Microelectronic Engineering 124 (2014) 30–36

Contents lists available at ScienceDirect

Microelectronic Engineering

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

Imprint lithography provides topographical nanocues to guide cell growth in primary cortical cell culture

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ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form 28 February 2014
Accepted 9 April 2014
Available online 18 April 2014

Keywords:
Nanoimprinting
Nanostructure
Neuronal cell spreading
Directional guidance

ABSTRACT

In this paper, we describe a technology platform to study the effect of nanocues on the cell growth direction in primary cortical cell culture. Topographical cues to cells are provided using nanoscale features created by Jet and Flash Imprint Lithography, coated with polyethylenimine. We investigated nanoscaffolds with periodicities ranging from 200 nm to 2000 nm, and found that the samples with a period between 400 nm and 600 nm and a height of 118 nm showed highly ordered regions of neurites in a newly formed network with a preferential alignment tendency for astrocytes. Live/dead staining results showed that different materials, such as silicon, glass, and imprinted resist are rendered biocompatible by coating with polyethylenimine. This coating therefore allows for a free choice of scaffold materials and promotes good cell-substrate adhesion. From our results we conclude particular length scales of nanoscaffold can impose a degree of order on cell spreading behavior in a complex cellular brain-on-a-chip network, which could thus be used to emulate ordered brain regions and their function *in vitro*.

1. Introduction

In nature, ordered brain regions are formed during development [1]. Understanding this coordinated growth mechanism will become paramount in the design of brain-on-a-chip disease models of the brain. One of the most important factors for *in vitro* neuronal cell culturing on-chip is to provide a proper environment. The function of the neuronal cells is influenced by the so-called extracellular matrix (ECM) that provides a physical scaffold for the cells to adhere to as well as chemical cues for neuronal cell growth and morphological changes [2]. Researchers have made advances in studying the effect of surface topography on neuronal cells *in vitro* and seek to emulate the properties of the ECM with the help of micro- or nano-fabrication techniques. In these previous works the authors described that the confinement provided by micro scale patterns can induce neuronal cell attachment and direct neurite outgrowth [3–5]. In addition, nanostructures could be utilised as interconnecting spots to study neuronal cell adhesion [6,7]. These approaches provide us with various strategies to mimic the microenvironment of the ECM and study its influence on brain function. However, the aforementioned studies mostly focus on the interaction between substrates and cells of certain cell lines.

In this work, we present an *in vitro* study of primary cells from the cortex (CTX) of new born rats on nanoscaffolds. For the present study, we chose to fabricate the nanoscaffolds by Jet and Flash Imprint Lithography (J-FIL™), a cost-effective, high-throughput nanopatterning method (for details of the various nanoimprint approaches, see, for example, the review by Guo [8]).

It is the ultimate goal of our research to design a device that applies nanomechanical actuation to modulate brain functions. We hypothesise that the nanoscaffolds with the dimensions investigated, here, which can lead to ordered neurite outgrowth may also influence signal transduction in the neurite network via the mechanism of mechanotransduction at the cellular adhesion points, if they can be dynamically adjusted [9]. However, we must first understand the interaction of neuronal cells with specific pattern geometries, and determine which may be suitable to align CTX into ordered networks similar to those found in the naturally developed brain. To realise such a brain-on-a-chip concept, we must also identify suitable cell culture conditions, as well as dimensions and fabrication processes parameters for the nanoscaffold. Ideally, we can determine, and replicate, those properties of the natural ECM which influence directional neuronal cell

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http://dx.doi.org/10.1016/j.mee.2014.04.012
0167-9317/

spreading. In this study, we investigated imprinted nanoscaffold periodicities ranging from 200 nm to 2000 nm. Cell culture results have yet to be obtained for the complete variety of dimensions, including specific ridge-groove ratios, that we have fabricated, but our results so far demonstrate that the culture of CTX on poly-ethlenimine (PEI)-coated nanoscaffolds with length scales between 400 nm and 600 nm show that the nanogrooved surface provides clear directional guidance to neuronal cells in comparison to a flat surface as a control. In this paper we describe the experimental method for generating the nanoscaffold technology platform and demonstrate its potential for advanced brain-on-a-chip studies using cell viability and immunostaining techniques. We also employ scanning electron microscopy (SEM) to investigate the interactions at the sub-cellular scale.

2. Materials and methods

2.1. Nanoscaffolds

Grooved nanoscaffolds were fabricated by Jet and Flash Imprint Lithography (J-FIL^{TM}). Non-Silicon Monomat (Molecular Imprints Inc.) was used as a resist for J-FIL using an Imprio55 machine (Molecular Imprints, Inc.). A commercial quartz stamp (IMS Chips) fabricated by electron beam lithography and reactive ion etching was applied during J-FIL with a set of variations for the ridge-to-groove ratio (R/G) and pattern periods (P) previously designed and manufactured [10]. Table 1 displays the dimensions of the structures in the stamp used in the experiment.

