Nanopatterned substrate stiffness affects primary cortical cell network formation

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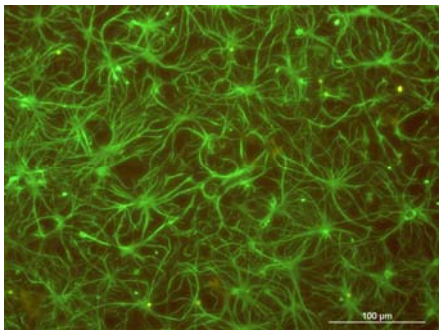
Networks of neurons cultured on-chip can provide insights into both normal and disease-state brain function. The ability to guide neuronal growth in specific patterns allows us to study how brain function follows form. Our goal is to combine microfluidics with tissue engineering to create a 'living' brain, generating realistic *in vitro* neural circuitry, which can be used to standardize experimental neuronal cell culture.[1]

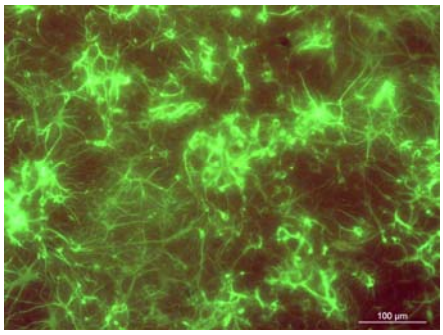
In previous work, we demonstrated that cultures of primary cortical cells (CTX) were sensitive to the specific nanoscale surface structure on silicon patterned with a nanoscaffold of resist (Non-Silicon Monomat). CTX grown on surfaces with grating periods between 400 nm and 600 nm and a height of 118 nm, showed highly ordered regions of neurites with a preferential alignment tendency for astrocytes.[2]

Cells are also sensitive to the mechanical properties of their environment. The extra-cellular matrix (ECM) influences the function of neuronal cells, providing not only a physical scaffold, but also chemical cues for cell growth and behavior.[3] The stiffness of the scaffold material has a significant effect on the number of primary neurons and astrocytes attached, as well as on the direction of their outgrowths.[4] Materials suitable for nanopatterning, have stiffnesses above that of the ECM[3,4], but the effect of higher stiffnesses has not yet been quantified for CTX. Here, we study two materials with large differences in stiffness, PDMS and Silicon (Si). PDMS is often used for cell studies on chip[5], and nanopatterned PDMS showed the differentiation of mesenchymal stem cells towards the neuronal lineage.[6] Si is a well-known material in nanopatterning technology.

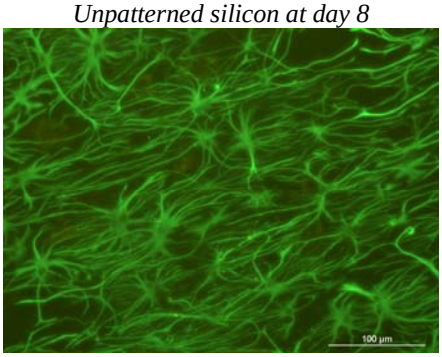
Our results show that both nanopatterned silicon and PDMS guide the outgrowth of astrocytes in CTX culture, but astrocytes tend to form more outgrowths from each cell body on the softer PDMS substrates than on the harder Si ones. Astrocytes thus 'sense' the nanoscaffold stiffness, which might have a profound effect on the neuronal network formed. Future studies will focus on neurophysiology, to analyze which of these networks is a more realistic model of the brain.



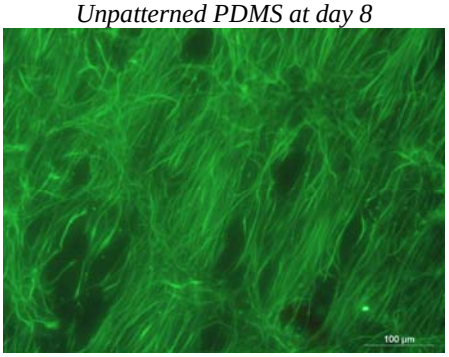
Unpatterned silicon at day 8



Unpatterned PDMS at day 8



Patterned silicon at day 12



Patterned PDMS at day 8

Immunostained astrocytes (GFAP-green) on PEI coated substrates with different stiffness. Patterned Si is etched with a pattern, period width, P, = 400 nm, ridge width, R, = 280 nm, depth, D, ≈ 200 nm. Patterned PDMS is replica molded, with P = 450 nm, R =270 nm, D ≈ 110 nm. Silicon nanopatterns were fabricated by Jet and Flash Imprint Lithography (J-FIL), followed by etching. All nanoscaffolds were coated with polyethylenimine (PEI), as PEI ensures good cell-substrate adhesion and viability.[2,6] Scale bar is 100 µm.

References

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Acknowledgements

This project is financially supported by the ERC, grant no. 280281 (MESOTAS). We thank B. Vratzov (MESA$^+$ Institute) for assisting in J-FIL.