Electronic Supplementary Information

Heike Hardelauf *et al*.

Micropatterning Neuronal Networks

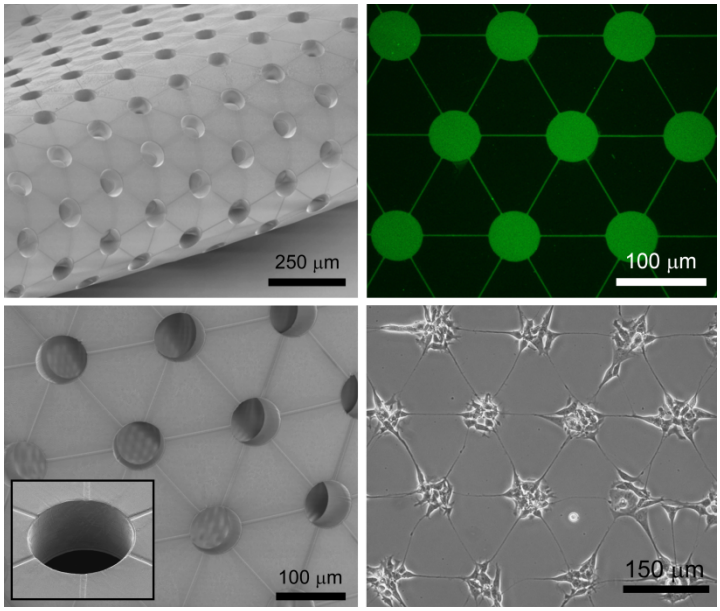
Heike Hardelauf[a]*, Sarah Waide[a]*, Julia Sisnaiske[b], Peter Jacob[a], Vanessa Hausherr[b], Nicole Schöbel[b], Dirk Janasek[a], Christoph van Thriel[b] and Jonathan West[a,c]

* Equal contribution

[a] Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., 44139 Dortmund, Germany

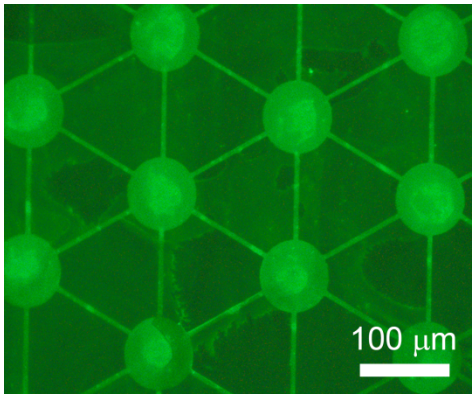
[b] Leibniz Research Centre for Working Environment and Human Factors – IfADo, 44139 Dortmund, Germany

[c] Institute for Life Sciences, University of Southampton, SO17 1BJ, U.K.

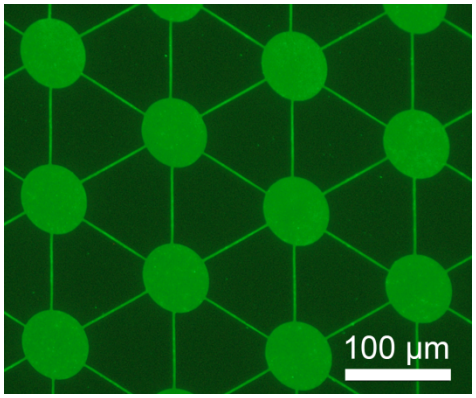


ESI Fig. 1 Bilayer plasma stencilling technique[1]: A flexible PDMS membrane with through-holes connected on the basal side by micron-scale channels (left). Plasma stencilling a PLL-*g*-PEG-coated substrate replicates the pattern for localised protein deposition (conjugated with FITC, top right). Neuron-like differentiated human SH-SY5Y neuron-like cells adhere to the nodes and extend outgrowths to form an interconnected neuronal network (bottom right). Modified and reproduced with permission of the Royal Society of Chemistry.

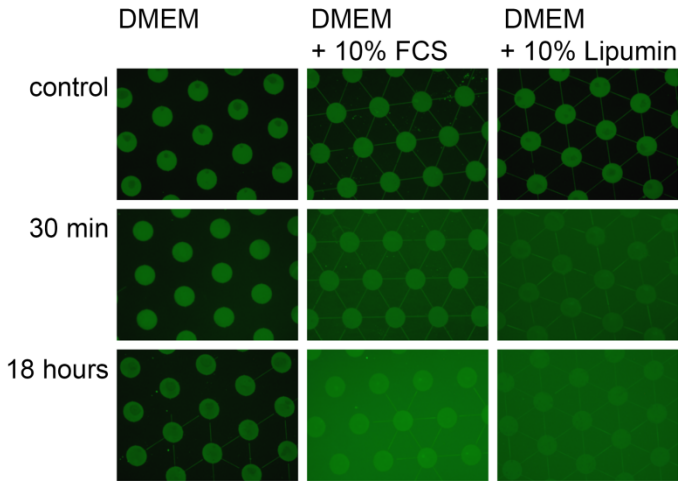
1. H. Hardelauf, J. Sisnaiske, A. Taghipour-Anvari, P. Jacob, E. Drabiniok, U. Marggraf, J. P. Frimat, J. G. Hengstler, C. Neyer, C. van Thriel and J. West, *Lab Chip*, 2011, **11**, 2763-2771.



