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Controlled protein adsorption on microfluidic channels with engineered roughness and wettability

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ABSTRACT

Plasma processing is demonstrated as a generic technology not only to fabricate, roughen, and control the wetting properties of microfluidic devices but also to control the protein adsorption in microfluidic channels intended for bio-analysis. After lithography on poly(methyl methacrylate) (PMMA) substrates, deep anisotropic O_2 plasma etching was utilized to pattern microchannels, at conditions where very rough bottom walls were obtained. Where desirable, the rough surfaces were hydrophobized by means of a C_4F_8 plasma deposition step through a stencil mask creating superhydrophobic and hydrophilic stripes. In such microchannels, protein adsorption was controlled by the surface roughness and wettability showing null adsorption on superhydrophobic stripes and greatly enhanced adsorption on rough hydrophilic stripes. Using the biotin–streptavidin system, we demonstrate application of rough microchannels in extremely sensitive detection of proteins with two orders of magnitude improvement compared to flat microchannels. Thus, such control in protein immobilization on the rough walls can be potentially applied in bio-analysis.

1. Introduction

The fabrication of microfluidic devices with feature size in the micrometer range is of great importance in many fields of analytical science, where a small quantity of sample is available, enhanced resolution and sensitivity in separation and detection is needed and increased functional integration is desired [1]. Even though silicon and glass were the structural materials traditionally used for microfluidic devices, the need for easy and low cost fabrication, as well as the wide range of desirable properties (optical, chemical, mechanical and biological) has recently shifted the attention on polymeric materials [1,2].

Microfabrication of polymeric microfluidics is routinely realized through master-based techniques such as hot-embossing, injection molding and microcasting [1]. The first two techniques are mostly appropriate for mass production of microfluidic devices, while the last one is appropriate for rapid prototyping. In the fabrication process of microfluidic devices, it is often desirable to control the surface wettability [3], thus facilitating liquid transport or imparting functionalities such as hydrophobic valving or capillary pumping [4,5]. Recently, deep plasma etching of polymers [6–15], through a lithographically determined or a stencil mask, has been proposed for the fabrication of microfluidic channels in various polymeric substrates (polyimide, polycarbonate, poly(dimethyl siloxane), poly(methyl methacrylate), poly(ether ether ketone), etc.), thus introducing a mold-free fabrication method. Moreover, plasma etching as a standard IC and MEMS technology can be used for both large scale (it is inherently a mass production technique) and also prototype fabrication. Its main advantage over other widespread industrial techniques such as injection molding is that it allows simultaneous to fabrication control of surface wettability, and thus post-processing to control the wall surface energy is prevented, alleviating a serious bottleneck in mass production of microfluidic devices [16]. In most works where plasma has been implemented for the fabrication of microfluidic devices, the main concern was the plasma-induced roughening of the surfaces [8], and efforts were made to optimize the process for minimal or controlled surface roughness [14,17]. At the same time, ordered three-dimensional (3D) structures have attracted the interest of the bio-analytical community, due to their increased surface to volume ratio which can lead in highly sensitive detection in applications related to DNA purification [18], immuno-diagnosis [19], cell cultures [20], etc. In all these applications, the increased surface area has been achieved either by means of patterning of the substrates [18,21] or by the use of inherently 3D materials such as porous silicon [22] or anodic aluminum oxide [19]. However, most of these

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materials and techniques are not appropriate for the fabrication of *purely* polymeric microfluidic devices for bio-analysis.

In our previous work [15], deep plasma etching through a lithographically defined mask has been proposed for the fabrication of microfluidic devices, and the simultaneous control of their wettability. Through intense roughening of channel surfaces, stable superhydrophilic channels have been demonstrated, and they have been implemented for capillary filling and passive valving, in combination with superhydrophobic patches in the microchannels. In addition, we have demonstrated the capacity of plasma-induced *rough* polymeric *open* surfaces to adsorb significant amounts of proteins, compared with the flat (untreated) surfaces [23–25]. To our knowledge, there is no other technique where patterning, surface wettability and protein adsorption control can be achieved simultaneously using a single technique. In the present work, microfluidic channels fabricated on PMMA polymeric substrates by deep etching and roughening in O_2 plasmas are implemented in microfluidic devices intended for bio-analysis. To this end, the mechanical stability of rough structures is evaluated upon immersion in water and the adsorption of proteins is studied as a function of roughness and surface wettability. Extremely sensitive detection of proteins flowing through rough microfluidic channels is demonstrated through a biotin/streptavidin binding assay. The ability to control protein adsorption on microchannels by means of the same technology implemented for the fabrication of the devices, i.e. plasma etching, is demonstrated, to the best of our knowledge, for the first time in the literature and, due to its advantages, it can be envisioned to find potential applications in the rapid and facile fabrication of bio-analytical microfluidic devices of enhanced sensitivity.

