Advances in 3D Neuronal cell culture

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In this contribution, we present the latest advances for a fully functional hybrid bioreactor coupled to commercially available microelectrode arrays (MEAs) to study 3D neuronal networks in controlled environments for analysis of cellular physiological and pathological responses. Previously, we have shown the ability to culture 3D neuronal cells within hybrid PDMS bioreactors that can be reversibly coupled to commercially available microelectrode arrays MEAs[1]. In addition, we have demonstrated the ability to guide neuronal outgrowths from rat primary cortex (CTX) cells on nanogrooves. We showed that nanoimprint lithography, specifically J-FIL[2], is a robust fabrication technique to pattern various substrates for cell based assays (Fig 1a). The average percentage of 2D aligned outgrowths was above 85% for all substrates (Fig 1b)[3].

However, for a more biomimetic *in vivo* representation of neuronal cell cultures, 3D culturing is required to reproduce the tissue like brain microenvironment. To further our understanding of uniquely nanopatterned interfaces, we added Matrigel atop the primary CTX cells cultured on the nanogrooves. We assessed the impact of the guiding effect in 3D and evaluated the influence of nanogroove guidance on culture height. Here, we show that primary astrocyte outgrowths keep 65% of their alignment capability when growing 6 µm from the nanogrooved surfaces (Fig 2), suggesting that outgrowths can still align at distances 6 times their own diameters (assuming the average outgrowth is 1 µm in diameter).

With the view to incorporate these guided culture interfaces in our hybrid PDMS bioreactor[1], we have also optimized 3D cellular cultures by analysing the effect of different gel matrices (Matrigel, Puramatrix and collagen) on the neuron model cell line SH-SY5Y. We report major differences between 3D and 2D neuronal cultures, primarily in respect to cellular size (Fig 3a and b). We report a 50% decrease (from 40 µm to 20 µm) in cell size for 3D cultures when compared with 2D cultures. Fig 3c highlights the randomness of a unpatterned surface-soft gel interface when compared to nanogrooved surfaces (Fig 1b and 2b) where cells and outgrowths grow in uncontrollable fashion.

Taken together, the ability to guide neuronal growth in specific patterns and within a bioreactor coupled with MEA as well as in 3D will allow us to study how changes in cell morphology translate to changes in function and will lead to the development of improved *in vitro* assays to understand, mimic and treat brain disorders such as epilepsy.

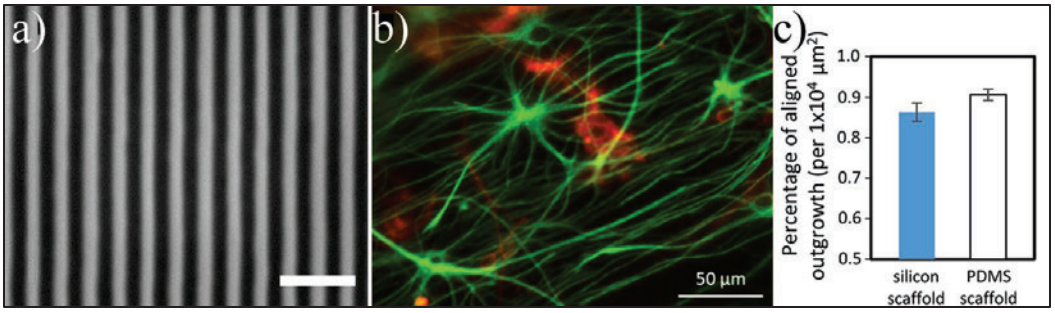

Figure 1: a) SEM image of structured nanoscaffolds generated by J-FIL. Scale bar: 1 µm. b) Immunostaining of CTX cells (astrocytes in green) on a PEI coated nanoscaffold after 12 DIV (nanoscaffold with 380 nm ridge width, 600 nm pattern period). c) Average percentage of aligned astrocyte outgrowth per 100 x 100 µm on nanoscaffolds with different stiffness. The "aligned outgrowth" is defined as a deviation of the grooves direction within an angle of less than 30° (n=8).

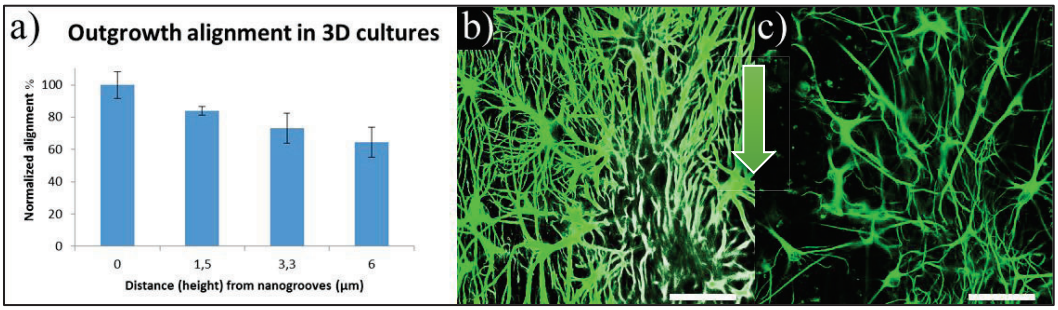

Figure 2: a) Normalized outgrowth alignment against 3D culture height showing that at 6 µm from the nanogrooves, 65% of alignment is still observable. The "aligned outgrowth" is defined as a deviation of the grooves direction within an angle of less than 30° (n=3). Example of CTX primary astrocytes aligned to the nanogrooves at b) 0 µm and at c) 6 µm are shown (groove direction indicated by arrow. Scale bar: 60µm).

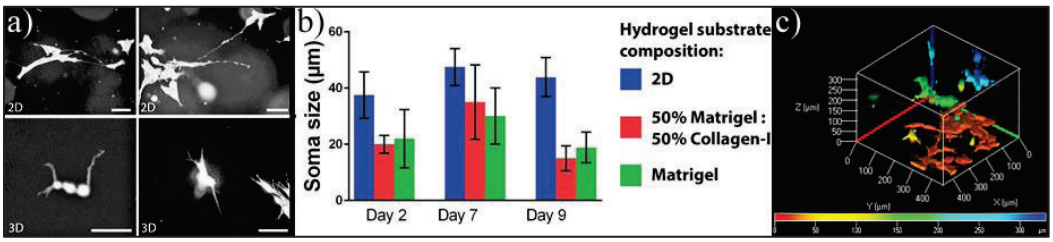

Figure 3: a) SH-SY5Y differentiated cells on 2D polystyrene surfaces (top), in Matrigel hydrogel (bottom left) and 50% collagen-I : 50% Matrigel hydrogel (bottom right). Scale bars 50 µm. b) cell size is equally affected regardless of gel composition confirming that neurons adopt a more *in vivo* morphology when cultured in 3D. c) Confocal stack of SH-SY5Y differentiated cells showing variation in cell morphology with height (from red to blue) but also highlighting the randomness of a unpatterned surface-soft gel interface.

References:

1. B. Schurink and R. Luttge. J. Vac. Sci. Technol. B **31**(6), (2013).
2. S. Xie and R. Luttge. Microelectronic Engineering **124**, 30–36 (2014).
3. S. Xie, B. Schurink, F. Wolbers, R. Luttge, and G. Hassink. J. Vac. Sci. Technol. B **32**, 06FD03 (2014).