Microelectronic Engineering 141 (2015) 173–177

Contents lists available at ScienceDirect

Microelectronic Engineering

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

SU-8-based nanoporous substrate for migration of neuronal cells

Eunhee Kim [a,b], Seung-Jun Yoo [c], Cheil Moon [c], Bradley J. Nelson [a,b,d], Hongsoo Choi [a,b,*]

[a] DGIST-ETH Microrobot Research Center, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, Republic of Korea
[b] Department of Robotics Engineering, DGIST, Daegu 711-873, Republic of Korea
[c] Department of Brain Science, DGIST, Daegu 711-873, Republic of Korea
[d] Institute of Robotics and Intelligent Systems, ETH Zurich CH-8092, Switzerland

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 5 March 2015
Accepted 13 March 2015
Available online 19 March 2015

Keywords:
Nanosphere lithography (NSL)
Nanoporous surface
SU-8
Cell viability
Cell mobility

ABSTRACT

Most polymer-based biomedical implantable microscale devices have a smooth surface, so that cell seeding is suppressed in the absence of an adhesive material coating on the surface. SU-8 is a negative photoresist, and is widely used for the fabrication of micro-/nanoscale biomedical devices. A physical surface modification technique was introduced in this study to enhance cell viability and mobility on a SU-8 substrate. To characterize cell viability and mobility, four types of SU-8 substrate were prepared: flat bare substrate, poly-L-lysine (PLL)-coated flat bare substrate, nanoporous substrate, and PLL-coated nanoporous substrate. R*at pheochromocytoma* (PC12) cells were cultured on these substrates, and nerve growth factor (NGF) was added to induce differentiation of the PC12 cells. On the seventh day of cell culture, PC12 cells on the nanoporous SU-8 substrate showed 24.3% cell differentiation (neurite outgrowth) versus 1.1% cell differentiation on the flat bare substrate. It was also found that cells had a tendency to move from a flat surface to a nanoporous region. These cellular activities on the nanoporous SU-8 substrate suggest that nanopores can be used to regulate cellular activities and can be applied to SU-8-based microscale biomedical devices.

1. Introduction

In recent decades, many nanofabrication technologies, such as nanoimprint lithography (NIL) [1,2], electron beam lithography (EBL) [3,4], and self-assembly with block copolymers [5–7], have been developed for various purposes, including changing the surface roughness of a substrate. Nanopatterned surfaces are known to affect cell behavior, such as attachment [8,9], migration [10], and differentiation [11–14]. Thus, nanopatterns can be used on the surface of an implantable medical device to control cellular activities for different biomedical applications. Regulation of cellular activities on an implantable biomedical device is an important issue in the biomedical engineering field to control the stability and durability of the implanted device.

Some recent studies have shown that cell activities can be affected by nanopatterned surfaces, with pore sizes varying from 150 to 400 nm [11,15]. Block copolymers can be used to produce various nanopatterns such as holes, pores, and wrinkles on a substrate by simple fabrication methods [7], but the size of the nanostructures is limited to ~10–15 nm. Nanofibrous scaffolds made of biopolymers, such as poly(L-lactic acid) (PLLA) [16] and poly(ε-caprolactone) (PCL) [10], have been used due to the their highly porous nature that is considered desirable to allow cell migration [17]. However, because this biopolymer is biodegradable, it would be difficult to apply to the surface of an implantable micro device that requires a continuous interaction between cells and the device. Nanostructured tissue-culture polystyrene (TCPS) [18] can also be fabricated by NIL and supports the migration of PC12 cells. Using a similar methodology, nanopatterned polyurethane acrylate (PUA) substrates [19] can be fabricated by nanoimprinting. For this stamping method, the substrate should be flat. Thus, the nanoimprinting method may have limitations in application to implantable micro devices that have a three-dimensional structure. SU-8 is an epoxy-type resin that has been used in implantable devices in many studies [20–24]. It is known to be biocompatible when used as an implantable micro-electrode for a brain machine interface (BMI) [21,25]. Accordingly, we chose SU-8 as a substrate to fabricate nanostructures for possible applications on implantable micro-devices, such as microrobots and micro-electrode arrays. It is challenging to have a layer of SU-8 hundreds of nanometers thick that is useful for a biocompatible coating for materials. However, SU-8 was desirable for the purpose of this study because we propose using it as part of implantable micro devices.

