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Photolithography and plasma processing of polymeric lab on chip for wetting and fouling control and cell patterning

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ABSTRACT

We propose a planar technology for fabrication and surface modification of disposable, polymeric, microfluidic devices, and show applications in cell patterning. By planar technology we mean lithography directly on a polymeric plate followed by plasma etching of the plate. In this context, we developed lithographic processes directly on the polymeric substrate, employing easily strippable photoresists, for which stripping is performed without attacking the polymeric substrate. We then applied oxygen plasma etching to transfer the pattern and chemically modify the polymeric substrates, followed by optional fluorocarbon plasma deposition through stencil masks, producing microfluidic channels with desired geometrical and wetting characteristics. We tested various organic photoresists such as AZ 15nXT, AZ 9260, maP-1275, a silicon containing resist Ormocomp® on an organic strippable underlayer (LOR®), as well as metal masks in order achieve high resolution (1–4 μm), plasma etch resistance, and stripability for photoresists with thickness of ~5–20 μm (for details see Supporting information). We selected the Ormocomp® stack as the best candidate to define microfluidics with plasma etching onto PMMA, PEEK and COP substrates containing hydrophilic and superhydrophobic areas. We demonstrate significantly increased cell attachment on the plasma treated PMMA areas compared to untreated ones, and highly selective cell attachment (on–off) onto hydrophilic versus the superhydrophobic areas using a particular cell line. Such control of cell attachment and growth on plasma nanotextured surfaces can be applied to creation of microdevices aiming to cell patterning, cell isolation, as well as cell arrays.

1. Introduction

The fabrication of polymeric disposable microfluidic devices is of great importance in many fields of bio-analytical science [1]. Traditionally, the fabrication of polymeric disposable microfluidics is done by injection molding or hot embossing on thick polymeric substrates [2,3]. These technologies do not modify the surface of the polymer, and thus surface modification – if required – should follow fabrication by employing some wet or dry (e.g. plasma) chemical treatment.

Alternatively, fabrication of disposable microfluidics may be done using lithography, or lithography and plasma etching. In this case fabrication is based (a) either on lithography of thick photoresists on hard substrates, or (b) lithography of thick photoresists on a polymeric substrate followed by etching of the substrate [2–7]. In the first case (a) the photoresist will form the two walls of the microfluidic, while in the second case (b) it will often be stripped-off leaving a microfluidic engraved in the substrate.

Thus, there is a need to develop both, thick photoresist lithographic processes often on sensitive and/or transparent polymeric substrates, as well as plasma etching processes for such polymeric substrates.

Today, progress has been achieved for case (a) when the photoresist is used as a structural material. The epoxy photoresist SU-8 [8–10] is the most widely used material for microfluidic structure fabrication, but also polyimides (PI) [11], and benzocyclobutene (BCB) have been applied [12,13]. Dry film laminated photoresists have been also used [14,15].

Less work has been done for case (b), where the photoresist is used to transfer the pattern using deep etching of the polymer: here, the following requirements are posed: (1) the photoresist developer as well as the stripper should not attack the polymeric substrate; (2) the photoresist must have (a) enough resolution, (b) good plasma etch resistance, (c) good selectivity with respect to the etched polymer, and (d) must be easily stripped. We note that while use of lamination sealing may allow one to bypass

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requirement 2d, this cannot be bypassed if thermal sealing is done [16].

Deep plasma etching of polymers [7,17–20] has already been proposed for the fabrication of polymeric microfluidics, thus introducing a mold-free method. In this respect, recently, we also have proposed the use of lithography directly on the polymeric substrate followed by plasma etching as a mass production technology for both patterning and surface modification of polymeric substrates [5,7]. Plasma processing allows control of the wetting properties from superhydrophobic to hydrophilic [5]. We have so far demonstrated hydrophilic channels with stable hydrophilicity used for capillary filling [5] and passive superhydrophobic valves [21]. We have also achieved patterning of proteins flowing through nanotextured microfluidics with hydrophilic/superhydrophobic patches [22]. Finally, we have also demonstrated highly intense and sensitive plasma nanotextured microarrays on polymers [39–41]. Here we will present a detailed comparison of various photoresists used for patterning and etching polymeric microfluidics, and we will apply our technology for cell patterning.

Several methods have been developed for cell patterning, or creation of fouling and anti-fouling areas, including microcontact printing [23], photolithography [24,25] and photoactivation of functional groups [26]. Surface properties including wettability and topography have been also documented as critical factors that can affect cell behavior [27,28]. One of the methods used is based on the extreme contrast of wettability. Ishizaki et al. [29] reported that NIH 3T3 fibroblast cells easily adhered on superhydrophilic surfaces, whereas they adhered on superhydrophobic ones only after extensive cultivation time. On the other hand, Senesi et al. [30] showed that nanostructured plasma-deposited, teflon-like superhydrophobic coatings actually increased adhesion, spreading and growth of 3T3 fibroblasts cells. Geyer et al. [31] reported a UV-initiated photografting method for the fabrication of cell arrays on surfaces consisting of superhydrophilic microspots separated by superhydrophobic barriers. Khorasani et al. [32] showed that attachment of BHK fibroblast cells was statistically lower on superhydrophobic PDMS surfaces, treated by CO_2 pulsed laser, compared to untreated ones.

