DEVELOPMENT AND CHARACTERIZATION OF HUMAN IPSC-DERIVED 3D NEUROSPHERES FOR DRUG DISCOVERY

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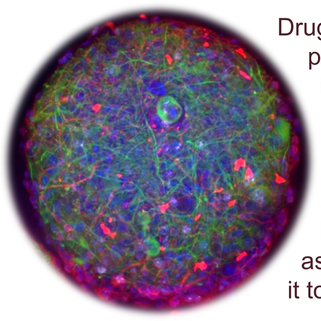
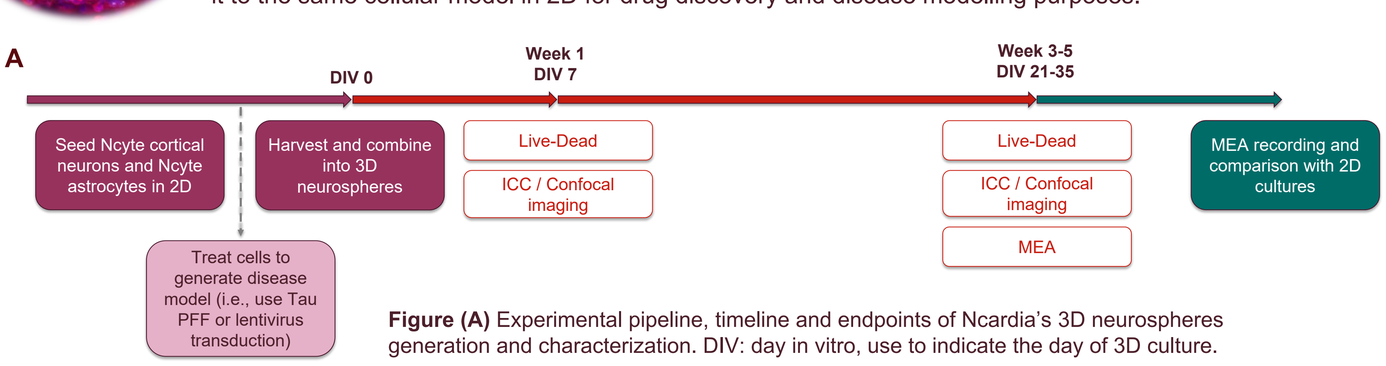
Background



Drug attrition rates remain high, with 90% of drugs tested in Phase I trials failing to reach the market. This is even more pronounced for central nervous system (CNS) targeting drugs. One major reason for this is the use of inappropriate preclinical models, which fail to predict successfully the outcome of clinical trials in terms of efficacy and toxicity.

However, induced pluripotent stem cells (iPSCs) and iPSC-derived 3D models offer potential solutions. iPSC-derived 3D neurospheres can be generated from human cells and better recapitulate the biology (maturity), morphology and molecular dynamics observed in human in vivo. Additionally, these models can be developed from patient derived iPSC, thus increasing the relevance and predictability of the model.

Ncardia generated 3D neurospheres from a co-culture of in house developed hiPSC-derived Ncyte cortical neurons with Ncyte astrocytes and microglia. Ncardia characterized the model in respect of cell composition, maturation and function and compared it to the same cellular model in 2D for drug discovery and disease modelling purposes.



