All-in-polymer injection molded device for single cell capture using multilevel silicon master fabrication

S. Tanzi, S. T. Larsen, M. Matteucci and R. Taboryski

*Department of Micro- and Nanotechnology, Technical University of Denmark, DTU Nanotech, Building 345B, DK-2800 Kongens Lyngby – Denmark; simt@nanotech.dtu.dk

ABSTRACT

This work demonstrates a novel all-in-polymer device for single cell capture applicable for biological recordings. The chip is injection molded and comprises a "cornered" (non planar) aperture. It has been demonstrated how cornered apertures are straightforward to mold in PDMS [1,2]. In this study we demonstrate cornered apertures made in a thermoplastic polymer. One of the advantages of cornered apertures is the ease of microscopy under a standard inverted optical microscope, when using transparent materials. After the part is injection molded, the sealing of the chip is performed by thermal bonding to a polymer foil, so the complete device results from only two parts. It differs from similar devices in the novel material and fabrication platform that enables high reproducibility and inexpensive mass production. Optimization of the fabrication scheme has been carried out in order to avoid defects during demolding. Capturing of single PC12 cells has been demonstrated.

Keywords: injection molding, polymer, single cell capture, UV LIGA, dry etching

1 INTRODUCTION

For the lab-on-a-chip systems, polymers are an obvious choice of materials when cost efficiency and implementation for mass production are taken into consideration. Within all the mass replication technologies, injection molding is receiving more and more attention because of its high production efficiency, and potential to fabricate patterns of down to nanometer scale. In our case, we enroll injection molding as final step of a UV LIGA process [3]. The patterns are first transferred into a silicon sample by means of standard UV photolithography and dry etching and then replicated to a metallic shim by using electroplating. This shim is inserted into the molding tool for injection molding polymer replicas that are exact copies of the Si master. The chips are sealed by UV-assisted thermal bonding to a 100 μm thick polymer foil. The requirement for a good mold is having a positively tapered sidewall profile, which will allow an easy demolding. Therefore, it is very important to fabricate templates with a suitable etching profile. Furthermore, mask undercut, which can prevent the demolding process, should be avoided or at least controlled. Within this framework, we demonstrate an all-in-polymer chip for single-cell capture.

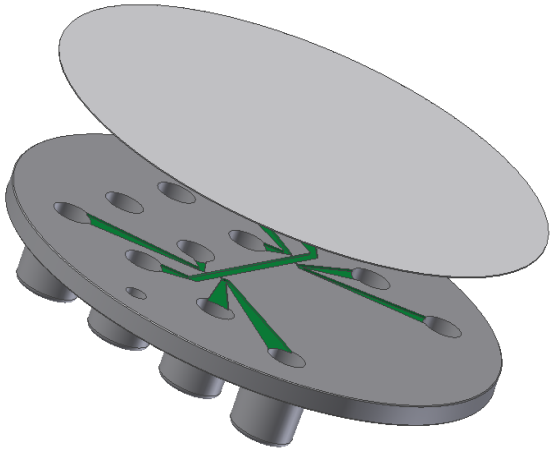

Figure 1: The figure shows a 3D representation of the device concept. The final device results from thermal bonding between an injection molded polymer part and a commercially available foil. The green colour shows the microfluidics open system.

2 CHIP DESIGN AND MOTIVATION

Reported methods of cell capture are mechanical trapping, electric trapping and optical tweezers [4]. Standard mechanical trapping uses fluids to flow through a microchannel and it utilizes either silicon [5] or PDMS [4] pillars to catch cells. Although electric trapping and optical tweezers have been demonstrated, they might alter or damage cells due to high electric field or heat generated by the laser. Therefore, mechanical trapping remains one of the most reliable and non-invasive alternatives. Our device presents a different approach for mechanical capture, where a single cell flowing in a carrier channel can be trapped to a small capturing micro hole by applying suction to it, see Fig. 2. The chip comprises a "cornered" (non planar) aperture based on a microfluidic junction between a large chamber for cell delivery and a lateral capillary for cell

trapping. Zanetti et al. have reported that mammalian cells can be trapped to a micro hole using this kind of apertures in PDMS [1, 2]. In this study we demonstrate that cornered apertures are straightforward to mold in a thermoplastic polymer. One of the advantages of cornered apertures is the ease of microscopy under a standard inverted optical microscope. The chip design combines channels with different heights in a multilevel microfluidic system. Despite such devices have been reported in PDMS, the mold fabrication and the demolding represents a challenge when injection molding is used for fabrication. The main challenge comes from combining, in the same design, channels with depths that differs in order of magnitudes. The silicon master contains both suction channels (~5 µm wide, ~4 µm deep) and carrier channels (~200 µm wide, ~50 µm deep). The critical dimensions of the suction channels are comparable with defects that can occur during the demolding. The smaller the size of the structures, the more critical this issue becomes. A multilayer fabrication scheme has been adopted here, using a silicon oxide mask to define the small features.