Fig. 2.1(1–4) shows the schematic outline of the J-FIL process. Before imprinting, a layer of 60 nm bottom anti-reflective coating (DUV30J; Brewer Science) was spin-coated on the wafer at 3000 rpm and baked at 120 °C for 120 s to improve the adhesion of the resist. Then, droplets of liquid imprint resist (Non-Silicon Monomat) were dispensed on the pre-coated wafer according to the optimised J-FIL process previously developed for this stamp [10]. From previous work the residual layer thickness is known to be 40 nm, while the imprinted resist height corresponds to approximately 100 nm, matching the height of the stamp. Both 100 mm dia. P-type ⟨100⟩ double-sided polished silicon (Si) (Okmetic) and 100 mm dia. borofloat glass (Schott) wafers were used as substrates for nanoscaffolds. When the stamp was pressed onto the substrate, the liquid resist droplets formed a continuous thin layer and filled the structures of the stamp because of capillary force (Fig. 2.1(2)). After leveling the contact force between the stamp and the substrate, the resist was polymerised by UV exposure while the template was still in contact with the resist film (Fig. 2.1(3)). The stamp was detached once the resist was fully hardened (Fig. 2.1(4)). Each of the processed wafers was imprinted by step and repeat with a centered 2×2 array. Subsequently, the patterned region of the wafer level nanoscaffolds were cut into 9×9 mm pieces to fit a standard 24-well tissue culture plate.

Table 1
Dimension of nanostructures in the quartz stamp.

No.	Ridge width (nm)	Groove width (nm)	Pattern period (nm)	No.	Ridge width (nm)	Groove width (nm)	Pattern period (nm)	No.	Ridge width (nm)	Groove width (nm)	Pattern period (nm)
1	770	230	1000	10	370	230	600	19	120	480	600
2	470	130	600	11	220	380	600	20	570	230	800
3	420	180	600	12	170	430	600	21	400	400	800
4	500	500	1000	13	150	150	300	22	200	200	400
5	100	100	200	14	120	180	300	23	120	280	400
6	180	130	300	15	270	130	400	24	1340	660	2000
7	220	780	1000	16	370	130	500	25	170	580	750
8	270	180	450	17	120	380	500	26	220	580	800
9	170	280	450	18	570	180	750	27	660	1340	2000

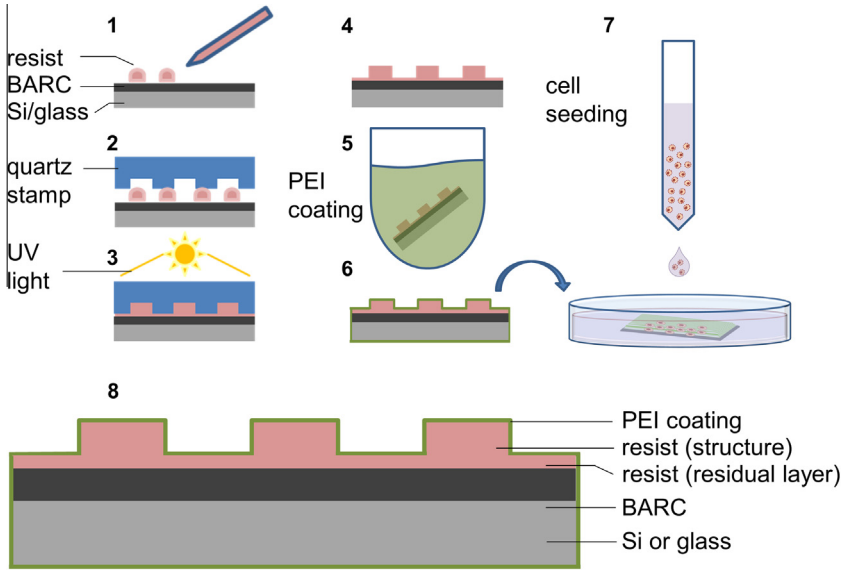

Fig. 2.1. Fabrication scheme and preparation of the nanoscaffold for cell culture. (1) Dispense the resist; (2) resist fills into the structures of the stamp through capillary force; (3) polymerise and harden the resist by UV exposure; (4) detach imprinted nanoscaffolds from stamp; (5) coat imprinted nanoscaffolds in PEI solution; (6) coated nanoscaffolds are ready to use; (7) seed CTX cell onto coated nanoscaffolds and start *in vitro* culturing; (8) profile of coated resist nanoscaffolds in detail.