ACCELERATING THE USE OF PATIENT-DERIVED hiPSC NEURONAL MODELS BY INCREASING EFFICIENCY OF REPROGRAMMING AND DIFFERENTIATION USING CELLRAFT TECHNOLOGY

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BACKGROUND

Establishing and differentiating patient-specific hiPSCs provides unmatched potential in developing accurate neuronal disease models. Despite the potential, the protocols associated with reprogramming somatic cells and differentiating hiPSCs are highly technical and require manual manipulation, which contributes to low efficiency, limited throughout, and lack of reproducibility. We hypothesized that CellRaft® technology could overcome technical challenges in reprogramming and differentiating hiPSCs into 2D and 3D neuronal models, providing an automated solution for these manual workflows. To evaluate reprogramming efficiency, electroporated fibroblasts were seeded in either a 6-well plate or in a CellRaft® Array. The CellRaft Array provides a novel culture environment combining flask-like culture with single cell separation, enabling precise monitoring of reprogramming efficiency, viability, and clonality. Using live staining for Tra-1-60 on the CellRaft Array, we identified more than 300 pluripotent clones, and fully characterized monoclonal hiPSC cell lines more than 1 month faster than the standard manual workflow which generated only 1 unconfirmed hiPSC colony. To optimize neural progenitor (NPC) differentiation, embryoid bodies were seeded on CellRaft Arrays in neural induction media to monitor formation of neural rosettes. Automated retrieval of CellRafts containing neural rosettes was performed using the AIR System, eliminating the need for manual dissection or rosette selection reagents. Post-isolation staining revealed 100% of CellRafts containing neural rosettes were Pax6 positive, indicating high fidelity of NPC differentiation. We also developed protocols for differentiating hiPSCs into neural organoids, including cerebral and choroid plexus. Using the CellRaft Array, segregated organoids, including clonal organoids, can be reliably imaged and analyzed over time, enabling temporal phenotypic selection of hundreds of single organoids, which is not possible using standard techniques. At desired timepoints, single organoids of interest can be retrieved from the array using the CellRaft AIR System for continued growth or downstream assays. Using software-guided selection, we have demonstrated the ability to reliably generate custom, reproducible single organoid assay plates for drug screening of normal and diseased hiPSCs. Together, these results demonstrate the ability of the CellRaft AIR Technology to provide an end-to-end solution for otherwise challenging workflows that will accelerate the utility and reproducibility of generating patient-specific hiPSCs and mature neuronal models.

APPLICATIONS

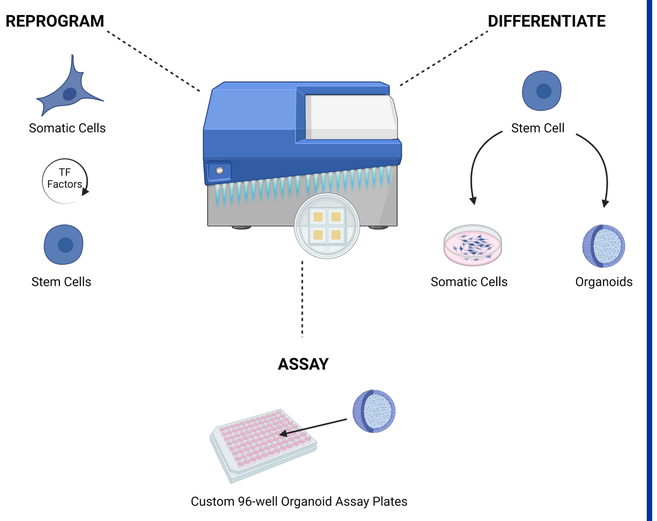


Figure 1: Graphical abstract of applications using CellRaft Technology. Using CellRaft Technology, we developed automated workflows to overcome technical challenges in reprogramming and differentiating hiPSCs into 2D and 3D neuronal models. In addition to these methods providing an automated solution to these technically challenging workflows, we have demonstrated that the CellRaft technology increases efficiency and reproducibility, compared to manual methods.

