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## Single-step UV-photolithography Fabrication of SU-8 Honeycombs with Microchannels for Cells Positioning on Silicon Oxide-based Nanopillars

F. Larramendy\*, M.C. Blatche\*\*, P. Temple-Boyer\*\* and O. Paul\*



LAAS-CNRS

**IMTEK** 

\*Microsystem Materials Laboratory, Department of Microsystems Engineering (IMTEK), University of Freiburg, Germany \*\*Laboratory for Analysis and Architecture of Systems (LAAS-CNRS), University of Toulouse, France

## **Objectives**

We report on the **fabrication**, **functionalization**, and **testing** of SU-8 microstructures for cell culture and positioning over large areas. The microstructure consists of a **honeycomb** arrangement of **cell containers** interconnected by micro-channels and centered around **nanopillar arrays** designed for promoting cell positioning. The structures are fabricated using a **single ultraviolet photolithography exposure** on the SU-8 resist. The proof of concept is given by the successful growth of **interconnected PC12 cells**.

# Cell pre-positioning on nanopillars by interconnected cell containers

Design 1 and 2 cell container structures were realized around 3 sizes (types A-C) of nanopillar arrys to study the influence of nanopillars on cell growth. PC12 cell were cultured on chips with nanopillars and SU-8 cell containers for 5 days.



## Introduction

Honeycomb structures interconnected by microchannels are fabricated using a single UV-photolithography exposure. With optimized process parameters i.e, focus depth  $d_f$  and exposure dose  $D_{exp}$ , microchannels of various aspect ratios are produced after photoresist dissolution.



**Figure 1:** Principle of focus depth  $d_f$  variation for producing different types of microchannels in negative photoresist; (a,c) focus in the middle of the photoresist of thickness  $t_{PR}$ , (b,d) focus depth shifted downward; darker and clearer shades in photoresist indicate lower and higher exposure doses.





**Figure 5:** SEM of neurons pre-positioned on nanopillar arrays of type B by Design 2 honeycomb arrangement of cell containers, with neurites extending across microchannels



### Honeycomb structure characterization

Choosing  $d_f < 0$  allows us to focus the reticle in the lower part of the SU-8 film. As a consequence, in Fig. 3(b) pairs of neighboring SU-8 columns are connected in their upper parts by arches and separated in their lower parts by microchannels.



SEM of (a) Figure 4: а nanopillar and (b) hexagonal of 91 array nanopillars termed type C. Smaller arrays have 37 (type B) and 7 (type A) nanopillars, as outlined in (b). (c) A neuron on a nanopillar array of type B with neurite growth.

**Figure 6:** Percentages of cells connected to nanopillar arrays of types A to C on silicon substrates without SU-8 structures (darker green bars) and embedded in interconnected honeycomb structures of Designs 1 and 2 (lighter green bar).

#### Conclusions

UV stepper projection lithography was used in a non-standard way to fabricate SU-8 based cell containers arranged in a honeycomb structure and connected by microchannels around nanopillars. Thanks to cell culture, we have proven that the interconnect SU-8 cell containers significantly increase the fraction of cells connected to nanopillars.

### Acknowledgements

**Figure 2:** Measured height (a,d), width (b,e) and length (c,f) of microchannels as a function of (a,b,c)  $D_{exp}$  for  $d_f = -50 \ \mu m$  and (d,e,f) as a function of  $d_f$  for  $D_{exp} = 1100 \ J/m^2$  for Designs 1 (red symbols) and 2 (blue symbols) with  $t_{PR} = 50 \ \mu m$ . **Figure 3:** SEM of cell container honeycombs of Design 2 obtained with 3 different values of  $d_f (d_{f(a)} < d_{f(b)} < d_{f(c)})$ . Technical support by L. Mazenq, A. Laborde and A. Lecestre at LAAS-CNRS (France) and by Prof. S. Takeuchi (IIS, the University of Tokyo, Japan) and his team and financial support by project EUJO-LIMMS (no. 295089) funded by the EU 7th Framework Programme are gratefully acknowledged.

### **Contact & Download**

Florian.larramendy@imtek.uni-freiburg.de temple@laas.fr, paul@imtek.de



