacrylate) spin-coated on a clean silicon wafer. It is apparent from the AFM image that the film has low surface rougness (< 0.2 nm) The PMMA film undergo symmetry break-up and is transformed into hemispherical droplets of uniform size (ca. 60 ± 3 nm) after the microwave treatment as shown in Fig. 2b. The observed phenomenon bears superficial resemblance to film dewetting [5], but differ in a number of important details among them the absence of air interface and the fact that the symmetry break-up was successfully induced even on thick (ca. 0.5-10 μm) polymer resists (cf. Fig. 1c) that are optimized against dewetting. Indeed, both thin PMMA and

thick photoresist films on silicon did not dewet when heated in air above their glass transition temperature. Figure 1d-1f shows that unlike block co-polymers templating and colloidal lithography, it is possible to direct the organization of the polymer into well-defined hierarchal pattern. The technique is easy to implement and does not require the expensive equipment required for processing block co-polymers template and requires shorter processing time (< 1000s) compared to colloidal lithography (> 10000s). It also appears that the technique is chemistry independent and applies to broad selection of polymers.
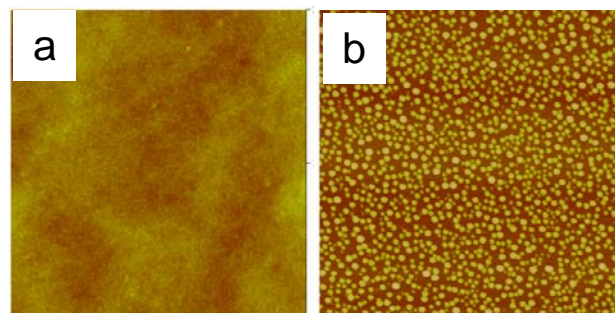

Figure 2: AFM pictures of (a) spin-coated and (b) microwave irradiated PMMA film (2 μm x 2 μm).

A thin uniform SiO$_2$ layer of 21 ± 8 nm was spin-coated onto the polymer nanostructure shown in Fig. 2b. The polymer core was removed by air calcination. The rapid oxidation of the polymer results in an eruption that creates a single crater hole in the thin SiO$_2$ shell as shown in Fig. 3a. The heating condition was adjusted so that each nanobottle has a single opening. The size of the bottle mouth depends on the mass of the polymer core, the thickness of the SiO$_2$ shell and the heating rate. The prepared nanobottles have a uniform bottle mouth of 15 ± 2 nm that was enlarged by selective etching to 58 ± 11 nm as shown in the figure. The calcined SiO$_2$ is amorphous and only the signals from Si and O atoms were detected by Energy dispersive X-ray spectroscopy (EDXS, Oxford Inca Energy TEM200 System) and X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5000). Organic carbons were absent from both XPS and FTIR (Perkin Elmer GX 2000) spectra. The FTIR spectrum displayed characteristic signals belonging to Si-O-Si stretching vibration of SiO$_2$ network at around 1100 cm^{-1}. The storage volume of the nanobottles is dictated by the size of the polymer core.
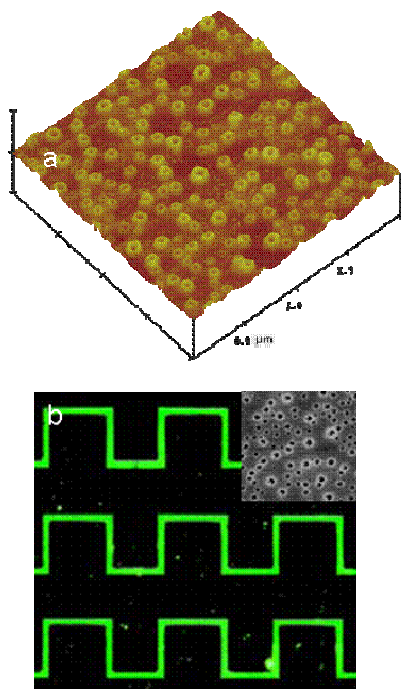

Figure 3: (a) AFM picture of glass (SiO$_2$) nanobottles on Si and (b) BSA-FITC immobilized on square-wave patterns of nano-bottles. Figure inset is an AFM picture of nanobottles with immobilized BSA.

The complex micropattern of nanobottles shown in Fig. 3b was fabricated by traditional photolithography. The picture in Fig. 3b was taken by a fluorescent microscope after the FITC conjugated bovine serum albumin was stored in the nanobottles by the simple incubation procedure. The fluorescent signal from the BSA-FITC proteins is concentrated on the square-wave pattern. No adsorbed protein molecule was detected on the surface of the nanobottles as shown in the figure inset. This demonstrates that large biomolecules such as protein can be stored inside the nanobottles. A separate study showed that enzymes including peroxidase (horseradish peroxidase), lipase (triacylglycerol acylhydrolases, EC 3.1.1.3) and invertase derived from *S. cerevisiae* (EC 3.2.1.26) were successfully stored in the glass nanobottles and were shown to be remarkably active towards different substrates.

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