A three-dimensional neuronal culture technique that controls the direction of neurite elongation and the position of soma to mimic the layered struure of the brain.

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Statement of Purpose: In this study, we therefore developed a 3D culture method using orientation techniques with collagen fiber and a polydimethylsiloxane (PDMS) microchamber. We demonstrated that this method can produce 3D neural networks by controlling the position of somata; therefore, it closely resembled the cell density of living tissue, and we controlled the direction of neurite outgrowth in the 3D gel. We also confirmed that multiple layers (series of cell blocks) could be constructed, and its 3D neuronal network function was analyzed using Ca^{2+} imaging. The morphology and activity of 3D neural networks were assessed by immunostaining and Ca^{2+} imaging. We constructed a 3D neuronal network from two cell blocks on a multi-electrode array (MEA) chip, which facilitated the noninvasive monitoring of electrical activity. We measured functional synaptic transmission between the interlayers using pharmacological experiments, the interlayer propagation time of the action potential (AP) during spontaneous activity, and the evoked responses to electrical stimulation. This method is the novel technique that is capable of reconstructing the 3D layered structure of the nervous system. This technique has many potential applications in clinical tissue engineering, as drug screening models, and in understanding the layered structure of the nervous system.

Methods: To fix the position of somata and to layer the cells, we used microfabrication techniques in order to prerare 500μm×4000μm×2000μm(final volume, 4×10^9 μm^3) PDMS microchambers, which were spaced 500μm apart. The PDMS sheet was attached to a 35 mm non-treated polystyrene dish. The insides of the PDMS microchambers were coated with 50 mg/ml^{-1} poly-D-lysine solution and washed two times. Cell suspensions were dropped into the PDMS microchambers using micropipettes and incubated at 37 °C with 5% CO2 for 30 min. The PDMS sheet was removed from the dish leaving cell blocks with multiple layers. To control the orientation of the collagen fibers, 80 μl of 2 mg ml^{-1} liquid rat tail collagen at 4 ℃ (Life Technologies) was dropped onto the dish, which was inclined at 45°, and the collagen was gelled in a 37℃ incubator where the dish was inclined at 45° for 5 min. The cells were cultured in the collagen gel with culture medium in a flat culture dish.

Results: We constructed 3D neural networks by controlling the cell positions and the direction of neurites. Using PDMS microchambers, we constructed cell blocks with multilayers and achieved neurite outgrowth direction control using the collagen fiber orientation technique. Fig. 2A shows the reconstructed 3D neuronal network at 10 DIV, which shows that the position of the somata area was fixed and that the neurites grew in one direction in the collagen gel after immunostaining using the nuclear marker Hoechst 33258 and the neuronal marker MAP2 (Fig.1). We also confirmed that neurons with multiple cell layers elongated their neurites in one direction in the 3D space. Morphological analysis of the 3D neuronal network, which was stained with H&E, confirmed the construction of multilayered cells. H&E staining of specimens using cross-sections of the somata demonstrated the order of the layers in the somata and their density. Three cell layers were constructed with 50 mm high collagen gel, and the cell density in the somata area was 43,000±8,000 cells per mm^3 (n = 4).

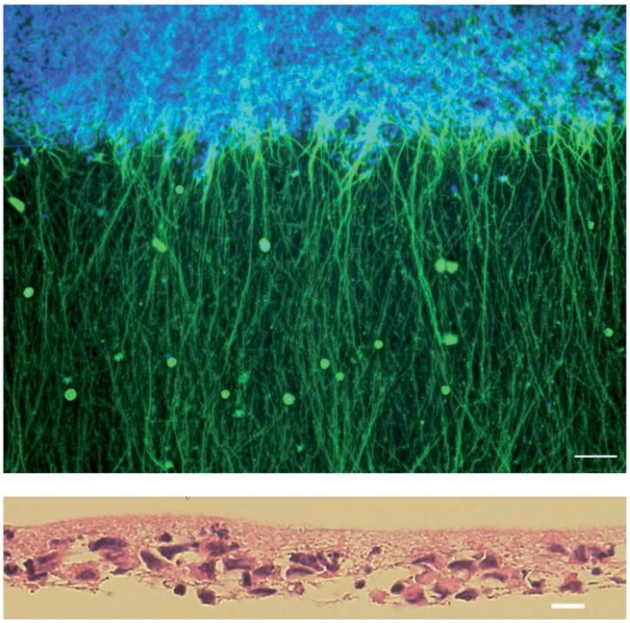

Figure 1. The 3D culture of the neuronal network after controlling the cell position and direction of neurites.

Conclusions: We developed a novel technique for constructing 3D neuronal networks with a layered structure on the basis of the orientation of collagen fibers and PDMS micro-chambers. This method allows the control of the cell position and the direction of neurite elongation in a 3D gel. Using this method, we mimicked the 3D layer structure of a cerebral cortex at the same cellular density as natural brain tissue, which controlled the direction of neuronal processes in straight lines in the interlayers. Furthermore, we confirmed that AP propagation occurred via a chemical synapse, and that it occurred at the same velocity as that recorded in natural biological tissue. Our technique may facilitate the fabrication of neuronal tissue grafis, the next generation of regenerative medicine, and drug screening assays using iPS cell-derived neurons.

Reference: A. Odawara, et al. RSC Advances. 2013; 3: 23620-23630.