* Corresponding author at: Department of Robotics Engineering, DGIST, Daegu 711-873, Republic of Korea. Tel.: +82 53 785 6212; fax: +82 53 785 6209.
E-mail address: mems@dgist.ac.kr (H. Choi).

http://dx.doi.org/10.1016/j.mee.2015.03.016
0167-9317/

In this work, we prepared nanopatterned SU-8 surfaces with polystyrene (PS) nanoparticles by nanosphere lithography (NSL) [26,27] to investigate cellular activities on the nanoporous SU-8 substrate. PC12 cells (a rat pheochromocytoma cell line; ATCC, Manassas, VA, USA) were used to assess cell differentiation and cell migration on the nanopatterned and normal flat substrates. The results were analyzed by several methods: scanning electron microscopy (SEM) for differentiated cells on the nanoporous substrate, atomic force microscopy (AFM) for the terminal part of neurites on the nanoporous substrate, and live confocal laser scanning microscopy for cellular mobility at the interface between the nanoporous region and the flat region. Interestingly, we found that the nanoporous SU-8 substrate had favorable effects on cell differentiation and cell migration compared with the normal flat substrate. Thus, the nanopatterned surface could have positive effects on cell viability for various biomedical applications and could be adapted for implantable biomedical devices.

2. Experimental details

The fabrication method for nanopores on the SU-8 substrate with PS nanoparticles is shown in Fig. 1. A 4-inch glass wafer (Borofloat 33, Schott, Jena, Germany) was cleaned chemically with isopropyl alcohol (IPA; JT Baker, Phillipsburg, NJ, USA) and washed in deionized water. A 40-μm-thick SU-8 (SU-8 2075, MicroChem Corporation, Newton, MA, USA) layer was coated on the glass wafer at 4000 rpm for 45 s and a monolayer of PS nanoparticles (0.3 μm in diameter, 5.0% w/v, Spherotech, Lake Forest, IL, USA) was coated on the SU-8 surface (Fig. 1(a)). PS coating was performed by transferring a PS monolayer, made on the water–air interface, to the SU-8 surface in a water bath [27]. The size of the PS nanoparticles was then reduced to about 240 nm with oxygen plasma using reactive ion etching (RIE, FabStar, Top Technology Ltd., Korea) to obtain ~60-nm spaces between adjacent nanoparticles. A chromium (Cr) layer, as a metal mask for etching SU-8, was then deposited by an electron beam evaporator (SRN-200, Sorona Inc., Korea) on the spaces among the deposited PS nanoparticles (Fig. 1(b)). PS nanoparticles were dissolved in a toluene solution to partially open the SU-8 surface for RIE using the nanohole-patterned Cr film as an etching mask (Fig. 1(c)). Finally, the residual Cr film was removed from the SU-8 surface with a Cr etchant (CR-7, etch rate: 500 Å min^{-1}; Cyantek, Fremont, CA, USA), and the fabrication process result is shown in Fig. 1(d). A field emission scanning electron microscope (FE-SEM, Hitachi S-4800, Japan) was used to observe the surface at each step in the fabrication process (Fig. 2).

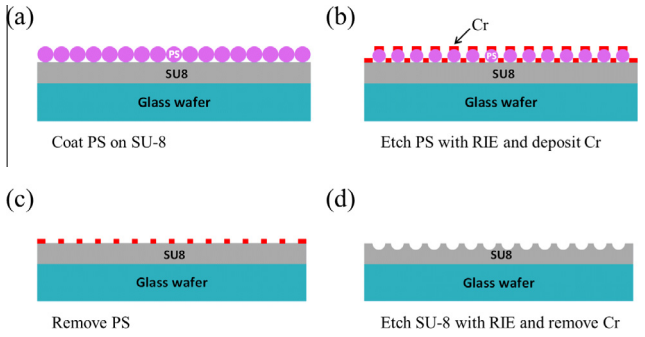

Fig. 1. Fabrication process of the nanoporous SU-8 substrate. (a) A polystyrene monolayer was formed on a SU-8-coated glass wafer, (b) a chromium thin layer was deposited as an etch mask, (c) polystyrene nanoparticles were dissolved in toluene, and (d) dry etching of the SU-8 substrate was performed and the nanoporous SU-8 substrate was the result after removing the chromium layer.

