Merging Femtosecond Laser-based 3D Printing and Soft-lithography: a Hybrid Fabrication route for Organ-on-Chips

Gulden Akcay, Regina Luttge
Neuro-Nanoscale Engineering, Department of Mechanical Engineering/Microsystems and Institute of Complex Molecular Systems, Eindhoven University of Technology, 5600 MB Eindhoven, The Netherlands
g.akcay@tue.nl

Organ-on-Chips (OoCs) rely on microfluidics in modeling organ function and their diseases[1]. Amongst many other recently developed techniques, historically, photolithography was the premier technique in making microchannels in glass[2]. As reviewed in Scott et al.[3] microfluidics also employs polydimethylsiloxane (PDMS) soft-lithography and PDMS microfluidic architectures can be sealed by joining to a further substrate by oxygen plasma[4]. Flat microscope glass slides often suffice as a sealing substrate in OoCs but patterning the glass would allow additional capabilities. Mechanical cues affect neuronal responses [5] hence we investigated whether our previously designed PDMS-based membrane actuator chip[6] could improve thanks to innovative femtosecond laser-based 3D printing. The updated design (Alphacam software) consists of five channels at a distance of 1.2 mm each with a depth of 40 µm and a width of 50 µm, and at either end of the channels air-tube insertion regions with a diameter of 1 mm as shown in Figure 1a. We utilized 3D printing enabled by FEMTOprint®[7]. In this process, femtosecond laser light locally inks the glass for the selective removal in 45% KOH applying ultrasound agitation at 85°C for 9 hours. Figure 1b shows one of the air-tube insertion regions under an 80° viewing angle. Figure 1c and 1d illustrate the channels before and after etching. After etching, the glass is cleaned with acetone and deionized water, followed by an ultrasonic bath in ethanol for 1 hour and air-drying. Figure 2a shows the result of the patterned glass slide. Figure 2b displays the updated PDMS devices acting as culture chamber. It is made by replication (1:10 (w/w) base:curing agent) from a 3D-printed mold created by a Formlab SLA 3D printer using clear V4 resin. A mold release spray aids demolding. Additional microscale features can now also be incorporated in the sidewall of the reservoir layer to foster further fluidic functions, e.g. pinning of gel layers or nutrient flow. As in our previous work, a silanized silicon wafer was used to spin-coat a PDMS layer at 2500 rpm for 2.5 minutes, resulting in a 10 µm thick membrane. Membrane and reservoir layers are cured overnight at 80°C in an oven and assembled by oxygen plasma-activated bonding (20 W, 30 sec). This assembly is stabilized at 80°C in an oven for 1 hour before punching the holes for inlets and outlet to the air channels via a laser-cut PMMA stencil. By a puff of an air gun, the removal of PDMS residues is ensured. Then, the oxygen plasma treatment as above is used to seal the channels in the glass slide with the PDMS constructs. The final chip (Figure 2c) must be kept in an oven at 80°C for at least 1 hour prior to its biological application in an OoC study.

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6. Xie, S., Gardeniers, J. G. E. & Luttge, R. *J. Micromechanics Microengineering* **28**, 3–6 (2018).
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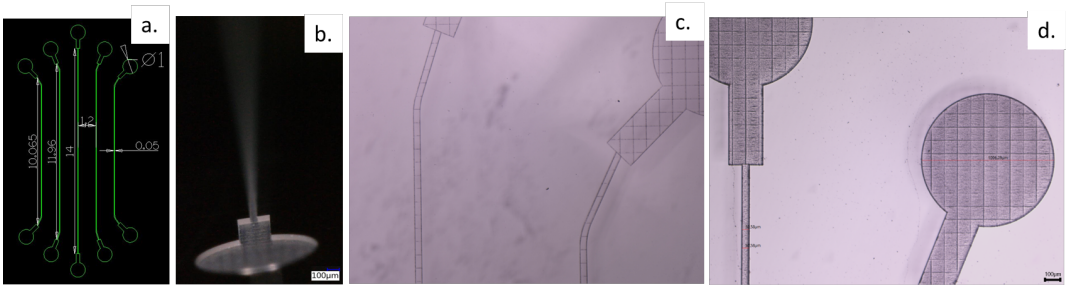

Figure 1: Channel Fabrication Process a.) Schematic design of the channels in Alphacam software, illustrating the layout of the microfluidic air-flow channels. b.) The image captures a view of a femtosecond laser-based 3D printed air-tube insertion regions with a diameter of 1 mm and channel structure from an 80° angle using a Keyence Digital Microscope. c.) Femtosecond laser-inked glass prior to etching. d.) Channels after the KOH etching process, showcasing the final structure, and confirming the successful completion of the 3D print.

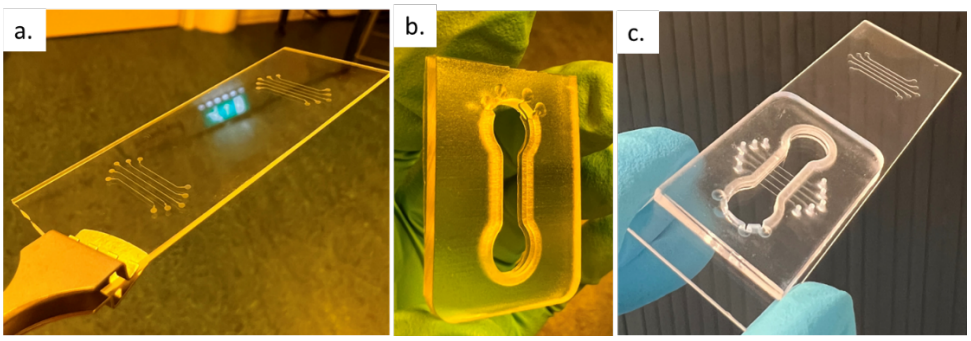

Figure 2: Microfabrication Steps of Brain-on-Chip (BoC). a.) Femtosecond Laser-based 3D printed channels in fused silica glass (Channel Layer). b.) PDMS reservoir layer serves as the container for hosting cells and the medium. c.) Fully assembled Brain-on-Chip (BoC) device. The integration of the Channel, PDMS Reservoir, and PDMS Membrane Layers results in a microfluidic system designed for studying neural cells under controlled mechanical stimuli.