Characterization of 3D microfluidics-generated cortical columns in vitro and in vivo

Mona Barkat[1], Luana Campos Soares[1], Elisa Marozzi[1], Mingyu Li[2], Yufan Xu[1], Marlene Lawston[1], Gretchen Greene[1], Gabriel Mose[2], Linna Zhou[2], Sally Cowley[3], Pawel Swietach[1], Hagan Bayley[2], Zoltán Molnár[1], Francis Szele[1]*

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK
[2]Department of Chemistry, University of Oxford, Oxford, UK
[1]Dunn School of Pathology, University of Oxford, Oxford, UK

The complexity of the cerebral cortex arises from the assembly of various cell types, including excitatory glutamatergic neurons and inhibitory GABAergic interneurons, into intricate circuits. Neurons in the lower cortical layers project primarily into subcortical and subcerebral structures, whereas upper-layer neurons establish intra- and interhemispheric connections. This complexity must be considered when developing treatments for neurological disorders. Although 3D culture methods, such as organoids, are considered a potential source of neural tissues, their relative homogeneity and lack of laminar organization restrict use as repair material after traumatic brain injury (TBI). We hypothesise that 3D-layered neuronal tissue, containing multiple cortical neuronal subtypes, may provide better integration and functional recovery when grafted in a murine model of traumatic brain injury (TBI).

Using calcium imaging, two distinct neuronal phenotypes were identified pharmacologically, notably glutamatergic and GABAergic. Time-lapse calcium imaging of neuronal activity revealed that early and late neurons produced distinct connections. The former generated stronger connectivity, moreover, late neurons had a higher integration and network robustness. To study how GABAergic cells modulate the activity of the ensemble neuronal network, the response of calcium signals to GABA application was compared against spontaneous activity. The network responded to GABA with a decrease in firing rate and the number of active neurons, indicating the presence of a mature, hyperpolarising-type of GABAergic cells. In contrast, glutamate treatment increased the number of active neurons and their firing rates. Collectively, our findings provide evidence of complex neuronal networks involving both glutamatergic and GABAergic neurons.

An in-house microfluidic technique was next used to fabricate two-layered 3D-neuronal tissue, comprising distinct upper- and lower-layer cortical neuronal compartments. These constructs showed long-term survival, proliferation, and differentiation into layer-specific fates in vitro. Analysis of neuronal activity in the constructs representing upper and lower cortical neurons revealed distinct neural activity signatures, mirroring patterns reported in monolayer cultures. Two clusters of neuronal activity, distributed within the two layers, could be detected, with some hubs being integrated into most of the subnetworks, indicating the formation of complex connectivity and inter-laminar neuronal circuit formation.

Implantation of the bioengineered cortical tissue into the cerebral cortex of immunocompromised SCID gamma mice led to the anatomical restoration of the cortical lesion. Within the implants, the neurons continued differentiation into layer-specific fates, with axonal extensions following layer-specific patterns: upper cortical neurons projecting intracortical and the lower cortical neurons projecting into subcerebral and subcortical regions. Potential vascularization of the constructs was observed two weeks post-implantation, and this vascularization persisted and was detectable up to two months, the maximum time point examined after implantation. Furthermore, the glial response, as indicated by reactive Iba-1 and GFAP-positive cells, showed a reduction over time. This vascularization and remodelling in glial reaction suggest a stabilization of the tissue environment following initial implantation.

Our findings suggest that microfluidic-fabricated cortical tissues are a promising avenue for anatomical and functional studies of the developing human cortical circuits. We propose their use as bioengineered tissue implants for TBI recovery.