Micro-nanofabricated platform technology for cell seeding experiments in neuro-nanobiology

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Pioneering research in the neurosciences utilizes micro-nanotechnology.[1] The exact definition of cell seeding environments and tuning of scaffold properties for dissociated primary neurons, however, remains a challenge. By means of a polydimethylsiloxane (PDMS) soft lithography, a micro-nanofabricated silicon template is applied to provide for a new cell culture platform technology, here introduced for experiments in neuro-nanobiology. The PDMS culture platforms contain micro-nanoscale structured wells offering cell guidance behavior. The use of similar silicon templates for nanobiology has been demonstrated earlier in the study of bone tissue regenerative medicine.[2] As in the previous work, a Laser Interference Lithography (LIL) pattern transfer process was applied to produce a nanostructure across the surface of a 4" prime silicon wafer and subsequent PDMS replication resulted into multiple micro-nanostructured cell seeding platforms. Figure 1 A and B show the micro/nanostructured silicon template and Figure 1 C, D, and E depict some examples of the PDMS replicated structures after cell seeding and network growth. Micro-nanostructured PDMS surfaces were assembled with rings also from PDMS to provide a larger cell seeding reservoir to contain the initial culture medium. To carry out a pilot cell culture repetitive micro-nanostructured platforms were inserted into the 24 wells of a standard tissue culture plate and primary neurons dissociated form the brains of newborn rats were seeded and visually followed over 8 weeks. In-vitro cell culture medium was refreshed every two days. Figure 1 D and E specifically show that the neurons are sustainable during prolonged culture on the PDMS micro-nanostructured platforms. The network-forming growth behavior of the primary neurons were preserved after seeding as staining the cells with a LIVE/DEAD ® viability/cytotoxicity kit for mammalian cells after completion of 8 weeks confirmed that a majority of cells were flourishing.[3]

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[1] Alberti M., et al. (2010) Microfluid Nanofluid 9:963-972
[2] Lamers, E., et al. (2010) Biomaterials, 31 (12), pp. 3307-3316.
[3] http://tools.invitrogen.com/content/sfs/manuals/mp03224.pdf

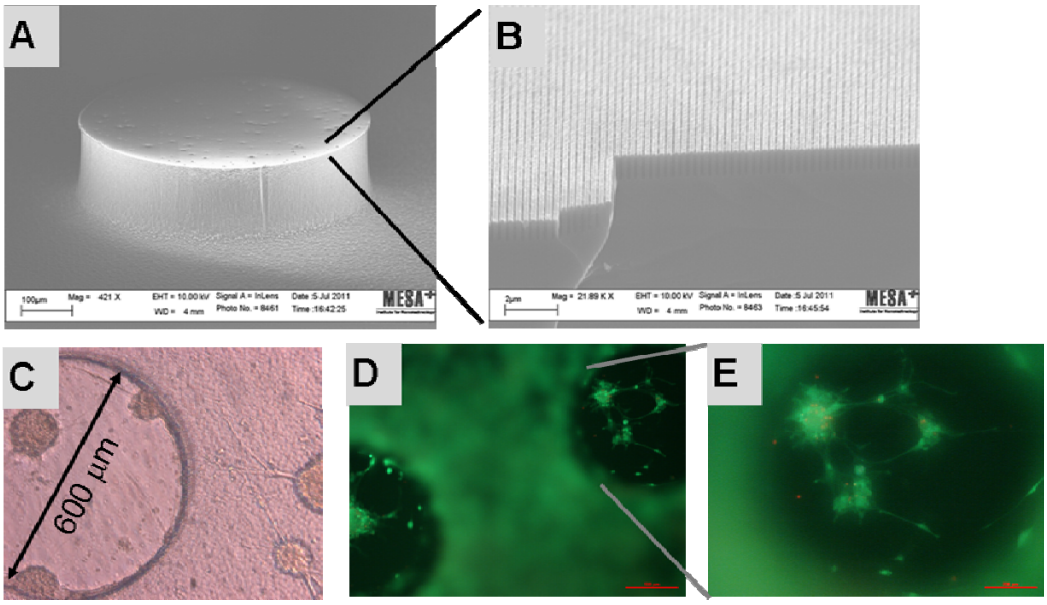

Figure 1: Micro-nanostructured silicon template for replication of PDMS cell seeding platforms for neuro-nanobiology: A. Pillar of microscale dimensions with a diameter of 600µm and a height of 100µm and a nanostructured top forming subsequently a nanostructured well in the PDMS cell seeding platform. B. Detail of the pillar top surface carrying a well-defined nanograting structure with a periodicity of 300 nm. C. Optical microscope image of a replicated PDMS platform with primary neurons seeded onto its surfaces. On both surfaces, atop the well-structure and inside of the wells neurons form clustered networks. The micro-nanostructured wells form a barrier to excessive cellular network growth, while the unstructured platform surface provides carpet-like cellular network growth. The neuronal clusters inside of the well are interconnected with the neurons atop. D. Fluorescent optical image of two wells after 8 weeks of cell culturing depicting neuronal clusters after a live/dead staining. E. Detail of fluorescent image inside of one of the wells.