CELL Microsystems®

RAFTNOTE

Making the Impossible Possible: Platform and Protocols to Develop Clonal iPSC Derived Organoids

Reimagine your iPSC-derived Organoid Assays

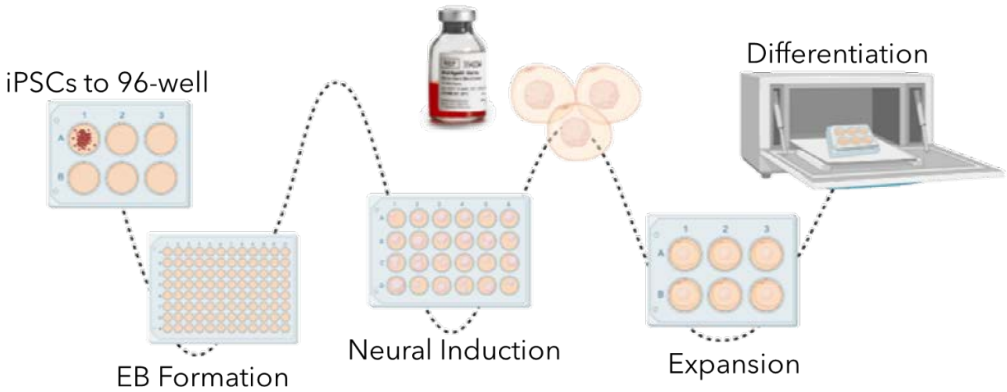
Introduction

Lack of relevant *in vitro* models of human neurodevelopmental and degenerative disorders has been alleviated with the advancements in induced pluripotent stem cells (iPSCs) and organoid technology. In the last decade, several protocols and commercial kits have been launched to help differentiate iPSCs into multicellular, neuronal organoids that closely resemble human brain development, including region-specific cellular composition and functional physiology. However, the adoption of these organoid models is still limited to relatively low throughout applications, as the workflows are hampered by challenges in reproducibility and scalability, as well as being manually intensive. Here we report the use of the CellRaft® Technology, to develop and enable streamlined, reproducible organoid workflows that offer reliable imaging, software-guided selection, and automated isolation of single organoids for downstream applications.

Key Highlights:

1) Track and trace growth and differentiation of hundreds of iPSC-derived organoids
2) Fully automate isolation of individual viable, intact organoids of interest
3) Generate customized 96-well plates for organoid-based assays, such as clonal propagation, drug or toxicity screening, or target discovery

Traditional Workflow



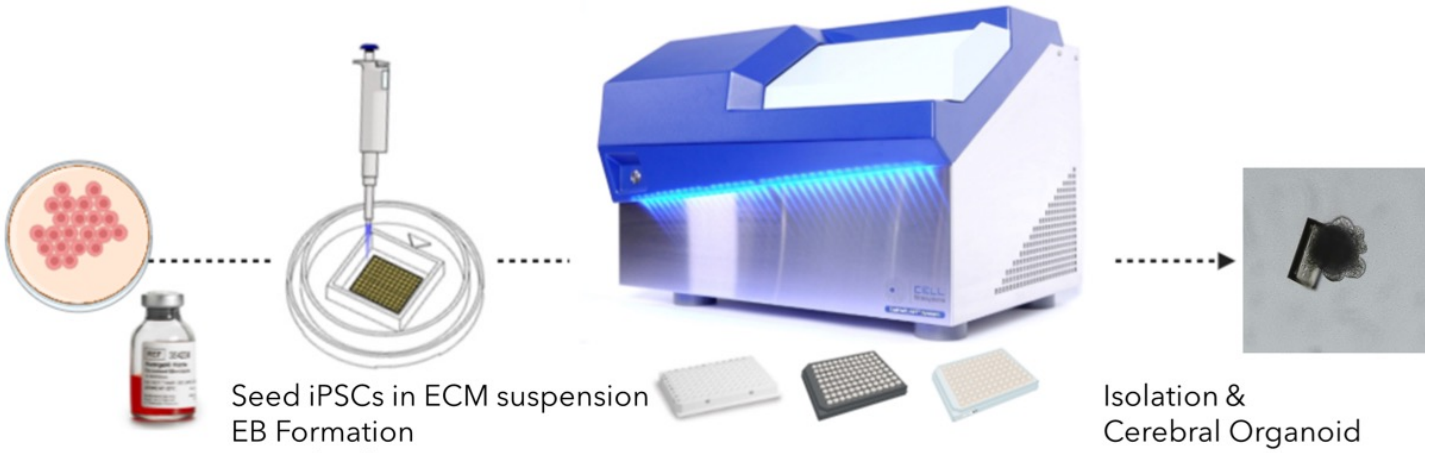
Questions this RaftNote Answers

1. How can iPSCs be reliably differentiated into tissue-specific organoids?
2. What is the best protocol for developing clonal organoids?
3. Can the differentiation of individual organoids be monitored over time?