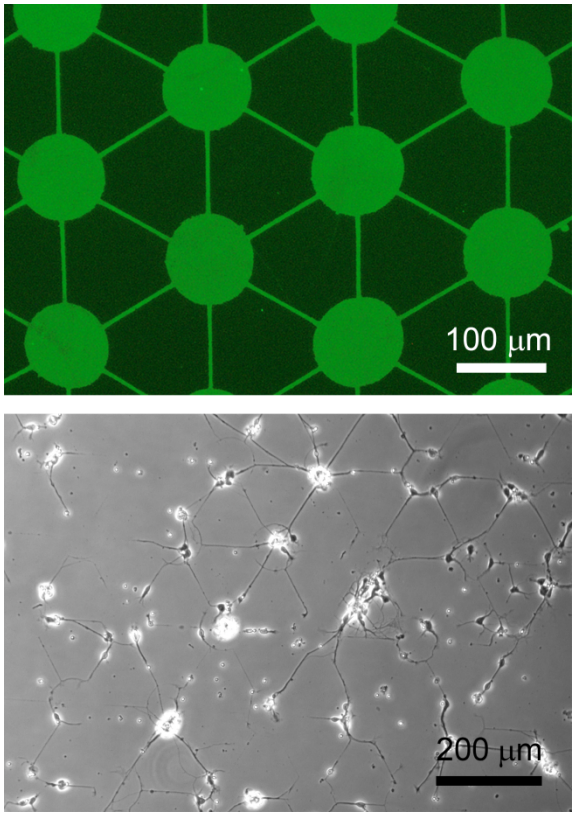
ESI Fig. 2 The addition of PLL-FITC to a plasma patterned surface coated with PLL-*g*-PEG. The PEGylated surface does not prevent the adsorption of PL.



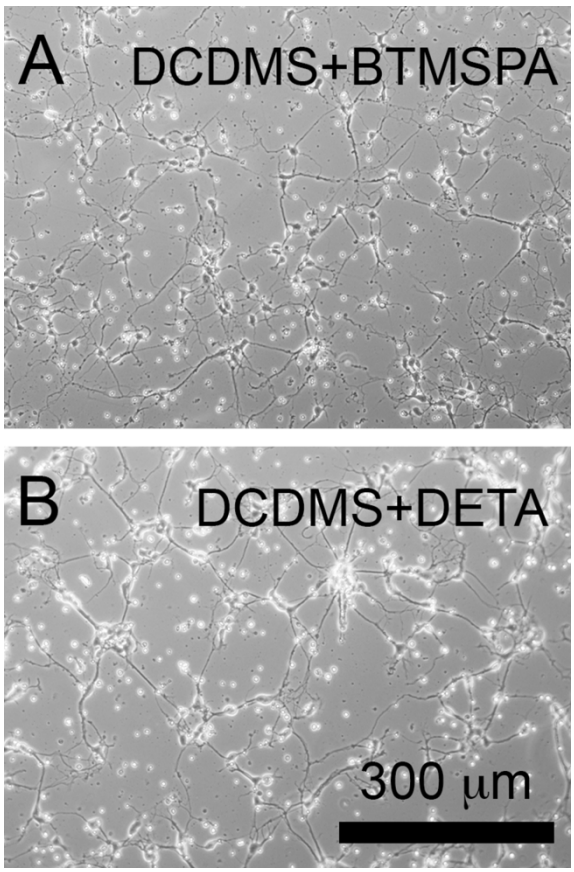
ESI Fig. 3 TCS-PEG/PLL-FITC patterns. Immediately after back filling with PLL-FITC, patterning was observed. However, these patterns were not reproducibly transferred across the entire surface and were of low stability, rapidly decaying while stored in buffers or cell media.



