3D Parkinson's disease model of human iPSC-derived dopaminergic neurons for novel drug candidates screening

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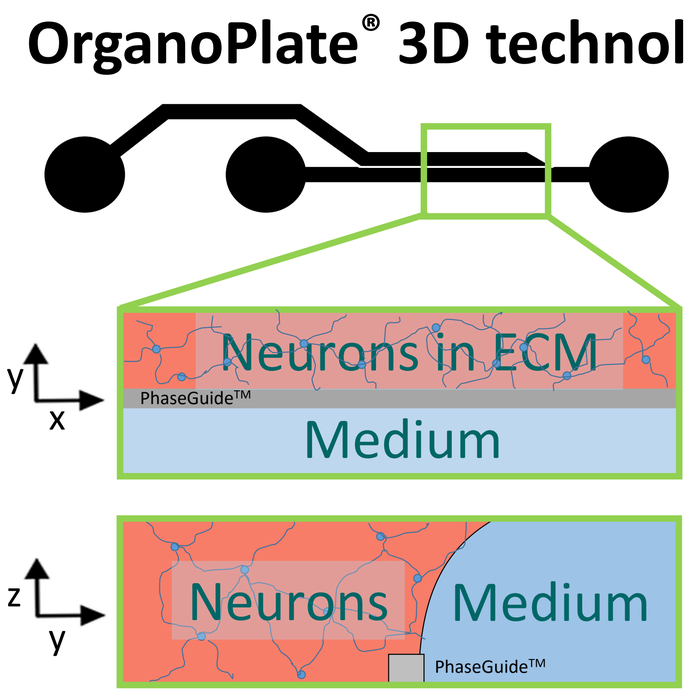
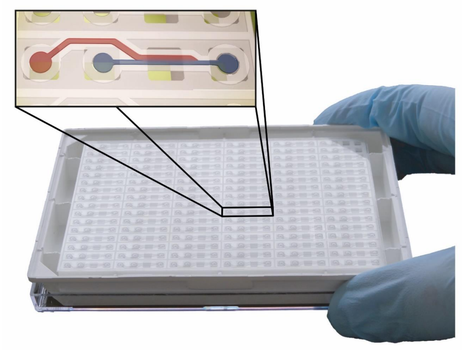
Overview

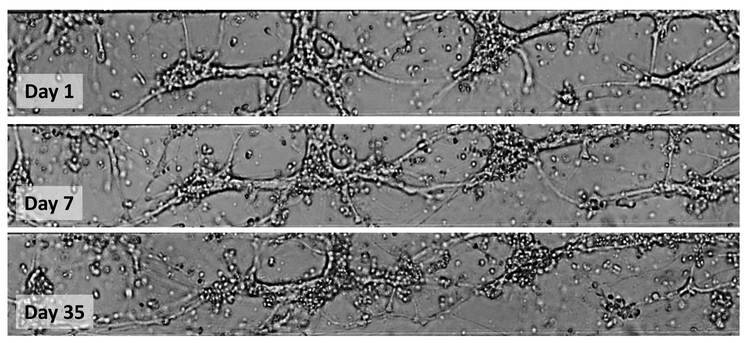
Parkinson's disease (PD) is a neurodegenerative disorder which is characterized by motor dysfunction and progressive loss of dopaminergic neurons in the substantia nigra. Important advances made in understanding of pathogenic pathways have not yet been translated into any neuroprotective treatments[1]. The high heterogeneity of the disease and the lack of preclinical models that adequately recapitulate PD pathology prohibit successful development of neuroprotective therapies.

Here, we show the development of a 3D PD model based on patient-derived induced pluripotent stem cells. Functional dopaminergic neurons were obtained after 6 weeks of differentiation and can be used to identify novel drug candidates.

OrganoPlate® 3D technology



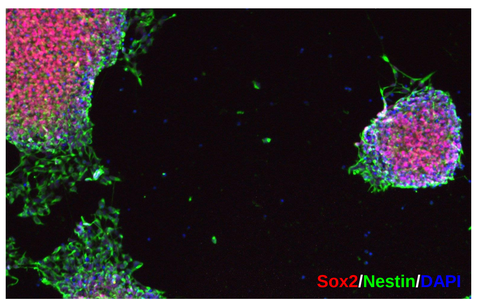




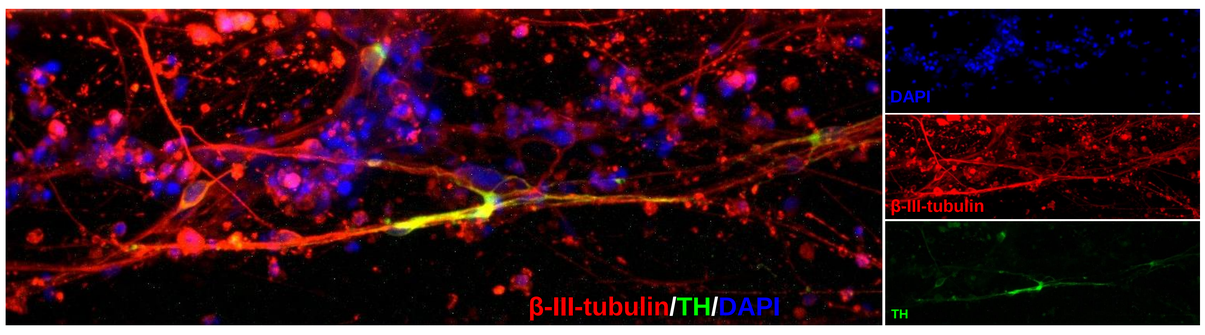
The OrganoPlate® is a high-throughput platform that combines the most recent advances in 3D cell culture and microfluidics[2]. The OrganoPlate® contains 96 tissue chips and is compatible with standard laboratory equipment. The intermediate human neuroepithelial stem cells (NESCs) were seeded in an extracellular matrix (ECM) in the gel channel (pink) and differentiated towards functional dopaminergic neurons in 6 weeks. The adjacent medium channel (blue) supplies the neurons with nutrients.