Experimental Design

Two edited (RFP or GFP) iPSC lines were cultured in mTeSR Plus (StemCell Technologies) using standard methods. iPSCs were differentiated into cerebral and choroid plexus organoids using adapted protocols of commercially available kits (StemCell Technologies). First, iPSCs were dissociated into single cells or small fragments and seeded in a 1:1 GFP+/RFP+ ratio on the 500µm CellRaft Array in a suspension of extracellular matrix in embryoid body formation media. An initial scan to capture images of single cells, or small fragments of cells, was performed 4 hours after cell seeding using the CellRaft AIR® System. The arrays were scanned every 24 hours to monitor organoid formation and differentiation, and media changes for neural induction and expansion were performed at timepoints recommended by the manufacturer. The CellRaft Cytometry™ software was used to identify CellRafts containing single-iPSC-derived organoids based on diameter (100-300 micron). CellRafts with single organoids were identified and isolated into a 96-well plate with cerebral maturation media on day 10. Organoids were maintained in cerebral maturation media out to day 43 with media changes every 2-3 days. On day 28, non-GFP+ organoids were stained using NeuroFluor NeuO (StemCell Technologies) to visually confirm neural differentiation. On day 43, single, mature cerebral organoids were treated with a 6-point dose curve of ethanol (6.25-50 mM) for 6 hours to simulate acute alcohol exposure. To evaluate alcohol-induced apoptosis, relative caspase activity was measured using the Caspase3/7-Glo Assay (Promega).

CellRaft Technology Workflow



Results

We successfully generated and captured time-course images of hundreds of iPSC-derived neuronal organoids on each array. Mono- and dual-fluorescent cerebral and choroid plexus organoids were established from single cells, or multiple cells, using our amended protocols (Figure 1). Using the CellRaft Cytometry software, we were able to quantify single-cell organoid forming efficiency across three replicate arrays for each organoid type (Figure 2). Clonal iPSC organoid formation was achieved with a high degree of efficiency (>17%). Using the AIR System, automated isolation of CellRafts with single organoids was performed to deposit organoids that were selected based on diameter (100-300 micron) into 96-well collection plates. This process preserves organoid morphology and viability, and organoids were subsequently cultured off-array in cerebral maturation media out to day 42 (Figure 3). Significant three-dimensional and neural outgrowth (>98%), which stained positively for live neurons, was observed in cerebral organoids by day 25 (Figure 4A). Images were captured of each organoid on day 40, after the cerebral organoids were fully mature. By selecting organoids for isolation based on size on day 10, we were able to generate a 96-well plate with single mature organoids that are relatively similar in size for our downstream toxicity screen. Using day 43 cerebral organoids, we performed an ethanol dose-curve to evaluate the effect of acute alcohol exposure on mature cerebral organoids. At the highest doses, we observed a dose-dependent activation of caspase activity, an indicating alcohol-induced initiation of apoptosis (Figure 4C).

