Microelectronic Engineering 86 (2009) 2334–2341

Contents lists available at ScienceDirect

Microelectronic Engineering

journal homepage: www.elsevier.com/locate/mee

An alternative method for fabricating microcontact printing stamps

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ARTICLE INFO

Article history:
Received 21 November 2008
Accepted 10 April 2009
Available online 19 April 2009

Keywords:
Cell patterning
Microcontact printing
Self-assembled monolayers (SAMs)
Photopatternable silicone

ABSTRACT

In this paper, we describe the development of microcontact printing stamps from photopatternable silicone. The photopatternability of this material enables convenient and fast stamp fabrication, and allows rapid patterning of substrates for culturing biological cells. Microcontact printing stamps made of the photopatternable silicone with linewidths as small as 2 μm were fabricated and reliable cell patterning results were obtained by optimizing the stamping process. An optimal stamp surface was obtained by optimizing the photolithographic process. Our successful demonstration of patterning cells using the photopatternable silicone stamps establishes this alternative approach for fabricating microcontact printing stamps.

1. Introduction

Anchoring cells in predefined patterns on a surface has become very important for the development of cellular biosensor technology, tissue engineering applications, and understanding fundamental cell functions [1–3]. Realization of predefined neural networks *in vitro* is especially important to achieve high-resolution analysis at the electrical, metabolic and structural levels [4,5]. Mammalian neural cells naturally rely on an *in vivo* support network called the **e**xtra**c**ellular **m**atrix (ECM) for survival. Therefore, to pattern these cells in an organized way, their growth can be controlled by patterning the support structure they require. Most tissue engineering applications begin with a specific chemical, controlling where the ECM is affixed, and ultimately where the tissue is able to grow. Alternatively, a chemical which behaves like the ECM can be directly patterned on the substrate, and cell growth can still be controlled.

In order to pattern cells in specific patterns, a common approach is to create cell-attractive regions separated by cell-repulsive regions, so that cells will be bound to the cell-attractive regions without spreading over the adjacent cell-repulsive regions. At present, microcontact printing (μCP) is the most commonly used technique to pattern SAMs or proteins (and thereby cells) on a micrometer scale, and **p**oly**d**i**m**ethyl**s**iloxane (PDMS) is the most frequently used material for microcontact printing [1,6,7].

By printing proteins or self-assembled monolayers (SAMs) on surfaces using PDMS stamps, microcontact printing has become a routine technique for fundamental biological research. For example, PDMS stamps have been used to imprint alkanethiols (forming hydrophobic regions) and thiolated **p**oly**e**thylene **g**lycols (PEGs) (forming protein repulsive regions) on gold, followed by coating with an ECM component (e.g., fibronectin) in the hydrophobic area, which creates non-adhesive regions unsuitable for the cell growth separated by adhesive islands of defined shape and size [8]. Some researchers have also used PDMS stamps to print poly-D-lysine and PEG silane (protein repulsive SAM) directly on a glass surface. Poly-D-lysine is a cell-attractive protein and suitable for the growth of certain cell types, such as hippocampal neurons [9]. There are great advantages for microcontact printing using PDMS stamps, such as low cost and rapid prototyping, however, PDMS stamps have some drawbacks. For instance, PDMS stamps are easily deformed because of their low Young's modulus [10,11].

In this paper, we report the use of a novel material, WL-5351 photopatternable silicone (Dow Corning), to create stamps for microcontact printing. The main motivation for exploring this material is that its photopatternability would enable a simpler fabrication process, without a molding step. Compared with PDMS, this photopatternable silicone has a higher Young's modulus [12]. Its high Young's modulus is expected to reduce the deformability of the stamps. In our previous work, we have formed the photopatternable silicone stamps with a minimum line and spacing resolution of 25 μm [13], which is not fine enough for cell research, where a typical size of mammalian cells suspended in culturing medium is about 10 μm in diameter [14]. We have improved the

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doi:10.1016/j.mee.2009.04.013

resolution of the photopatternable silicone stamps down to 2 μm. We also present here an optimization of the fabrication process for photopatternable silicone stamps based on statistical design of experiments related to the flatness of the contacting surface. Precise cell patterns were thus obtained using stamps made of photopatternable silicone.

2. Materials and methods

The fabrication process for the photopatternable stamps and subsequent patterning of cells on glass slides previously treated by microcontact printing are schematically presented in Fig. 1. First, we fabricated the photopatternable silicone stamps using a photolithographic process. Then, a hydrophobic self-assembled monolayer was printed on a glass substrate using the photopatternable silicone stamp to prevent cell growth. Finally, a hydrophilic SAM suitable for cell growth was coated on the part of the glass surface that was not already hydrophobic, to promote cell growth. Immortalized mouse hypothalamic neurons (GT1-7) were cultured, *in vitro*, on the chip prepared in this fashion. Patterned cells, with or without fluorescent staining, were visualized by an inverted microscope. The details of the individual steps are presented below.