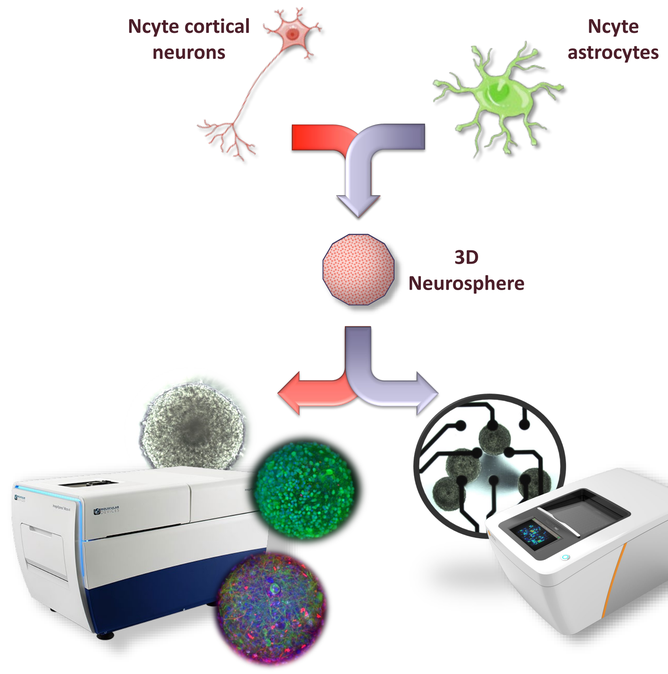


Figure (A) Experimental pipeline, timeline and endpoints of Ncardia's 3D neurospheres generation and characterization. DIV: day in vitro, use to indicate the day of 3D culture.

1. Generation and characterization of 3D Neurospheres

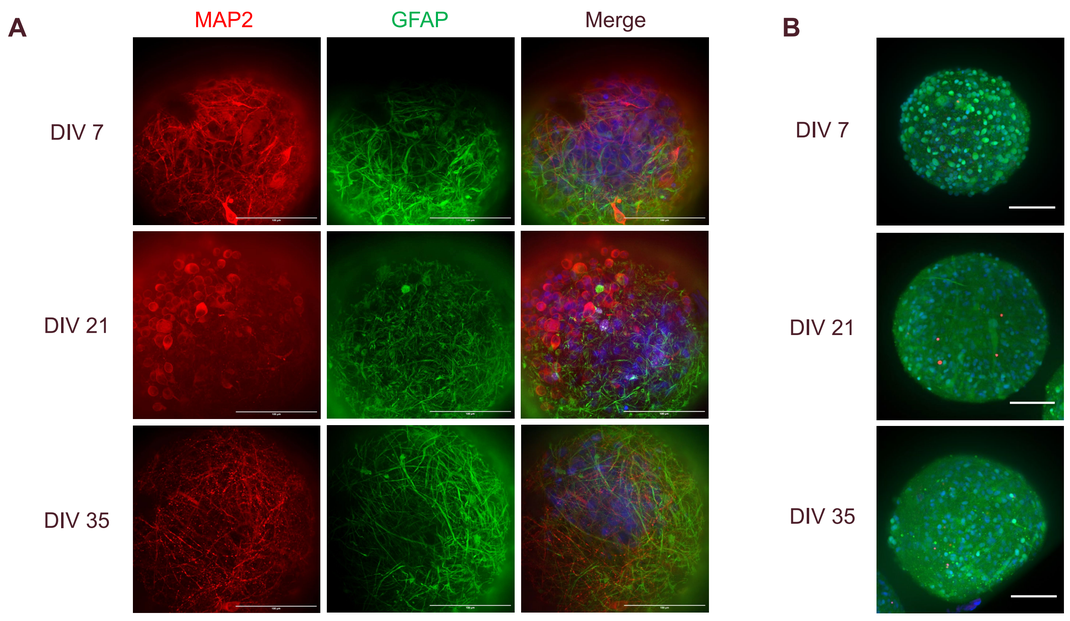


Figure 1 (A) Immunofluorescence for cellular markers. 3D Neurospheres were stained after 1 week in 3D culture (DIV 7), 3 weeks (DIV 21) and 5 weeks (DIV 35) for neuronal marker MAP2 (in red) and astrocyte marker GFAP (in green). Nuclei were stained with DAPI (in blue). The neurospheres show a consistent expression of MAP2 and GFAP throughout time. **(B) Live-dead staining.** 3D Neurospheres were stained with Calcein-AM (in green), which stains live cells, and with PI (in red), which stains dead cells, at DIV 7, 21 and 35 showing good viability at all time-points. Max projection. Scale bar 100 µm.