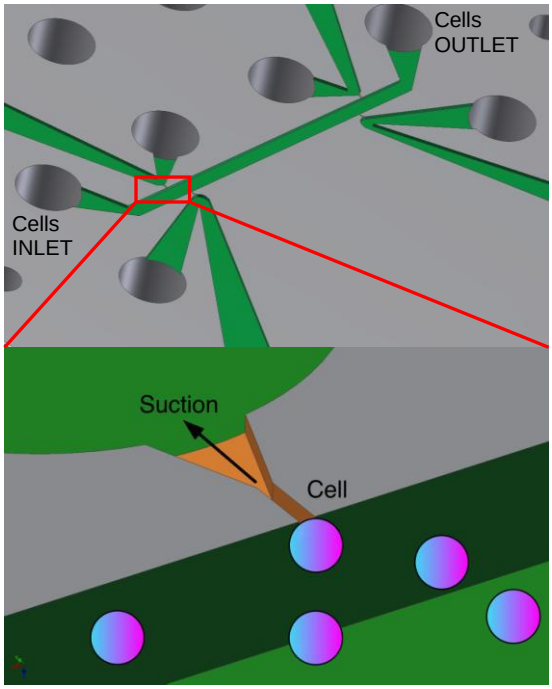

Figure 2: The figure shows chip design with "cornered" apertures based on microfluidic junctions between a large chamber for cell delivery and a lateral capillary for cell trapping.

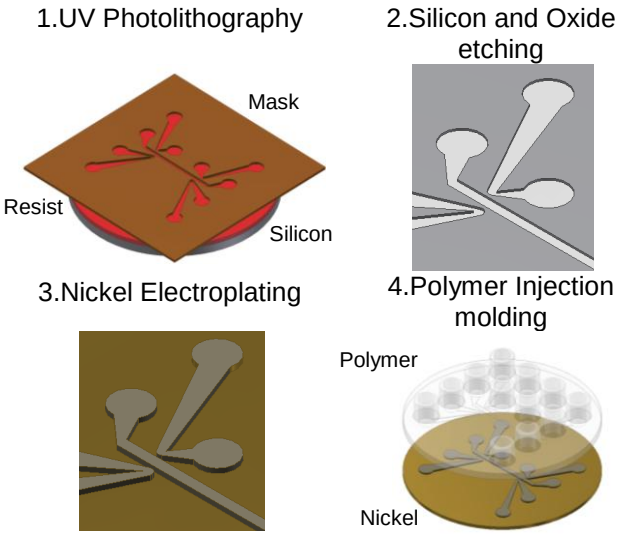

Figure 3: The figure shows the main steps in a UV-LIGA process. Photolithography and dry etching for Si master origination, Electroplating for Nickel shim fabrication and Injection molding for replication of the master.

3 FABRICATION

A two steps lithography process is needed to fabricate the Si master. We start from a 100 mm, <100>, single side polished, 525 µm thick silicon wafer. A 200 nm thick thermal SiO2 hard mask is grown at 1050°C in a Tempress horizontal furnace. A chemical treatment with Hexamethyldisilazane before both the spin coatings is used to promote the adhesion for photoresist. The photolithography process is done by Karl Suss Mask Aligner MA6 (exposure wavelength 365nm) in hard contact mode, front side alignment at 7 mW/cm2. A 2,2 µm thick AZ5214E positive resist is spun on the top side of the wafer using a Maximus 804 SEE Sister Semiconductor Equipment spinning system, baked at 90°C for 90 seconds and patterned by the first lithography step in order to define the smaller features that will originate the lateral apertures. A silicon oxide etch process is conducted to transfer the mask design into the oxide layer by using RIE (reactive ion etching) from Surface Technology Systems STS. Then, a 6,2µm thick AZ4562 positive resist is spun on the top side of the wafer using the same spinning system, baked at 100°C for 100 seconds and patterned by the second lithography step in order to define the carrier channels. A RIE oxide etch process is therefore conducted to transfer also the second mask design into the oxide layer and a DRIE (deep reactive ion etching) etching is performed by a Pegasus STS (Surface Technology Systems) system to etch the deep features into silicon wafer. A continuous process of silicon etching using a SF6/O2 plasma tapered sidewalls and smooth surface. A final RIE silicon etch process is conducted to etch the shallow channels into silicon wafer. This silicon master, which is an exact copy of the final chip, needs to be electroplated in order to fabricate a Ni insert to