2.1. Photolithographic process for the photopatternable silicone

The photolithographic process for the photopatternable silicone (WL-5351, Dow Corning), which acted as a negative photoresist, was as follows: prior to use, the photopatternable silicone was allowed to equilibrate to room temperature from its storage temperature (−15 °C). The substrate used in this experiment was a 4 inch diameter silicon wafer. First, the silicon wafer was cleaned by a commercial cleaning solution (Nanostrip, Cyantek Inc) for 30 min, then rinsed and dried. Next, the wafer was dehydrated at 150 °C for 30 min in a convection oven. The photopatternable silicone was spin-coated at 500 rpm for 15 s with a ramp rate of 100 rpm/s, and then ramped at 200 rpm/s to a speed of 2500 rpm for 40 s. To reduce edge bead formation, the photopatternable silicone was finally spin-coated at 1500 rpm for 65 s with a decelerating rate of 200 rpm/s. After spin-coating, the film was soft-baked at 110 °C for 4 min on a vacuum hot plate and then exposed to broadband UV with a dose of 1200 mJ/cm^2 using a contact aligner EV620 (EV Group Inc, Albany NY). Following the UV exposure, the film was subjected to a post exposure baking (PEB) at 140 °C for 2.75 min. It is during this step that the UV-irradiated portions of the film undergo a crosslinking process rendering those areas insoluble in the developer [15]. After the photopatternable silicone film was developed in WL-9653 developer (Dow Corning) for 4 min and rinsed in isopropyl alcohol (IPA) for 4 min, the elastomer stamp was hard-baked at 180 °C for 60 min.

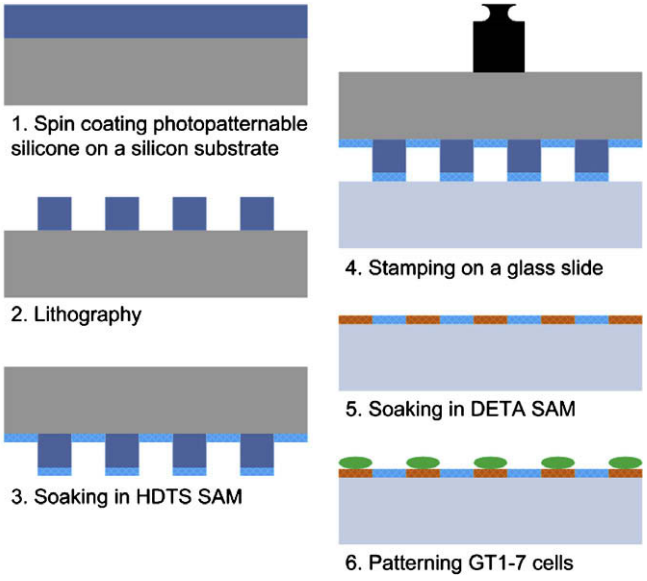

Fig. 1. Schematic of patterning of GT1-7 cells on glass slides by microcontact printing using photopatternable stamps.

2.2. Microcontact printing with photopatternable silicone stamps

The first step after the completion of the stamp fabrication process was to cut photopatternable silicone stamps on a silicon substrate to size (3 cm^2 in area), manually using a diamond scriber. The printing process was as follows: **h**exa**d**ecyl**t**richloro**s**ilane (HDTS), which forms a hydrophobic surface and prevents cells from growing, was inked onto stamps by soaking them in a 7.2 mM solution of HDTS (Gelest Inc., PA) in toluene for 30 s, and then the stamps were blown dry with a stream of nitrogen. The stamps were placed in contact with piranha-cleaned (70% H_2SO_4 and 30% H_2O_2) glass slides. In order to enhance the physical contact of the stamps, a load was applied to this sandwich structure (Fig. 1) for 30 min. For the photopatternable silicone stamps, which have higher Young's modulus than conventionally used polymethyldisiloxane (PDMS) stamps [12], the applied loads tested were 0.67, 1.00, 1.30 and 1.67 MPa. The range of loads was applied in order to determine the minimum load necessary for clear and reproducible patterns. After 30 min, the stamps were carefully removed and the substrate was rinsed with ethanol and deionized water. The substrates were dried with a stream of nitrogen. Finally, a hydrophilic SAM derived from 3-trimethoxysilyl propyl-diethylenetriamine (DETA), a chemical replacement for ECM proteins to facilitate cell adhesion and growth [4], was coated on the regions not already coated with the hydrophobic HDTS SAM by immersing the glass substrate in a 23 mM solution of DETA (Gelest Inc., PA) in methanol for 1 h. The same rinsing and drying steps mentioned above were repeated twice.

2.3. Cell dissociation and culturing

Immortalized mouse hypothalamic neurons (GT1-7) were used to assess the effect of the SAM patterns on the cells' positioning and growth. The GT1-7 cells were maintained in 25 cm^2 flasks (Fisher Scientific, GA) at 37 °C in an incubator with humidified 8% CO_2. The culture medium contained **D**ulbecco's **M**odified **E**agle **M**edium (DMEM) (Gibco, NY), 1 mM sodium pyruvate, 10 mM sodium bicarbonate, 2 mM L-glutamine, 10 mM Hepes buffer and 10% **f**etal **b**ovine **s**erum (FBS, Gibco, NY) [16].

Glass slides patterned with alternating hydrophobic (HDTS) and hydrophilic (DETA) SAM regions were put into six-well cell-culture plates (BD Biosciences, CA). The GT1-7 cells were dissociated by incubating in 0.125% (w/v) trypsin solution at 37 °C for 8 min. Following trypsinization, the cells were pelleted by centrifugation at 750 rpm for 5 min and re-suspended in the culture medium. The cells were plated in the six-well plates at a density of 1×10^5 cells in 5 mL culture medium. The cells were maintained under standard conditions until patterns could be observed (typically 48 h) by optical microscopy.

2.4. Cell imaging by epifluorescent microscopy/phase contrast microscopy

The patterned GT1-7 cells were rinsed with **p**hosphate **b**uffered **s**aline (PBS) solution and placed in serum-free medium (containing