2. Materials and methods

2.1. Materials

Optically transparent 2 mm thick PMMA plates were purchased from IRPEN (Spain). Bovine serum albumin (BSA, Cohn fraction V, RIA grade) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Streptavidin labeled with AlexaFluor® 546 (AF546) and goat anti-rabbit IgG antibody labeled with AlexaFluor® 488 (AF488) were purchased from Molecular Probes, Inc. (Eugene, OR, USA). Biotinylated bovine serum albumin (b-BSA) was prepared according to a published method [26].

2.2. Methods of microchannel fabrication and surface characterization

A lithographic process with a thin Si-containing photoresist was implemented to define the etching mask, i.e. the area on the PMMA sheets around where microchannels would be formed by etching. The lithographic process is described in detail elsewhere [15] and depicted schematically in Fig. 1(a). Deep O_2 plasma etching of the polymeric substrates followed the lithography step. Plasma processing was performed in a Micromachining Etching Tool (MET) from Alcatel, equipped with a helicon source (at 13.56 MHz). A second capacitively coupled RF generator gives the ability to independently polarize the sample (situated on the electrode), and thus enhance the ion-bombardment without affecting the ion-flux or the neutral species concentration. The process conditions used in this work were: top (plasma) power 1900 W, pressure 0.75 Pa, gas flow 100 sccm, substrate bias voltage −100 V and substrate temperature −20 °C. Temperature was controlled via the backside cooling of the substrate holder (typically a Si wafer). The polymeric substrates were glued on the substrate holder with thermal paste to ensure good thermal transfer. Etching was performed under anisotropic conditions, i.e. vertical etch rate ≫ horizontal rate, ensuring nearly vertical channel profiles.

To obtain spatially controlled superhydrophilic (contact angle <10°) and superhydrophobic stripes (contact angle >150°, contact angle hysteresis <5°), after O_2 plasma etching, a polymeric stencil mask was used bearing open windows and was laminated on the polymeric substrate bearing the as-etched microchannels so as the open windows of the stencil mask crossed the microchannels at right angle (see Fig. 1(b)). Deposition occurred by employing C_4F_8 gas as Teflon-like film precursor, using an already optimized set of parameters: 900 W, 5.32 Pa, 25 sccm, 0 °C [27]. By such process parameters, a less than 15 nm Teflon-like film was deposited within 15 s, changing the wetting properties of the microchannel walls from superhydrophilic (as etched, and in areas covered by the stencil mask) to superhydrophobic (at the channel area within the opening of the stencil mask window).

For comparison reasons, microchannels with smooth walls were also fabricated by hot embossing on a hydraulic press (Carver model 3850 CE). A Si mold fabricated by lithography and plasma etching was used and pressed (with 30 kg/cm^2) against a PMMA substrate at a temperature of 120 °C. After pressing at 120 °C for 30 min, the press was cooled slowly to room temperature, and the patterned PMMA plate was separated from the Si mold.

The last step was the bonding of the polymeric substrates with lamination films to seal the open PMMA microchannels. Sealing was accomplished through application of adhesive films (3 M Advanced Polyolefin Microplate Sealing Tape 9795 from 3M Co.) by means of a laminator (Mega Photopolymer Laminators) at room temperature.

Roughness characterization was performed by means of a JEOL JSM-7401F FEG SEM scanning electron microscope (samples viewed top-down or at a tilt). The surface wetting properties were evaluated with water contact angle measurements performed on a GBX-DIGIDROP instrument at ambient conditions using 5 μl water drops.

2.3. Protein immobilization on microchannels of controlled wettability

Immobilization of goat anti-rabbit IgG antibody labeled with AlexaFluor® 488 (AF488) in closed PMMA rough microchannels was performed by injecting, immediately after the microchannel fabrication, 100 μg/ml of labeled antibody solution in 50 mM phosphate buffer, pH 7.4, and incubating for 1 h at room temperature (see Fig. 1(b)). Subsequently, the microchannel surfaces were extensively washed 5 times with 50 mM phosphate buffer, pH 7.4, 0.05% (v/v) Tween 20, and distilled water and dried under a N_2 stream. Fluorescence images were acquired with an Axioskop 2 Plus epifluorescence microscope (Carl Zeiss, Germany) facilitated with a Sony Cyber-Shot 8-bit digital camera and processed with the ImagePro Plus software (Media Cybernetics, Inc., USA). All images were taken at exactly the same conditions.

2.4. Protein spotting and detection on rough microchannels

Immobilization of b-BSA on open, aged, PMMA microchannel (contact angle has aged back to that of untreated PMMA, i.e. 65°) surfaces was performed by spotting 12-nl droplets of b-BSA solutions (2–200 μg/ml) in 50 mM phosphate buffer, pH 7.4, using a commercially available microarray spotter (BioOdyssey Calligrapher™ MiniArrayer, Bio-Rad Laboratories), and incubation for 1 h at 65% humidity (see Fig. 1(c)). After thorough washing for 3 times with 50 mM phosphate buffer, the samples were immersed in a 10 mg/ml BSA solution in 50 mM phosphate buffer, pH 7.4 (blocking solution), for 1 h at room temperature, in order to cover the remaining free protein binding sites of the surface. Again the