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**Title:**

**Single step UV-photolithography fabrication of SU-8 honeycombs with microchannels for cells positioning on silicon oxide-based nanopillars**

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**Abstract:** (Your abstract must use **Normal style** and must fit in this box. Your abstract should be no longer than 300 words. The box will 'expand' over 2 pages as you add text/diagrams into it.)

This paper reports a novel technique to realize honeycomb arrangements of cell containers interconnected by microchannels using SU-8, a commonly used epoxy-based negative photoresist. These structures are fabricated using a single UV-photolithography exposure. With optimized process parameters, microchannels of various aspect ratios are produced. The cell containers and microchannels are used for the organization of axonal growth between neurons. Each container is centered on a nanopillar array promoting cell positioning. The proof of concept is given by the successful growth of interconnected PC12 cells.

About 2- $\mu\text{m}$ -high and 500-nm-wide silicon oxide-based nanopillars are produced by deep reactive ion etching using a photoresist mask. They are passivated by a 10-nm-thick silicon nitride layer. The resulting surface is biocompatible. The nanopillar arrays define areas for neurite and cell adhesion, while appropriately enhanced individual nanopillars can later be used for the recording of intracellular potentials. Next, the honeycomb structures are fabricated by a technique making use of a single photolithographic mask, a single SU-8 layer and standard equipment for stepper-based, optical projection lithography. Tuning the focus depth to negative values allows us to focus the reticle in the upper part of the SU-8 film, leading to an UV exposure imbalance between its upper and lower parts. As a consequence, pairs of neighboring SU-8 columns are connected in their upper parts by arches and separated in their lower parts by microchannels with aspect ratios depending on the column design parameters.

Achieved microchannels widths range from 3 to 10  $\mu\text{m}$ , while lengths extend from 5 to 30  $\mu\text{m}$  for an SU-8 resist film thickness of 50  $\mu\text{m}$ . After oxygen plasma treatment, PC12 cells in solution were cultured on the chip during one week. Cells settled on the whole chip and grew neurites through the SU-8 microchannels, thus setting up interconnections between all cell containers.

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