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Fabrication of 3D Controlled *in vitro* Microenvironments

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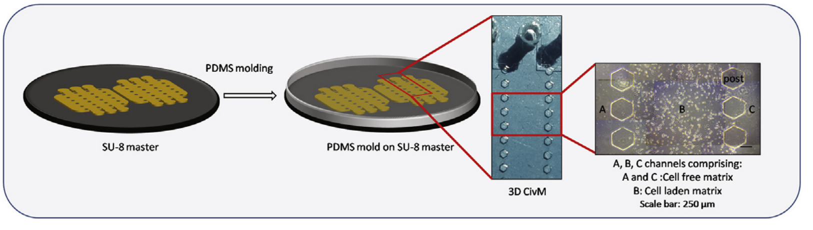
G R A P H I C A L A B S T R A C T



A B S T R A C T

Microfluidics-based lab-on-a-chips have many advantages, one of which is to provide physiologically relevant settings for cell biology experiments. Thus there is an ever increasing interest in their fabrication. Our goal is to construct three dimensional (3D) Controlled *in vitro* Microenvironments (*Civ*Ms) that mimic the *in vivo* microenvironments. Here, we present our optimized fabrication method that works for various lab-on-a-chip designs with a wide range of dimensions. The most crucial points are:

- While using one type of SU-8 photoresist (SU-2075), fine tuning of ramp, dwell time, spin speed, durations of soft bake, UV exposure and development allows fabrication of SU-8 masters with various heights from 40 to 600 µm.
- Molding PDMS (polydimethylsiloxane) at room temperature for at least two days instead of baking at higher temperatures prevents not only tears and bubbles in PDMS stamps but also cracks in the SU-8 master.
- 3D nature of the *Civ*Ms is ensured by keeping the devices inverted during gel polymerization.

A R T I C L E I N F O

Method name: Fabrication of microfluidics based lab on a chips

Keywords: UV lithography, Polydimethylsiloxane, Microfluidic device, Lab-on-a-chip, Microenvironment, Hydrogel, Matrigel

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Microfluidics-based lab-on-a-chips have many advantages [1]: Small volumes down to pL are used. Small volumes provide enhanced safety when dangerous or toxic chemicals or biological agents are used. Precise spatial and temporal control can be achieved. High throughput analysis is facilitated [2]. Fabrication costs are low. The devices are portable. Finally, the devices provide physiologically relevant settings for cell biology experiments [3–8]. Such advantages have resulted in an increased interest in the methodological details of fabrication of lab-on-a-chips [9–11].

Method details

UV lithography

UV lithography (UVL) which is also called photolithography is a parallel writing method for fabrication of 2D and 3D micrometer scale designs using photo-reactive materials, called photoresists [10]. There are two types of photoresists: Positive and negative. Positive photoresist is degraded by exposure to UV light followed by dissolution in a developer while negative photoresist such as SU-8, is cross-linked in the same process. SU-8 is widely used for fabrication of masters that are in turn used for both 2D and 3D structures of interest. SU-8 is an epoxy based negative photoresist. SU-8 is available in different viscosities and is categorized as SU-8 2000 and 3000 series. The higher the viscosity (and the number following 'SU-8'), the higher the thickness of the polymer spun on a surface. We fabricate SU-8 masters with heights between 40 and 600 μm using SU-8 2075. These masters can then be used for PDMS molding. PDMS molds in turn are used for fabricating 3D Controlled *in vitro* Microenvironments (C*iv*Ms). Some of our 3D microfluidic platforms have a set of microfluidic channels separated by an array of posts. Such systems are convenient for studying different hydrogels and cell types in the same device at predefined dimensions while mimicking *in vivo* conditions [2–5].

UV lithography is carried out in a Class 1000 clean room. Special lab overalls suited for clean room use are worn.

First improvement of our method is the ability to generate SU-8 layers with different thicknesses ranging from 40 to 600 microns using only SU-8 2075 through careful optimization of the steps of UV lithography, in particular the spinning step. Thus the users do not need to procure all different kinds of SU-8 in their laboratories.

Materials

Photoresist SU-8 2075 [!**Caution**: Wear protective gloves].
SU-8 developer (Stored at +4 °C)
Si wafer
Acetone
Isopropanol
Dust-free tissue paper
Aluminum foil
Paper towel
Designed mask
Tweezers

Equipment

Hot plate
Mask aligner
Spin coater [!**Caution**: Do not open lid until the spinner comes to a full stop]
Fume hood
Stereoscopic microscope

Spin coating of SU-8

Day 1. First set the hot plate to 65 °C at least half an hour beforehand to ensure uniform heating and place the SU-8 bottle on the bench so that its temperature equilibrates to room temperature.