- **Automate hiPSC reprogramming and 2D and 3D differentiation workflows**
- **Live stain for selection and confirmation**
- **Select for monoclonality, morphology, and phenotype with analytical software**
- **Reduce population heterogeneity and increase total yield**

Take a picture to learn more

hiPSC Reprogramming

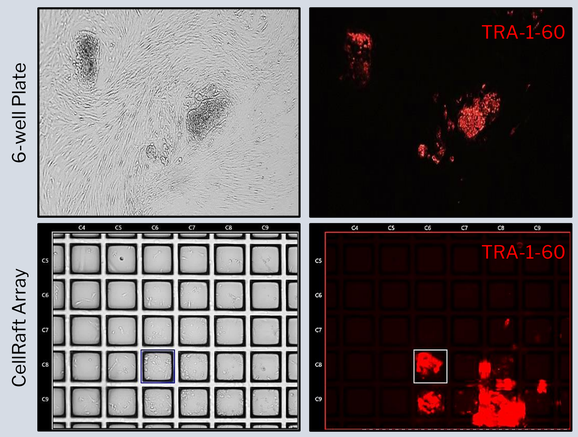


Figure 2: CellRaft Array enables segregation of reprogrammed foci and automated isolation. 6-well plate containing reprogrammed BJ cells in a lawn of non-reprogrammed BJ fibroblast (Top). 200µm CellRaft Array containing segregated reprogrammed BJ cells (Bottom) Representative images of brightfield and TRA-1-60 live staining (AF594.

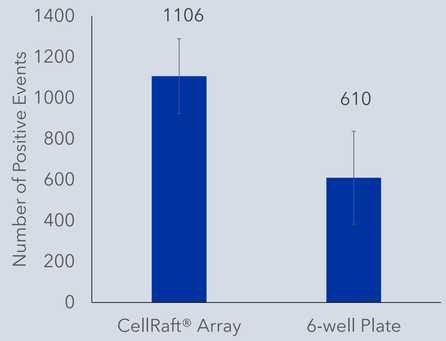


Figure 3: CellRaft Array workflow generates more pluripotent foci. Transfected BJ Cells (Epi5 Episomal iPSC Reprogramming Kit) were cultured for 15 days in N2B27 medium supplemented with bFGF in a 200µm CellRaft Array or in a 6-well plate. On D15, cells were live-stained using TRA-1-60 Alexa Fluor 594 Conjugate Kit and positive events were calculated using CellRaft® Cytometry for the Array and manually counted and averaged n=3 counts for the 6-well plate. CellRaft Array n=7 and 6-well plate n=4.

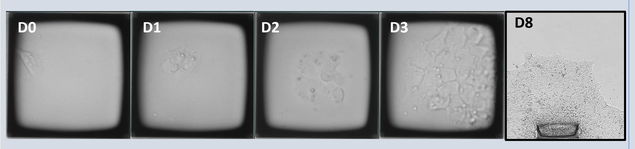


Figure 4: Efficient monoclonal iPSC generation using CellRaft Technology. Images of a CellRaft containing a clonal iPSC colony derived from bulk polyclonal reprogrammed cells with iPSC-like morphology (D0-D3 before being isolated into a 96-well plate). Post-isolation, the iPSCs grew off the CellRaft and a clonal colony emerged with iPSC morphology by D8.

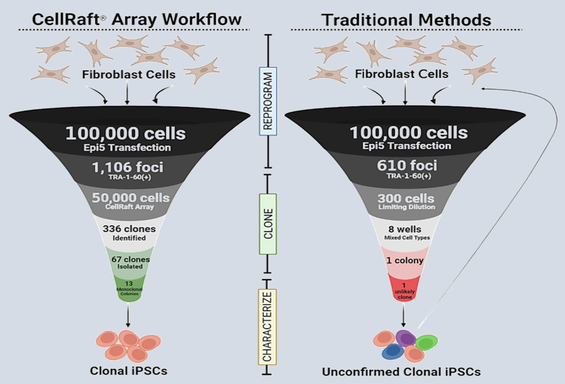
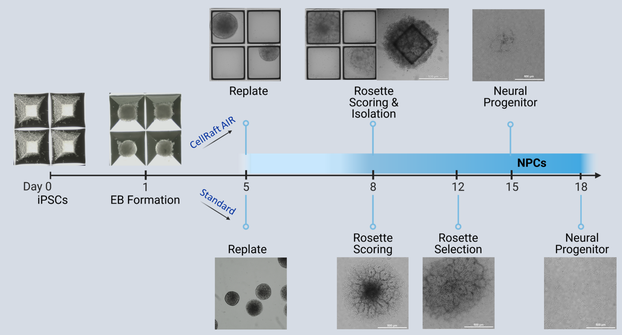


Figure 5. Comparison of the number of successful iPSC monoclonal lines that were generated by the CellRaft Technology workflow (left) compared to the traditional methods (right).