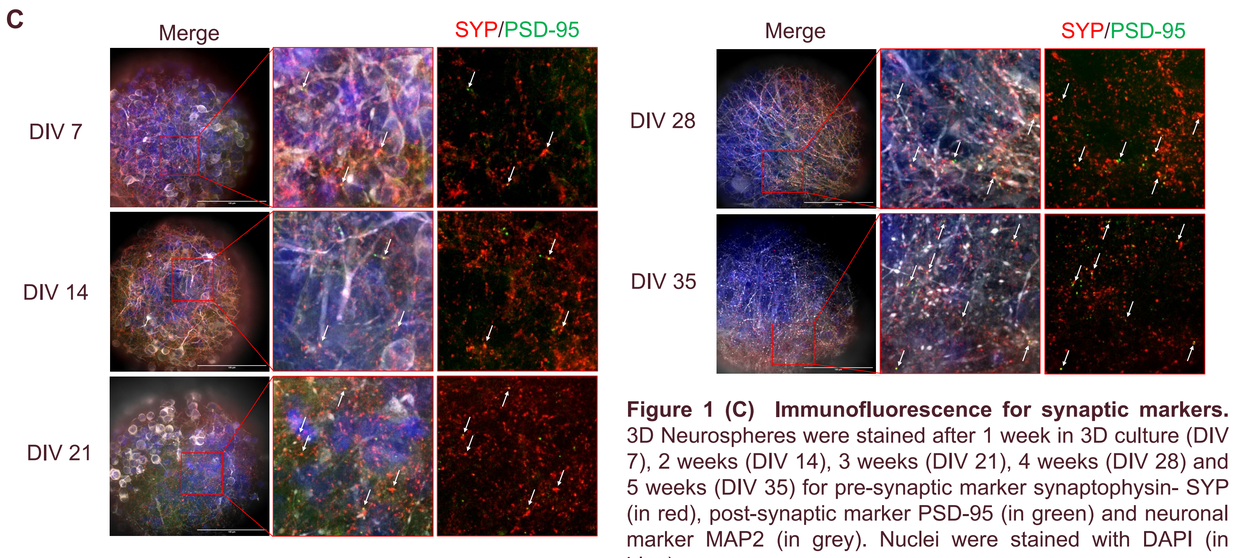


Figure 1 (C) Immunofluorescence for synaptic markers. 3D Neurospheres were stained after 1 week in 3D culture (DIV 7), 2 weeks (DIV 14), 3 weeks (DIV 21), 4 weeks (DIV 28) and 5 weeks (DIV 35) for pre-synaptic marker synaptophysin- SYP (in red), post-synaptic marker PSD-95 (in green) and neuronal marker MAP2 (in grey). Nuclei were stained with DAPI (in blue).

Arrows indicate synaptic structures, expressing SYP and PSD-95. SYP is highly expressed as early as DIV 7 and the expression is consistently maintained throughout culture. Post-synaptic marker PSD-95 is also expressed as early as DIV 7, though at a low level. Expression of PSD-95 is increased in time with highest expression reached at DIV 28. Colocalization of SYP and PSD-95 (in yellow) is also observed. Max projection. Scale bar 100 µm.