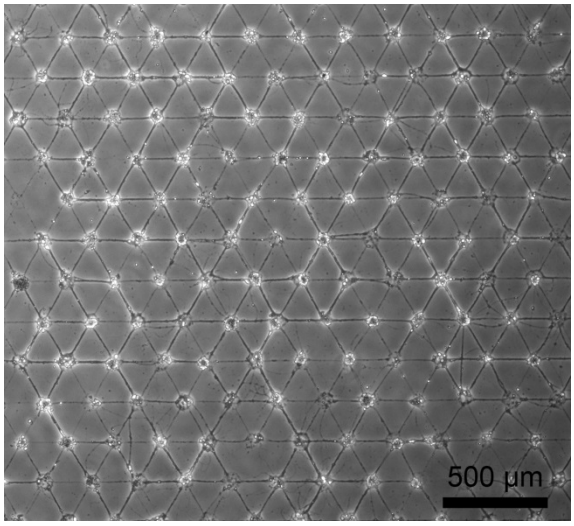
ESI Fig. 4 PLL-FITC patterns fabricated using surplus bilayer stencils were imaged immediately after preparation (control) and following incubation for 30 minutes or 18 hours in different culture media. The DMEM cell culture media has minimal effect on pattern integrity, whereas in the presence of essential growth supplements (10% (v/v) FCS or Lipumin) the pattern decays noticeably in 30 minutes, with significant non-specific adsorption following overnight incubation (18 h).



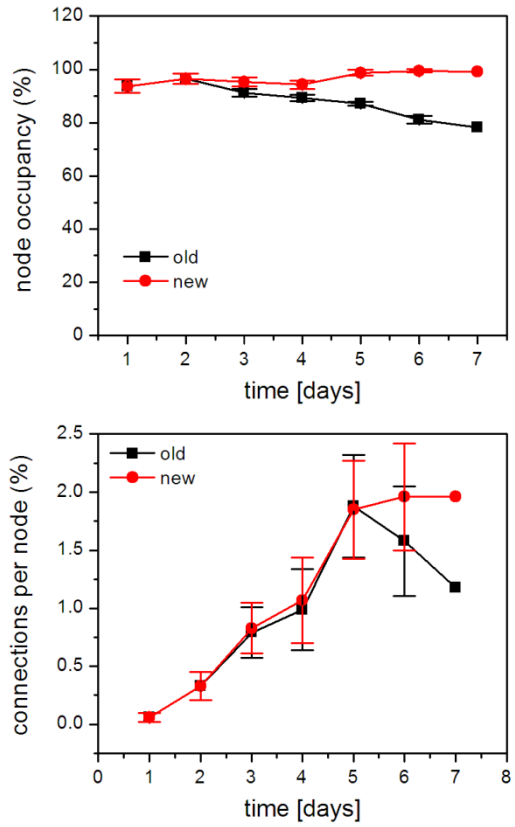
ESI Fig. 5 A DCDMS/PLL-FITC pattern imaged immediately after preparation (top) and choice results of rare patterns of cortical neurons following 5 DIV culture (bottom).



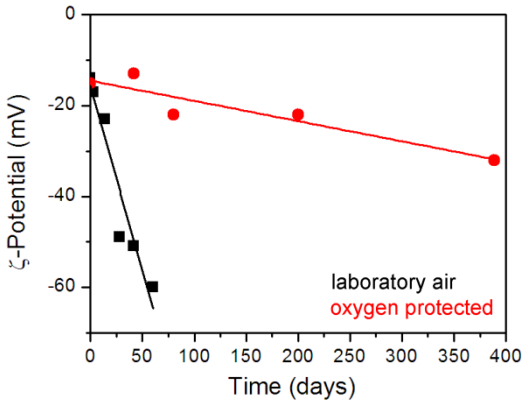
ESI Fig. 6 Cortical neurons adhering to a DCDMS-aminosilane pattern following 5 DIV culture.



ESI Fig. 7 Large area pattern compliance (156 nodes) of cortical neurons on PLL-*g*-PEG-coated substrates patterned with PL by microcontact printing. The spatial organisation of the neuronal networks was stable for 33 DIV (the experiment was concluded at this time point).



ESI Fig. 8 Redefining the occupancy and connections per node (cpn) terms. Clustering reduces node occupancy and consequently the cpn level (black). Expanding the node occupancy term to further include occupancy by neurite outgrowth results in maintained occupancy and cpn levels (red).



ESI Fig. 9 PLL-*g*-PEG stability. Zeta potential measurements demonstrate that the stability of PEG coatings decay significantly within 2 months when stored in air. In contrast, storage in a N_2 atmosphere (with oxygen displaced) prolongs the stability of the adlayer, with only a minor (~15 mV) drop in the zeta potential during storage for over a year. Similar preservation can be achieved by storing the substrates in argon at -80 °C.