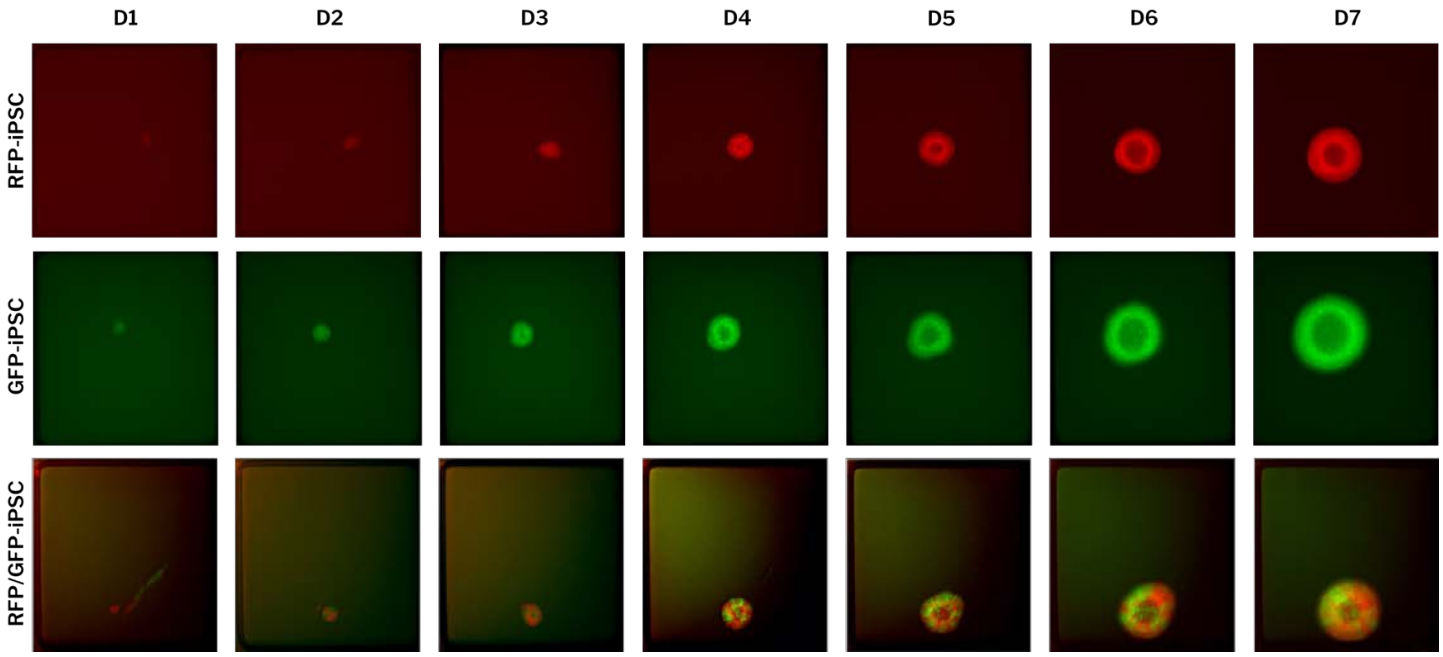


Figure 1. Edited single iPSCs are differentiated on the 500µm CellRaft Array to form mono- and dual-fluorescent neural organoids. RFP- and GFP-edited iPSCs were co-cultured on the CellRaft Array in dilute ECM and imaged daily for a week during embryoid body formation and neural induction for choroid plexus organoid differentiation.

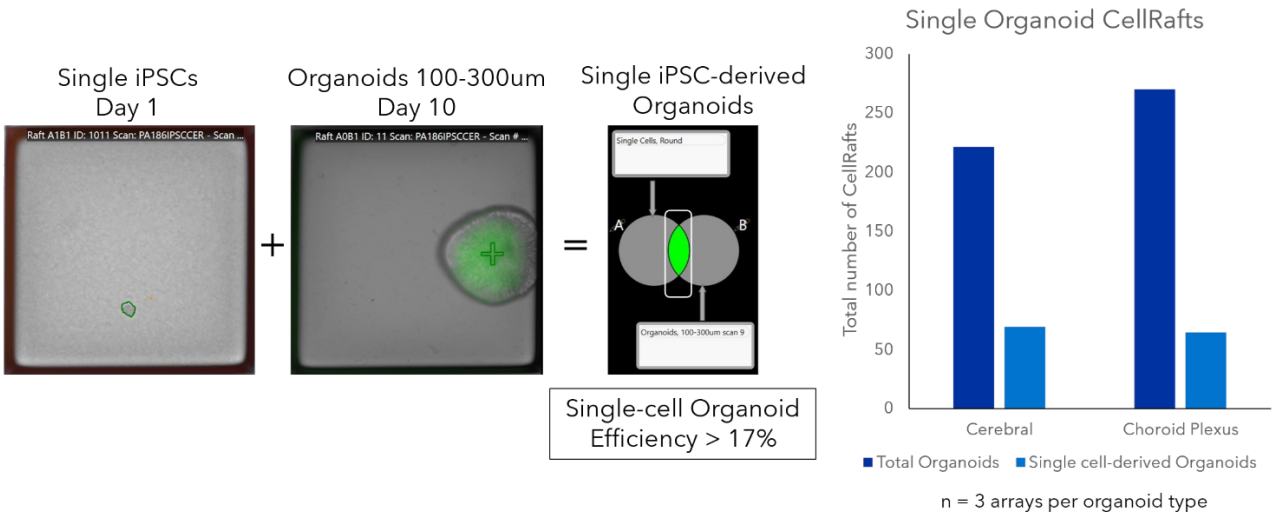


Figure 2. Image-based, software-guided organoid analysis. Using CellRaft Cytometry, user-defined populations can be built to identify CellRafts for isolation. For clonal workflows, populations identifying CellRafts with a single cell at day 1 and organoids at later timepoints can be overlayed to identify clonal organoids of interest, including fluorescence and morphological parameters.