2. Electrophysiological function of 3D Neurospheres vs 2D cultures

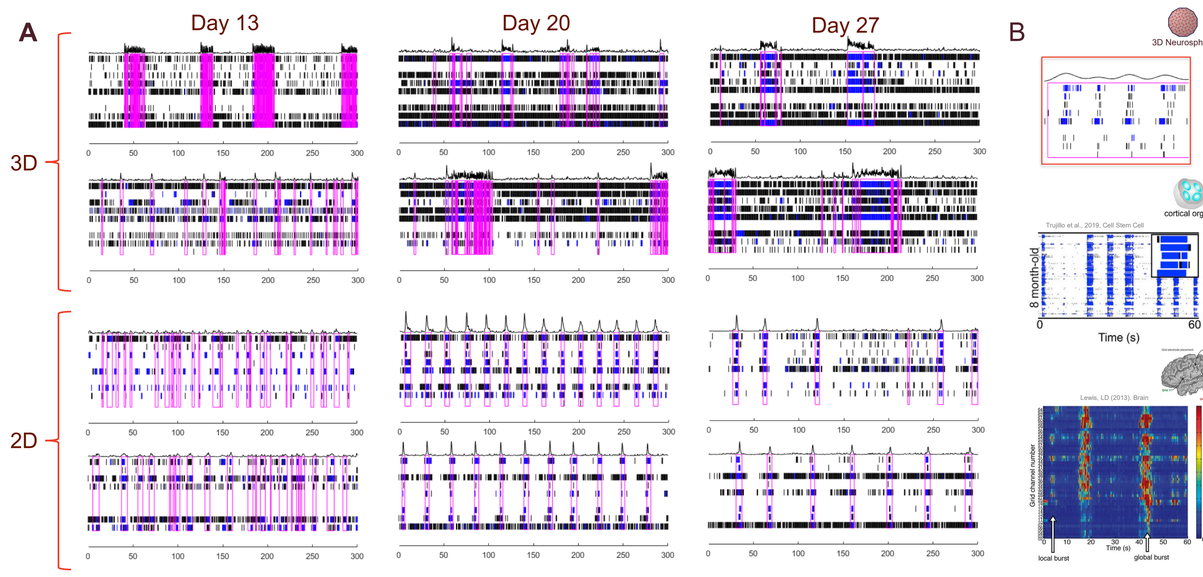
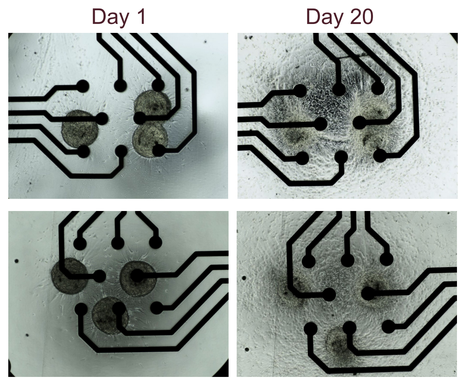
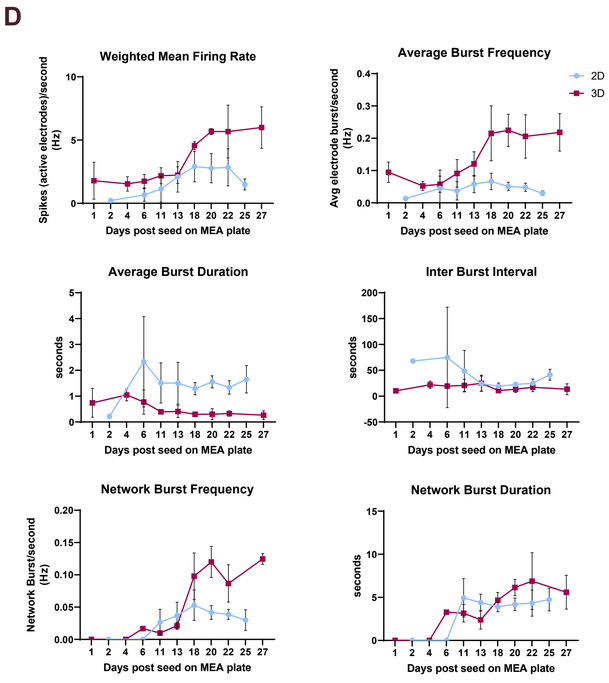


Figure 2 (A) MEA raster plot of 3D vs 2D cultures. Representative raster plots (300 seconds) of 3D vs 2D cultures in time. Both culture conditions show complex and structured firing pattern (bursts and network bursts) after 2 weeks of culture on MEA; 3D cultures, however, show a firing pattern that is more reminiscent of the pattern observed in EEG from brain. **(B) Raster plots comparison.** A close-up of Ncardia's 3D Neurospheres shows complex bursting patterns that are comparable to those observed in mature cortical organoids and human EEG.



(C) Bright field of 3D Neurospheres seeded on MEA plate. 96-well cytoview MEA plates pre-coated with PLO and laminin are seeded with DIV28 3D Neurospheres (Day 1). The neurospheres adhere to the plate and start to migrate outside the spheroid until they completely cover the electrode area (Day 20). **(D) 3D vs 2D neural metrics comparison.** Quantification of key neural metrics highlighted that 3D cultures are more active with a higher number of short and intense complex firing (bursts), and a pacemaker-like synchronicity (network bursts).



3. Example of 3D neurospheres application to disease modeling

The platform developed at Ncardia can be easily adapted to generate complex 3D disease models that recapitulate better the pathology of the disease observed in patients. At Ncardia we used the in house developed 3D Neurospheres to model Tau aggregation often observed in Alzheimer's disease.