Process	Parameters
Oxide deposition	1050°C dry oxidation ; t = 100 min
Spin coating	- HMDS vapor deposition - 2,2 µm AZ5214E - pre-bake: 90 °C for 90 sec
Photolithography	- hard contact mode, front side alignment W/A= 7 mW/cm^2 ; t = 9 sec - AZ351 developer; t = 90 sec
Oxide etch (shallow channels)	CF_4/CHF_3= 14/26 sccm; t = 4 min
Spin coating	- HMDS vapor deposition - 6,2 µm AZ5214E - pre-bake: 100 °C for 100 sec
Photolithography	- hard contact mode, front side alignment W/A= 7 mW/cm^2 ; t = 30 sec - AZ351 developer;t = 300 sec
Oxide etch (deep channels)	CF_4/CHF_3= 14/26 sccm; t = 4 min
Si etch (deep channels)	SF_6/O_2/Ar = 180/160/100; t = 2:40 min
Si etch (shallow channels)	SF_6/ O_2 = 32/8 sccm; t = 4 min
Oxide removal	BHF bath; t = 3 min

Table 1: The table shows the process parameters for the Silicon master creation.

be used in the injection molding machine. A 100 nm thick Ti seed layer is sputtered on the wafer by a Lesker CMS 18 sputter system. This is intended to increase the conductivity of the silicon wafer and facilitate the Nickel plating. An electrochemical deposition of Nickel is conducted on the silicon master by using Technotrans Microform 200, to obtain a final thickness 340 µm. Standard 28 wt% KOH silicon etch at 80°C for 7 hours is performed to dissolve the silicon wafer completely. The advantage of using this procedure is to exploit the flatness of the silicon wafer to define the flatness of the final polymer parts [6], since this is particularly important for the bonding with the top lid.
The resulting Nickel electroform is punched into an 85 mm diameter shim by using a customized hydraulic press. A standard epoxy glue resistant to high temperature is used to fill up the resultant cavities in the back side of the shim, and sand paper to polish it. The shim is inserted into a copper-based alloy mold, which defines the backside of the chip. Injection molding was done with an Engel Victory 80/45 Tech hydraulic injection molding machine equipped with an Engel pick up robot. Parts are molded from Cyclic Olefin Copolymer (COC) TOPAS grade 5013 from TOPAS Advanced Polymers GmbH with a glass transition temperature of 135^o C. The parts were produced using a mold temperature at 110^o C with a clamp force of 50 kN. Parts were released when mold was cooled down to 60°C. Chips are then bonded against a 100 µm thick TOPAS film using UV-assisted thermal bonding, as described by Matteucci et al. [7]

4 METHODS

Passage 12 rat pheochromocytoma (PC 12) cells were cultured on Collagen (type 1, SigmaAldrich) coated Nunclon T25 flasks (Nunc A/S). Cells were detached using trypsin, triturated to loosen cell clumps, centrifuged and resuspended in phosphate-buffered saline (PBS, Lonza). The cell solution was vortexed on a vortex mixer to distribute the cells evenly. Microscope images and videos were obtained using an AxioObserver A1 microscope with Axiovision software (Carl Zeiss GmbH).
Prior to experiments the fluidic channel system of the chip was filled with PBS, starting from the capillaries. Cells were introduced into the inlet port by using a 1ml syringe and they slowly flowed along the carrier channel. When a targeted cell was found within 100µm of the lateral aperture, it was attracted to it and trapped by applying a gentle suction (< 20 mbar) to the side channel using a VEMA piezo valve terminal (Festo) controlled with Labview software (National Instruments), see Fig. 4.

