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Research paper

Fabrication of elastomer pillar arrays with height gradient for cell culture studies

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ABSTRACT

We developed a method to fabricate dense elastomer pillar arrays with height gradient and uniform top surface by using photolithography and soft-lithography techniques. This method relies on formation of sloped tails aside pre-patterned stripes by spin-coating a low-viscosity resist and back side UV exposure through a Cr mask. PDMS pillar arrays were produced by casting to study migration of NIH 3T3 cells. With PDMS pillars of 2 μm diameter, 5.5 μm pitch size and variable heights in the range between 3.8 μm and 10.1 μm, we observed that NIH 3T3 cells migrated toward and then remained in the area of greater stiffness with smaller pillar heights. Thus, PDMS pillar arrays with height gradient and uniform top surface were shown to be useful in cell culture durotaxis studies.

1. Introduction

A wide range of biological processes, such as tissue formation, immune response, wound repair and tumor metastasis, rely on environmental stimuli. More precisely, cells respond to the chemical and physical signals of their neighbors, the extracellular matrix (ECM), the soluble factors, the shear stress, etc. [1–3]. For example, cells are sensitive to the stiffness and the surface morphology of the culture substrate [4,5], showing different organization of cytoskeleton [6,7] and different behaviors of migration [8] and differentiation [9]. However, these studies were limited to the use of homogenous material stiffness (either compositionally or structurally). Hydrogels of variable stiffness have also been used for cell migration studies, showing important effects of stiffness gradient [4,10–12].

To elucidate more clearly the relationship between cell migration and stiffness gradient, we fabricated elastomer micropillar arrays with height gradient but uniform top surface. Previously, micropillars have been used for cell force [13] and cell growth [14,15] analyses. Micropillar arrays with a step height variation have also explored to demonstrate preferential cell localization [7,16,17]. We expected that cells migrate on the elastomer pillar arrays along height gradient so that durotaxis effects can be studied more explicately. We first fabricated a mold with pillars of height gradient on a wavy substrate and replicated twice the mold features by polymethylsiloxane (PDMS) casting. We also cultured NIH 3T3 cells on the fabricated substrates and found that cells migrated toward and then remained in stiffer areas of the pillar substrate.

2. Materials and methods

2.1. Materials

Polydimethylsiloxane (PDMS) (GE RTV 615) was purchased from Eleco (France). SU8 photoresists (SU8-3005 & SU8-3010) and SU8 developer were obtained from CTS (France). DMEM, fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine and fungizone were purchased from Gibco (France). NIH 3T3 cells, fibronectin from bovine plasma, trimethylsilanechloride (TMCS) and all other chemicals without mention were purchased from Sigma-Aldrich (France).

2.2. Fabrication of the PDMS micropillars with height gradient

PDMS micropillars with height gradient were prepared by standard photolithography and soft lithography as shown in Fig. 1. Briefly, dot arrays with diameter 2 μm and period 5.5 μm were patterned on a blank Cr mask pre-coated with photoresist AZ 1518 using a micro-pattern generator (μPG101, Heidelberg, Germany). After development and chrome-etch, holes were obtained (Fig. 1a). SU-8 3010 resist of 15 μm thickness was then spun on the Cr mask (Fig. 1b) and exposed with another mask of absorber stripes of 10 μm line width and 160 μm pitch-size (Fig. 1c). After development (Fig. 1d), SU-8 3005 resist was spun on at 2000 rpm for 30 s and UV exposed to create wavy features (Fig. 1e and Fig. S1).

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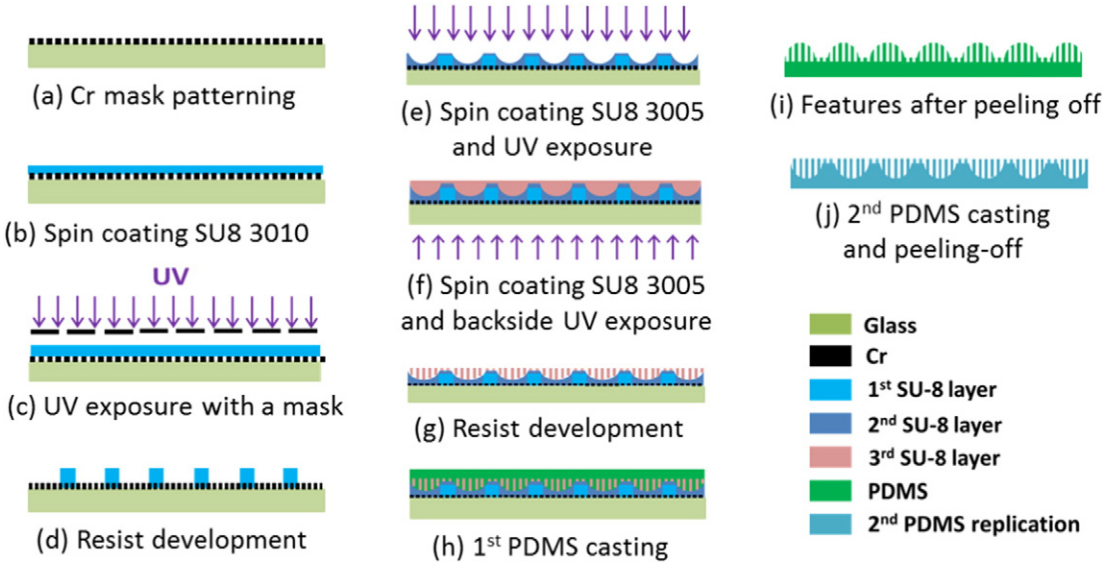