Ncyte cortical neurons are seeded in 2D and transduced with a lentivirus expressing Tau mutation P301L. After 24 hours, the cells are harvested and mixed with Ncyte Astrocytes in ultra low attachment v-bottom plates for spheroid generation.

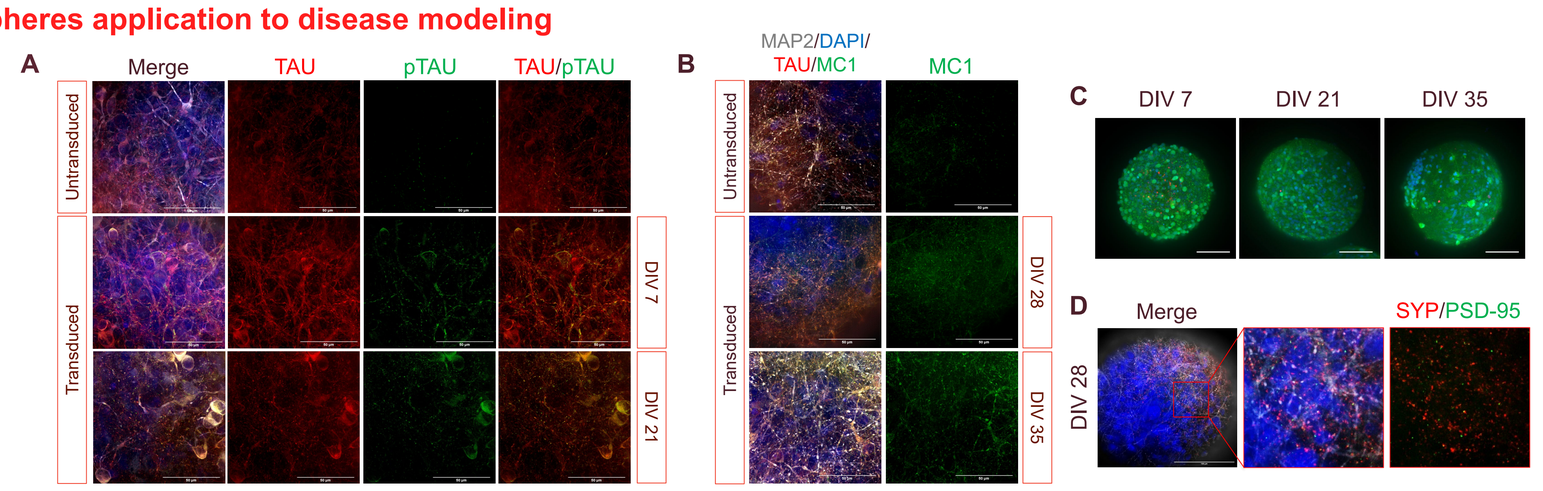


Figure 3 (A) Immunofluorescence for Tau/pTau. Transduced 3D Neurospheres were stained for neuronal marker MAP2 (in grey), Tau (in red) and pTau (in green). Nuclei are stained with DAPI (in blue). Untransduced neurospheres are used as control. The neurospheres show increased TAU and pTAU staining after transduction. **(B) Immunofluorescence for Tau/MC1.** ICC for neuronal marker MAP2 (in grey), Tau (in red) and MC1 (in green). Nuclei are stained with DAPI (in blue). Transduced neurospheres show expression of MC1. Max projection. Scale bar 50 µm. **(C) Live-Dead staining and (D) Immunofluorescence for synaptic markers.** 3D Neurospheres transduced with P301L lentivirus can be cultured for several weeks without affecting viability. They also show a good maturation level as shown by the expression of pre- and post-synaptic markers. MAP2 (in grey), SYP (in red) and PSD-95 (in green). Max projection. Scale bar 100 µm.

Conclusions

- Ncardia generated 3D neurospheres that can efficiently model CNS morphology and function in vitro, both in physiological and pathological conditions.
- This assay platform shows promise in modelling *in vitro* relevant neurodegenerative diseases like Alzheimer and Parkinson by integrating this system with hiPSC-derived microglia cells and/or combining it with Tau or α-Synuclein PFF.
- The developed protocol can be easily adapted to patient derived iPSC lines for disease modelling, target identification and screening to support you through your drug discovery process.