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Production of micro-patterned substrates to direct human iPSCs-derived neural stem cells orientation and interaction

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Abstract

High-resolution carbon micro-patterns have been produced by SU-8 negative photoresist photolithography process in order to obtain substrates with controlled morphology. Subsequently, substrates were subjected to pyrolysis treatment to obtain glassy carbon structures. iPSCs-derived Neural Stem Cells (NSCs) were cultured on the substrates to analyze the effect of the pattern on their morphology. Preliminary results show that the cells recognized the pattern and started to modify their orientation according to the imposed configuration. Moreover, cells developed cytoskeletal protrusions selectively in relation to the designed features of the substrate. Finally, NSCs seeded on parallel channels tend to communicate through the sidewalls.

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Keywords: Photolythography, pyrolysis, carbonization, patterning, neural stem cells.

1. Introduction

The capability to reprogram patient-derived somatic cells to induced Pluripotent Stem Cells (iPSCs) and direct their differentiation towards Neural Stem Cells (NSCs) has provided a renewable source of expandable patient-specific cells to generate a powerful platform for in vitro tissue engineering, regenerative medicine, disease modelling, and drug toxicity testing [1, 2].

Manipulating patient-derived stem cells fate is a key issue for improving the therapeutic options for neurodegenerative disorders and traumatic brain injuries. It is well known that NSCs require specific cell niches where biochemical signals and biophysical cues act together to regulate their self-renewal, proliferation, or lineage-specific differentiation [3].

The development of engineered micro-environments that provide, in addition to chemical growth factors, also mechanical supports, as a particular topography and surface texture, is crucial to obtain a more realistic in vitro disease-model [4-8].

Artificial conduits have been designed from biodegradable and non-biodegradable materials to be biocompatible, provide a specific guide for neurite growth and promote the transmission of biological signals [9]. In particular, surface topography has been shown to control NSCs spreading and differentiation by modulating intracellular signal transduction and gene expression [10]. The mechanical tension induced by topography stimulates nuclear mechanotransduction via cytoskeleton rearrangements, alterations of the nucleus shape and changes in the expression levels of genes that ultimately affect NSCs phenotype and function [4]. Patterned topographies, considering cell dimensions ranging from submicrometers to tens of micrometers directly influence the orientation of neural stem cells by changing cellular morphology along the patterns.

Several manufacturing techniques for the production of periodic high-resolution micro-structures have been

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developed to obtain substrates with controlled configurations that can modulate cells orientation and crosstalk during in vitro experiments [11]. In particular, defined configurations have been identified as key parameters to support long term maintenance of the undifferentiated phenotype or promote lineage differentiation [12, 13]. Specifically, groove micro-patterned surfaces have been shown to guide the alignment of neurites along the patterns, implicating enhanced neuronal differentiation [14].

Among several possible approaches for such micro-fabrication, lithography and laser micro-machining techniques have been used as stable strategies for fabricating uniform and tunable micro-patterns over large areas. Grooves of different dimensions generated via soft lithography are especially beneficial to promote NSCs differentiation [15]. On the other hand, laser micro-structuring on modified electrode surfaces with parallel grooves has been used to optimize the electrical stimulation of neural cells [16]. Indeed, electrical stimuli have been shown to influence the orientation of neurite outgrowth and promote the migration and differentiation of neural stem cells [17].

In this paper, the manufacturing process of conductive high-resolution structures with comparable dimensions to the cellular unit size is reported. The negative photoresist SU-8 polymer was used to produce different patterns by photolithography on silicon wafers. Later, the substrates were subjected to the pyrolysis treatment to obtain conductive glassy-carbon micro-patterns. The final morphology of the micro-structures was observed and characterized by Scanning Electron Microscopy (SEM) and laser probe profilometer. Through an application-based approach, two types of guidance micro-structures were used to investigate the ability of their configuration to modulate human iPSCs-derived NSCs cells morphology and orientation towards an enhanced neuronal differentiation.

2. Photolithography and pyrolysis

2.1. Photolithography

Two geometric configurations were designed for the photolithography masks that allow a selective cross-link of the polymer and the production of patterned wafers. The masks were designed as shown in Figure 1. Configuration A is characterized by rhombuses with longer (d1) and shorter (d2) diagonal equal to 25 µm and 35 µm, respectively, while the pitch is 25 µm. Configuration B is characterized by lines having a width (w_0) of 35 µm, and a pitch equal to 35 µm (Fig. 1).

The whole pattern of each configuration has a 5x5 mm^2 area.

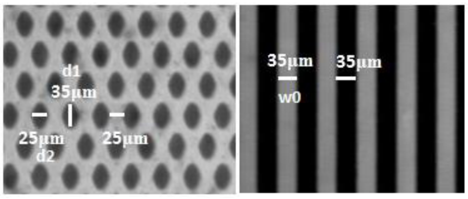

Fig. 1. Photolithography masks of configuration A (left) and B (right).

Silicon wafers without the dioxide layer SiO_2 and the SU-8 3050 negative photoresist were used for the photolithography. The SU-8 was spin-coated at 3000 rpm to obtain a film thickness of 50µm. The wafers were exposed to the UV in hard contact mode to minimize air gaps and the exposure time was set at 22 sec. A ramp of 2°C/min was used for the soft bake and the post-exposure bake to reach the final temperature of 95°C and the wafers were allowed to cool down to room temperature to ensure complete cross-linking and avoid stiction forces. All the parameters for the photolithography process were chosen considering the provided company (Microchem©) datasheets and were optimized to minimize the defects of a possible cross-linking reaction in the gaps between the resist structures.

2.2. Pyrolysis

The wafers were subjected to pyrolysis to obtain glassy-carbon structures from the SU-8 precursor. The pyrolysis process was performed in two steps. The wafers were pre-conditioned at 270 °C for three hours to avoid a thermal shock. Next, the substrates were placed in a furnace and a ramp of 10°C /min was applied for the pyrolysis at 950 °C in inert atmosphere. The stress due to the cross-linking of the polymer is released during the two-step heating process reducing the final stress on the carbon features of the pattern.

The entire fabrication procedure is shown in Figure 2.

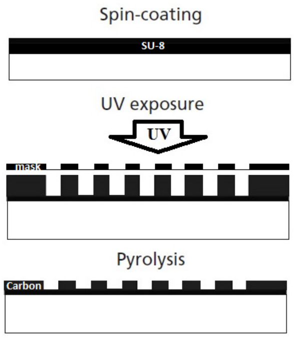

Fig. 2. Schematic illustration of patterns production process: the UV exposure of the 50µm SU-8 3050 base layer is followed by pyrolysis to obtain carbon structures. The reduction of the pattern thickness is due to the heat treatment occurring during pyrolysis.