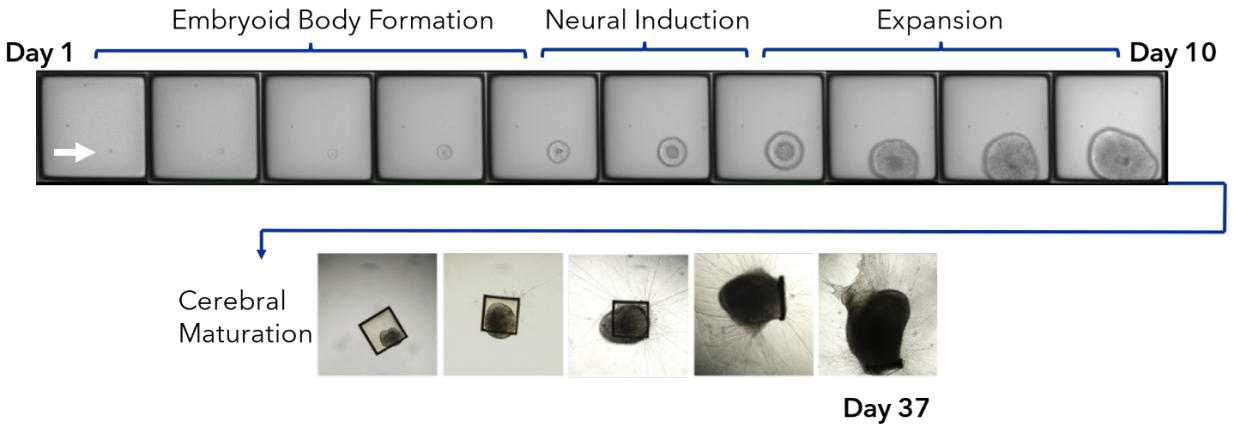
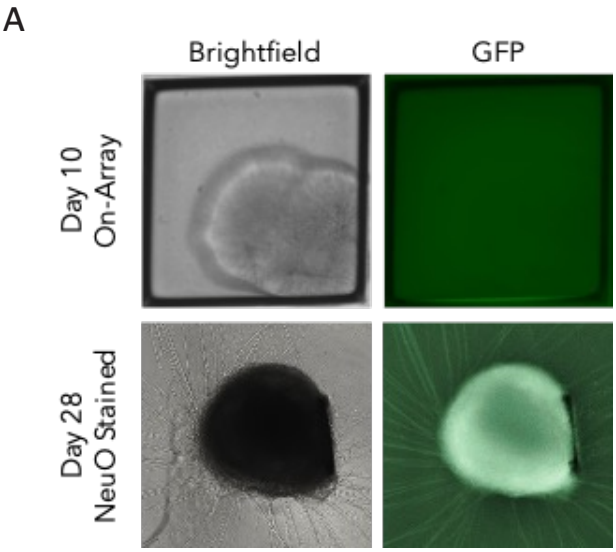
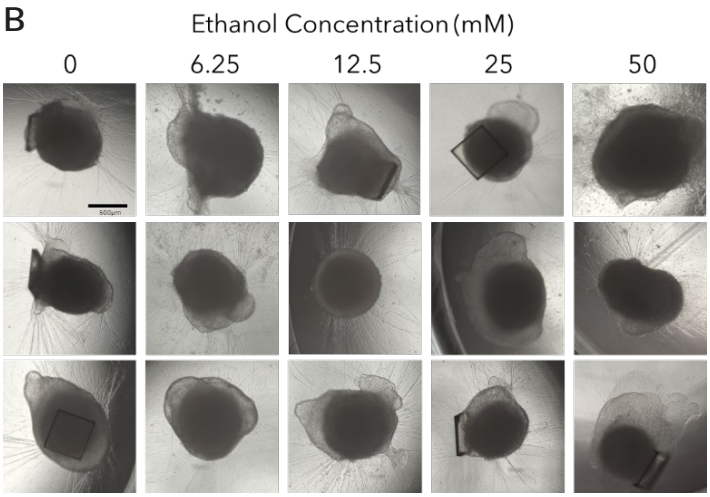


Figure 3. Clonal iPSC organoid neuronal differentiation. A mixed populations of edited (GFP or RFP) iPSCs were seeded on the 500µm CellRaft Array in a suspension of Matrigel. The first several stages of organoid differentiation, embryoid body formation (days 1-5), neural induction (days 5-7), and expansion days (7-10), were performed on-array by performing media changes. The array was scanned every 24 hours to monitor organoid differentiation. At day 10, single organoids were isolated into 96-well plates into maturation media. Cerebral organoids were maintained in maturation media to day 42.





