Neuronal cell network activity enhanced by nanogrooved substrates

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A new generation of advanced *in vitro* human brain models are vital to surpass the limitations of current cell culture platforms and animal cell lines in studying brain function and brain diseases. These new models can be achieved by means of brain-on-chip technology, employing micro- and nanofabrication to create well-defined, controllable and reproducible platforms with a cellular microenvironment that allows for *in vivo*-like, organized brain cell culture.
Previously, we investigated differentiation and network organization of the neuroblastoma SH-SY5Y cell line on nanogrooved substrates. Results showed that nanogroove guidance of neuronal outgrowths, neurites, is dependent on nanogroove dimensions. Also, increased orientation of neurites was positively correlated to differentiation of SH-SY5Y cells.[1] However, as mimicking brain structure alone is insufficient, we also investigated the electrophysiological function of the neuronal network. Preliminary experiments using calcium imaging were performed with differentiated SH-SY5Y on flat glass, flat polydimethylsiloxane (PDMS; Sylgard 184) and nanogrooved PDMS substrates to study network function. Nanogrooves were 104 nm high, periodicity was 993 nm and ridges were 269 nm wide as measured with atomic force microscopy. Calcium imaging recordings of 10 minutes at 0.1 Hz were made at 21 days *in vitro* to visualize spiking of calcium ion intake as indicator of electrical signaling by cells. Recordings were analyzed with in-house developed software[2]. Results show ~20% more cells with multiple spiking events per recording on both PDMS substrates as compared to flat glass. The number of synchronous spiking events was measured as indicator of network connectivity (Figure 1), showing ~2x more connections for nanogrooved PDMS compared to flat PDMS, and ~20x more connections for nanogrooved PDMS compared to flat glass.
Human induced pluripotent stem cell-derived cortical neurons (hiPSCs) were investigated as a more realistic alternative to SH-SY5Y cells. For now, preliminary experiments were performed to successfully maintain these cells on glass (Figure 2), PDMS and Ostemer® (#322 Crystal Clear) substrates.
As an outlook, we want to combine these results towards the investigation of electrophysiological activity of hiPSCs on nanogrooved PDMS and Ostemer®. These experiments will help elucidate the benefits of integrating nanogrooves for a more realistic form and function of brain models in brain-on-chip platforms.

[1] A.J. Bastiaens, S. Xie, and R. Luttge, J. Vac. Sci. Technol. B **36**, 06J801 (2018).
[2] E. Moonen, R. Luttge, and J.P. Frimat, Microelectron. Eng. **197**, 1 (2018).

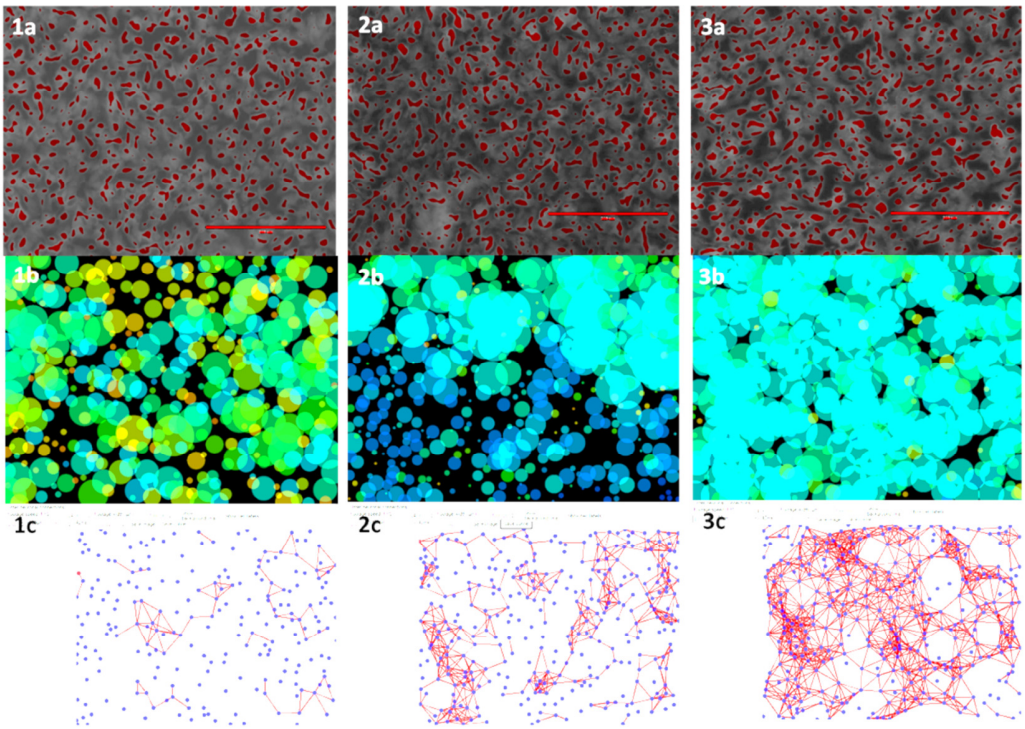

Figure 1: Spiking events in neuronal cell networks: Calcium imaging recordings were performed on differentiated SH-SY5Y neuroblastoma cells cultured on glass (*1*), flat polydimethylsiloxane (PDMS) (*2*) and nanogrooved PDMS (*3*). (*a*) Cell detection (red) superimposed on calcium imaging image. Scale bar 200 µm. (*b*) Spiking events visualized with circle size indicating the number of events and the color indicating the averaged time point at which spiking events occur. (*c*) Synchronous, correlated spiking (red lines) in cells (blue dots) at a maximum distance of 50 µm. Activity and connectivity increase for PDMS, in particular nanogrooved PDMS, compared to flat glass substrates.

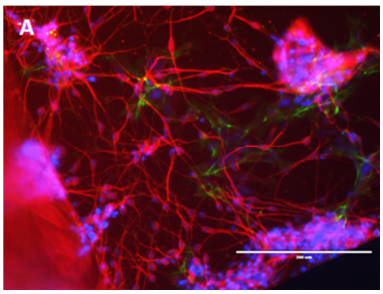

Figure 2: Neuronal cells on glass: (*a*) Human induced pluripotent stem cell-derived cortical neurons (hiPSCs) were cultured on a flat glass substrate according to manufacturer guidelines (AxolBio, #ax0015). hiPSCs were cultured for 11 days *in vitro*, then fixed and stained for MAP2 (red), F-actin (green) and cell nuclei (blue). Scale bar 200 µm.