Fig. 1. Schematic diagram of the fabrication process of PDMS pillar arrays with height gradient. Stripes of 10 μm width and 15 μm height were patterned on a pre-patterned Cr mask with dense anti-dot arrays. Then, a thin layer of photoresist was spin-coated, resulting in periodic wavy structures. Afterward, a new resist layer was spin-coated and backside UV exposed. The resulted micropillar arrays with height gradient were finally used as mold for replication. Positive-tone features of the mold could be obtained in PDMS by casting the replica of the mold.

Next, SU-8 3005 was spun on the wavy features at 2000 rpm for 30 s and UV exposed from back side (Fig. 1f), resulted in micropillars of SU8 of height gradient (Fig. 1g). Here, the SU8 pillars have the same top level on a waved ground.

For PDMS casting, the SU8 pattern was first exposed in a TMCS vapor to facilitate the mold release. A mixture of PDMS pre-polymer A and B components was prepared at weight radio 10:1 and degassed in vacuum for 20 min to remove air bubbles. After casting and curing in an

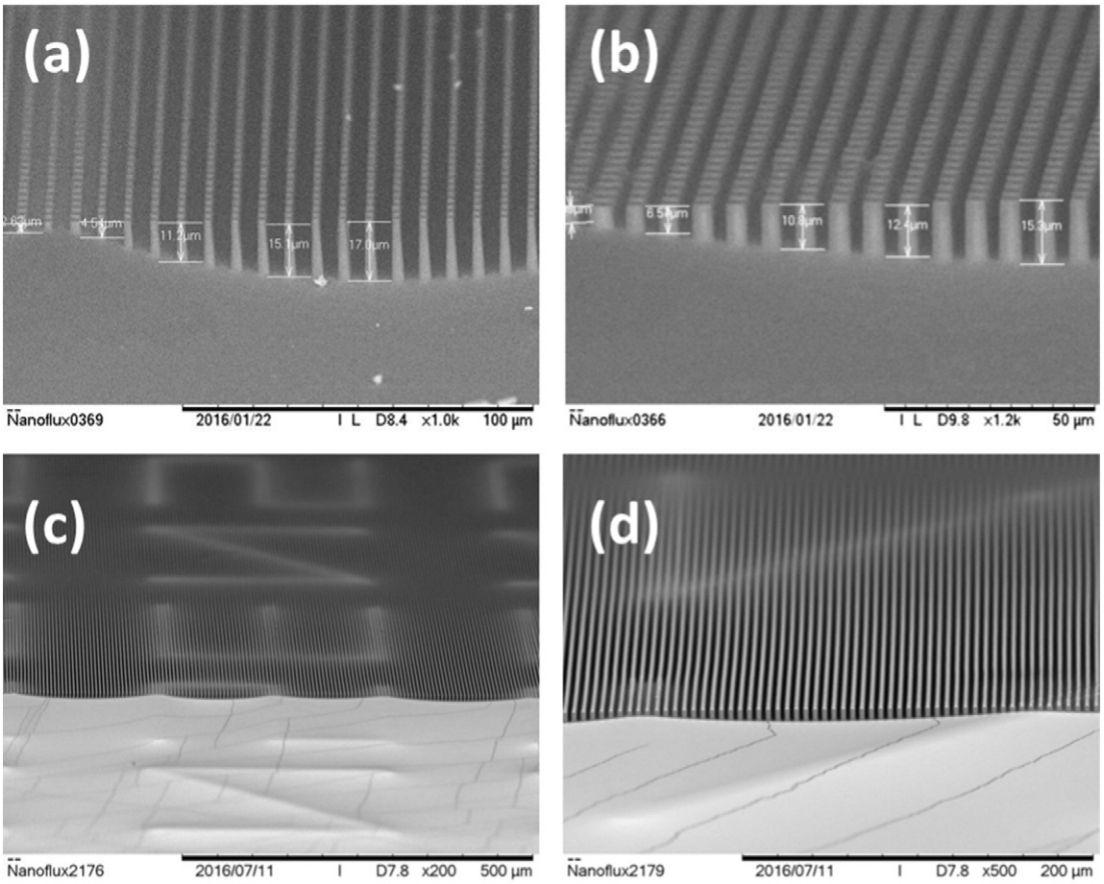

Fig. 2. SEM images of PDMS pillar arrays of different diameters and variable heights but uniform top surface. (a) High aspect ratio PDMS pillar arrays (3 μm average diameter and 17 μm height). (b) Large diameter and small spacing PDMS pillar arrays (5 μm average diameter and 3 μm spacing). (c, d) PDMS pillars of variable heights form letters with uniform top surface.