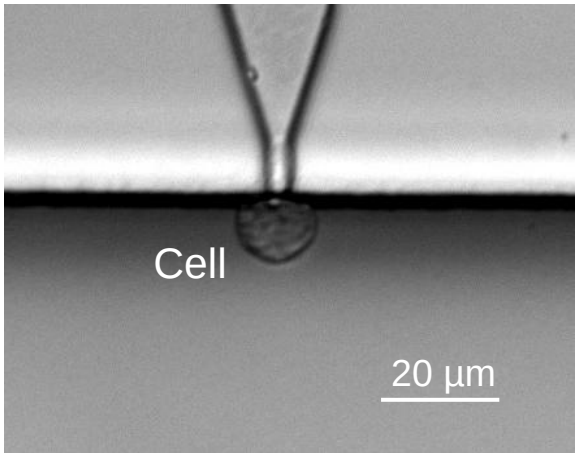

Figure 4: The figure shows a single PC12 cell being captured in the lateral aperture by by applying a gentle suction (< 20 mbar) to the side channel.

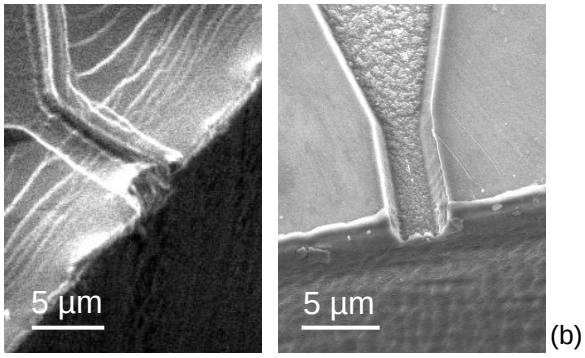

Figure 5: The figure shows SEM micrographs of lateral aperture in the final polymer part. To the left, a polymer part molded from a Si master etched with a standard Bosch process (a). To the right, a polymer part molded from a Si master etched with a continuous etching process enrolling Oxide hard mask (b).

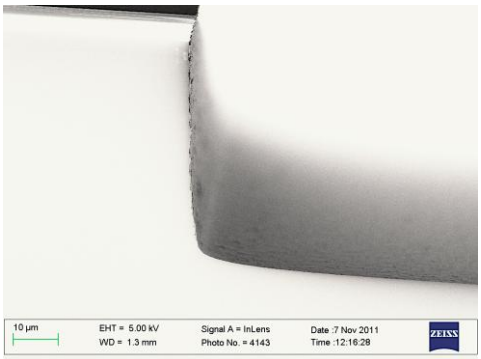

Figure 6: The figure shows SEM micrographs of lateral tapered sidewalls in the Silicon master during the fabrication. It shows a mask undercut smaller than 4 µm.

5 RESULTS AND CONCLUSION

Initially the fabrication process was designed to be as simple as possible and it enrolled a standard Bosch process optimized to realize vertical sidewall. Photo resist could be used as masking material and no hard masks were required, making the process flexible and faster. The polymer parts showed scratches of material from the sidewalls of the carrier channels that blocked the lateral apertures, see Fig. 5. We then performed the etching experiments with a continuous process by using the gases SF6, O2 and Ar, using an additional oxide layer for masking the Si.

This gave smooth tapered sidewall (>90°) and a mask undercut lower than the depth of the suction channel, see Fig. 6. The result was a perfect replica of the microstructures without any damages during the molding.

Experiments show that PC12 cells can be trapped to the lateral aperture simply by applying a slight negative pressure (< 20 mbar) to the small features.

In the future, this kind of device might be suitable for impedance measurements on a chip, where implementation with an electrode material will be required. This could be accomplished using Pedot:tosylate conductive polymer microelectrodes, which are integratable in the chip system presented here and exhibit good electrochemical properties [8]. Furthermore a reduction of the lateral aperture size, by means of etching process giving no undercut, will make the device suitable for electrophysiological recordings on single cells.

6 ACKNOWLEDGEMENTS

This work is supported by the Danish Advanced Technology Foundation through the Advanced Technology Project PILOC (grant no. 061-2010-1).

REFERENCES

[1] Seo, J. et al. (2004) *Appl. Phys. Lett.*, **84** , 1973-1975
[2] Ionescu-Zanetti et al. (2005) *PNAS*, **102**, 9112-9117
[3] E. W. Becker et al. (1982) *Naturwissenschaften* 69, 520–3
[4] Ling-Sheng Jang, Min-How Wang (2007) *Biomed Microdevices*, **9**, 737-743
[5] Andersson,H. et al. (2000) *Sens. Actuators*, **67**, *203–208*
[6] Taboryski, R. et al. (2010) *J. Micromech. Microeng.*, **20**, 055010
[7] M. Matteucci et al. *Submitted for publication*
[8] S. T. Larsen et al. (2012) *Analyst.*, **137**, 1831–1836