In this work, we elaborate our proposed mass-production amenable plasma technology for fabrication and surface modification of polymeric disposable microfluidic devices. To accomplish this goal, lithographic processes for thick and easily strippable photoresists were developed appropriate for patterning polymeric substrates such as PMMA, PEEK and COP. The rough superhydrophilic microfluidic channels fabricated on polymeric substrates by deep etching in O_2 plasmas are then processed in C_4F_8 plasma through a stainless steel mask to create superhydrophobic patches along the channels. First, increased cell attachment on nanotextured PMMA surfaces is shown. Second, highly selective attachment of cells on the hydrophilic versus the superhydrophobic areas is demonstrated. The ability to control cell attachment on microchannels by means of the same technology implemented for the fabrication of the devices, i.e. plasma processing, opens new frontiers in the rapid and facile fabrication of bio-analytical microfluidic devices.

2. Experimental

AZ 15nXT (negative) [33] and AZ 9260 (positive) [34] photoresists were kindly provided from MicroChemicals (a distributor of Clariant) and maP-1275 (positive) [35] was purchased from Microresist. Inorganic-organic hybrid polymer (Ormocer) [36] and lift off resist (LOR) [37] were purchased from Microresist.

Optically transparent 2 mm thick Poly (methyl methacrylate) (PMMA) plates were purchased from IRPEN (Spain), opaque gray 1.5 mm thick Poly (ether ether ketone) (PEEK) plates from RTP Company (USA), and Cyclo Olefin Polymer (COP) sheets (Zeonor-Film, ZF 14–188 μm) kindly provided by Zeon Corporation.

The lithographic exposures were carried out on a Karl-Suss MJB 3 STD Mask Aligner model using a broadband exposure lamp. The broadband lamp (without filter) intensity was 6.5 mW/cm^2.

All plasma processes were performed in a Micromachining Etching Tool (MET) by Alcatel, equipped with a helicon source (at 13.56 MHz) providing RF power up to 2000 W. 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 variables of this tool are: top (plasma) power (in W), total process pressure (in Pa), gas flow (in sccm), substrate bias voltage (in V) and substrate temperature (in °C). Temperature is controlled via He backside cooling of the substrate holder ensuring heat transfer to the electrode coolant. The sample is secured on the substrate holder with thermal paste. This is necessary since the reactor was designed for silicon wafers and not for polymer pieces. Under the selected etching conditions, etching is anisotropic i.e. the vertical etch rate is much higher than the horizontal, ensuring smooth channel profiles. However, since the source antenna is not electrostatically shielded, sputtering of the alumina dielectric cylinder inside the antenna causes micromasking and roughness formation at the nanoscale, which we have been referring to as nanotexture [38]. As we have shown in the past this feature is quite beneficial for wetting, and fouling control [22,39–41].

Device characterization was performed by means of a JEOL JSM-7401F FEG SEM scanning electron microscope (sample viewed top-down or at a tilt). The etch depth was measured with a stylus profilometer.

In order to evaluate the selective attachment and growth of cells on the hydrophilic areas over the superhydrophobic ones in the same microfluidic the fibrosarcoma cancer cell line HT1080 was used. The cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, and 1% (v/v) penicillin/streptomycin in tissue culture dishes at 37 °C in a water-saturated atmosphere of 5% CO_2 incubator until the cell culture attained 70–80% confluence. Then, the cell cultures were treated with a 0.25% solution of trypsin–EDTA solution in culture medium, to detach the cells from the surface of the dishes and use them for seeding onto the microfluidics.

The PMMA pieces bearing the open (not sealed) microfluidics were sterilized by exposure to ultraviolet light for 20 min, and then seeded with 4×10^5 cells/mL. Cells were cultured as described above for 24 h and then the PMMA channels were washed with 10 mM phosphate buffered saline, pH 7.4 (PBS), in order to remove the non-adhered cells. The attached cells were then fixed by incubating the surfaces with a 4% (w/v) paraformaldehyde solution (PFA) in PBS for 20 min. After fixation, cells were rinsed 3 times with PBS, and their membrane was permeabilized by treatment with a 0.1% (v/v) Triton X-100 solution in PBS, for 5 min. After gentle washing with PBS, the cells were incubated with 5 mg/ml BSA solution in PBS (blocking solution) for 1 h at room temperature. Then, the fixed cells were incubated with a 150 nM Phalloidin Atto 488 solution in PBS for 1 h, to visualize cytoskeleton (F-actin), followed by 3× washing with PBS. Cell nuclei was also stained by incubating the surfaces with a 50 ng/mL 4′,6′-diamidino-2-phenylindol solution (DAPI) in PBS for 5 min. After washing, the microchanells were observed with an epifluorescence microscope (Axioscop 2, Carl Zeiss). Cell adhesion experiments were performed three times in duplicate. In this first report only results on PMMA will be shown for cell patterning.