3D mature neuronal network

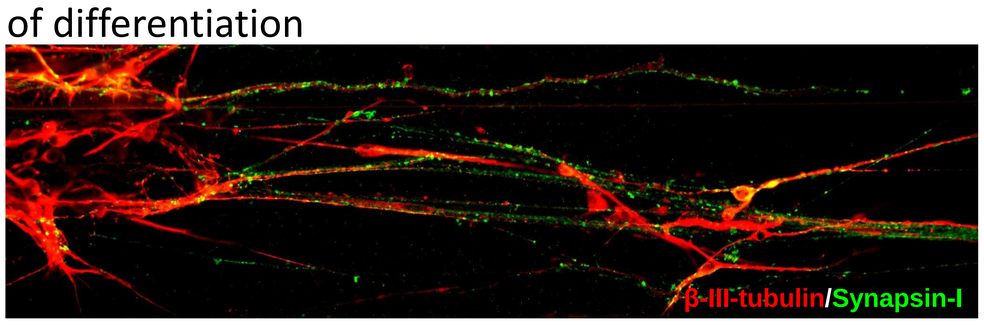
The NESCs, generated from healthy control and patient iPSCs, are maintained in their stem cell phase by activation of the SHH and WNT signaling pathways and express the stem cell markers SOX2 and Nestin[3].



NESCs are differentiated towards dopaminergic neurons to model the substantia nigra. Immunostaining confirmed the presence of dopaminergic neurons in a 3D environment (neuron marker, β-III-tubulin, and tyrosine hydroxylase (TH) for dopaminergic neurons).

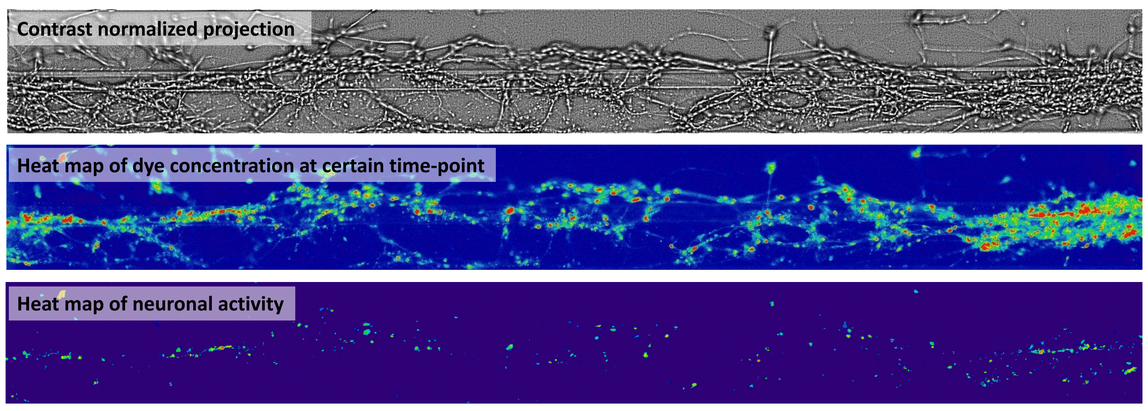


Neurons express the mature neuron marker Synapsin-I after 6 weeks of differentiation



Neuronal activity by calcium imaging

Neuronal firing causes an increase in intracellular calcium that can be detected as a rise in fluorescent signal using the calcium sensitive Fluo-4 dye.



Spontaneous neuronal activity

Differentiated neurons show spontaneous activity from 4 weeks differentiation.

- Time lapses are recorded at 20Hz on an ImageXpress Micro XLS-C system (Molecular Devices)
- Recordings are processed using ImageJ to extract calcium traces of individual neurons
- Varation in the fluorescent trace represents neuronal activity

Conclusions

- We developed a 3D Parkinson's Disease model for screening of novel candidate neuroprotective agents.
- Differentiated neurons express the dopaminergic marker TH and mature neuronal marker Synapsin-I.
- After four weeks of differentiation the neuronal network shows spontaneous firing.

Literature

Ref. (1) Schapira AHV et al., Lancet 384: 545–555.
Ref. (2) S.J.Trietsch et al., Lab on a chip, 2013, 8, 3548-3554.
Ref. (3) Edinson Lucumi Moreno et al., Lab Chip,2015, 15,2419.

MIMETAS the organ-on-a-chip company

SysMedPD

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