For the analysis of cell differentiation, bare flat and nanoporous SU-8 substrates were selected with or without poly-L-lysine (PLL) coating (Fig. 3). The substrates are referred to as flat bare SU-8 substrate (Bare), PLL coated flat bare SU-8 substrate (PLL), nanoporous SU-8 substrate (NP), and PLL coated nanoporous SU-8 substrate (NP + PLL). For the cell analysis, PC12 cells were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% horse serum (Sigma–Aldrich, St. Louis, MO, USA), 5% fetal bovine serum (FBS; Hyclone, Logan, UT, USA and Thermo Fisher Scientific, Rockford, IL, USA) and 1% antibiotics at 37 °C in 5% CO_2.

For the differentiation analysis, PC12 cells were seeded on the NP substrate (2.5×10^5 cells/mL) and treated with 50 ng/mL nerve growth factor (NGF; BD Biosciences, Bedford, MA, USA) in culture medium for 7 days (Fig. 4). Fresh NGF-supplemented medium was changed every 2 days. Using an optical microscope, three randomly selected substrate regions were observed. Student's *t*-test at a significance level of 95% was used in the analysis.

Live confocal laser scanning microscopy (LSM 7 Live, Carl Zeiss, Planegg, Germany) was used for cell migration analysis (Fig. 5). To observe a wide area of the substrates, nine panoramic photos were obtained at ×100 magnification and 70% zoom out (1.2-fold wider than the original). For the live-cell images, PC12 cells were seeded on a surface (2.5×10^5 cells/mL) that had both nanoporous and flat bare regions. The substrate was not coated with PLL to assess the effects of the bare nanoporous and flat bare regions. The experiment was performed with live cell microscopy, equipped with a chamber to maintain 37 °C and 5% CO_2 conditions. Thus, the PC12 cells were not damaged during the live cell experiment.

The water contact angle with respect to the volume of the water drop (~10 μL) on different SU-8 surfaces was measured using a contact angle measurement system (DSA100, Kruss GmbH, Germany). Detailed information regarding the wettability is provided in Supporting Information and Fig. S1, with mean values and standard error for five samples for each surface.

3. Results and discussion

The surface morphologies of the SU-8 substrate during the fabrication process were investigated by SEM (Fig. 2) for the PS-coated SU-8 substrate (Fig. 2(a)), etched PS on SU-8 substrate (Fig. 2(b)),

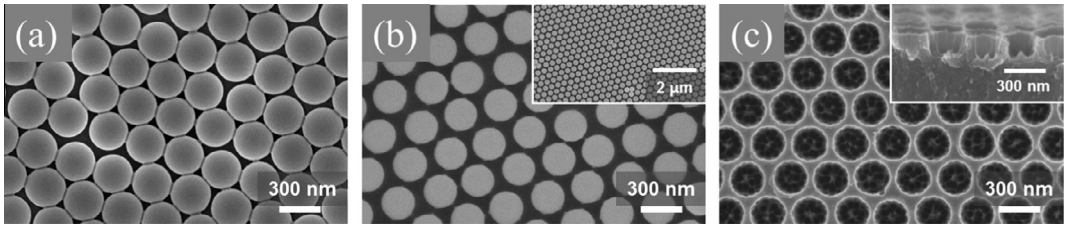

Fig. 2. SEM images during nanoporous SU-8 substrate fabrication. (a) A monolayer of polystyrene nanoparticles on the SU-8 substrate, (b) etched polystyrene nanoparticles on the SU-8 substrate and the inset showing uniformly etched PS nanoparticles over the SU-8 substrate, and (c) fabricated nanopores on the surface of SU-8 and the inset showing the cross-sectional view of the nanoporous SU-8 substrate.