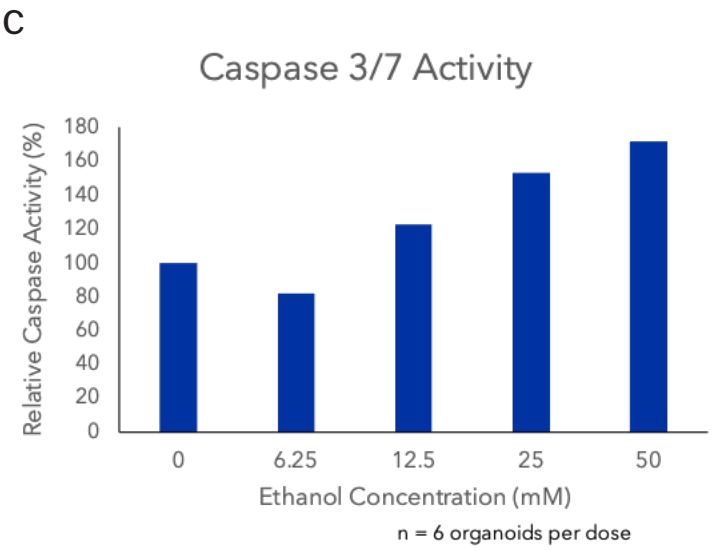


Figure 4. Alcohol induced apoptosis in neuronal organoids. On day 28, non-GFP organoids were stained using NeuroFluor NeuO, a membrane permeable fluorescent probe for detecting live neurons, as a visual confirmation of neural differentiation **(A)**. Images of mature cerebral organoids on day 40, prior to treatment with ethanol, from three of the six replicate wells demonstrating the ability to generate well-to-well reproducibility it the downstream assay on day 40 by selecting organoids based on size at day 10 **(B)**. On day 43, mature cerebral organoids were exposed to a 5-point dose curve of ethanol (6.25-50mM, n = 6 wells per dose) to simulate acute alcohol exposure. After 6 hours of treatment, organoids were evaluated for alcohol-induced apoptosis using the Caspase-Glo 3/7 Assay (Promega). We observed a dose-dependent increase in caspase activity, supporting activation of apoptosis at the highest doses of ethanol exposure **(C)**.

Discussion

Using the CellRaft technology, we have demonstrated an efficient, user-friendly workflow for growing and isolating hundreds of single iPSC-derived neuronal organoids. The CellRaft Array provides a unique platform for organoid culture that allows for spatial segregation of cells and reliable imaging over time, enabling clonal organoid propagation that is not possible using standard methodologies. A special feature of the platform is CellRaft Cytometry, which offers software-guided selection of CellRafts containing single organoids based on desired phenotypic, morphologic, and fluorescent properties for isolation using the AIR System. Our data demonstrates that the CellRaft Technology solves several challenges in cerebral organoid workflows, including higher yield and a less manual workflow, the ability to phenotypically characterize a heterogenous organoid population, and automated isolation of individual organoids of interest into 96-well plates for downstream assays. Altogether, the CellRaft Technology unlocks the potential for generating 96-well plates of custom, edited iPSC-derived organoids that can be used for any downstream application.

Conclusion

To accelerate the utility of iPSC-derived organoids into pre-clinical applications, there are three main challenges that must be addressed- scalability, reproducibility, and automated solutions for streamlining otherwise manual workflows. Using the CellRaft Array, hundreds of organoids can be grown in a single focal plane using less media and ECM reagents, compared to standard culture methodologies. Increasing organoid number, while reducing reagent usage, allows researchers to increase the scalability of generating organoids and ultimately increases the throughput of downstream assays. In addition, using the image-based software-guided selection tools, we have shown that selecting organoids based on size and other phenotypic characteristics improves well-to-well reproducibility for downstream assays and allows for normalization across multiple arrays and experiments. Lastly, the CellRaft AIR System provides an automated solution for isolating individual pre-characterized organoids into 96-well plates for downstream use. This, paired with more streamlined workflows for generating organoids, fills an unmet need for automating the process of generating reproducible 96-well plates for downstream organoid screening assays. Altogether, the CellRaft Technology offers a solution to move these more physiologically relevant organoid models into drug discovery and pre-clinical pipelines in a more efficient, cost-effective, and reproducible way.

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