Neural Progenitor Differentiation



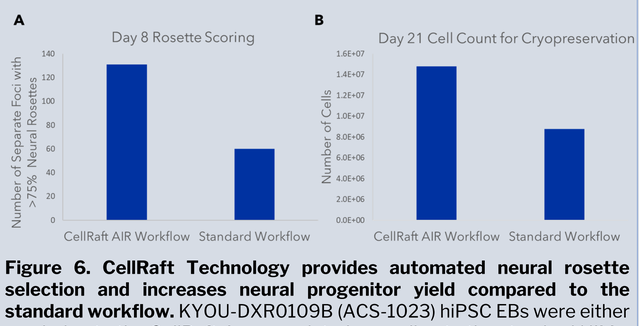


Figure 6. CellRaft Technology provides automated neural rosette selection and increases neural progenitor yield compared to the standard workflow. KYOU-DXR0109B (ACS-1023) hiPSC EBs were either seeded onto the CellRaft Array or plated according to the standard NIM + SMADi workflow. On day 8 (A) the CellRaft Array contained more than double the number of neural rosettes with >75% neural rosette morphology, compared to the companion 6-well plate. Additionally, the CellRaft workflow resulted in >1.6-fold increase in total number of cells available for cryopreservation on Day 21 after 2 passages in Neural Progenitor Media (B).

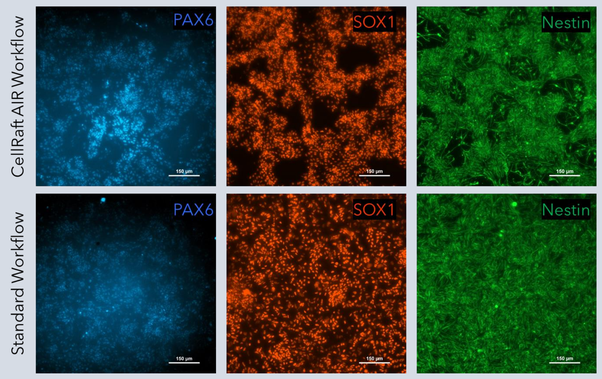


Figure 7: Differentiated neural progenitor cells demonstrate canonical neural marker expression. NPCs (p2, Day 24) generated using the CellRaft AIR and standard workflows were stained for neural progenitor markers PAX6, SOX1, and Nestin to validate neural induction.

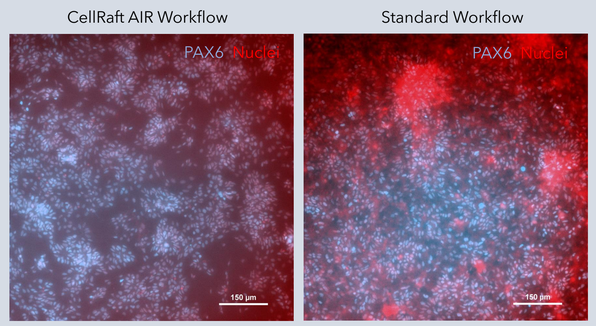


Figure 8: The CellRaft AIR workflow generates a homogeneous neural progenitor cell population compared to the standard workflow. NPCs (p2, Day 24) generated using the CellRaft AIR and standard workflows were stained for neural progenitor marker PAX6 and counterstained with a NucRed (647) nuclear dye. The CellRaft AIR population exhibits canonical rosette morphology, as well as more consistent overlay of PAX6 positive nuclei and total nuclei indicating a more homogeneous population of cells compared to the standard workflow.

Custom Neural Organoid Assays

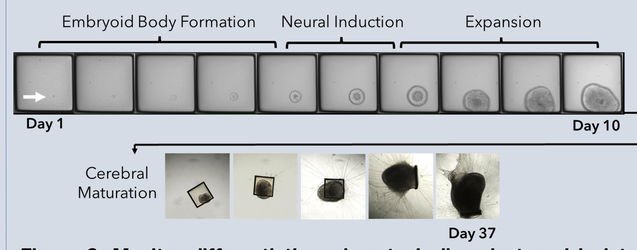


Figure 9. Monitor differentiation, phenotypically select, and isolate individual organoids to generate custom assay plates for downstream screening. hiPSCs were seeded on the 500µm CellRaft Array in an ECM suspension and imaged daily to monitor embryoid body formation (days 1-5), neural induction (days 5-7), and expansion days (7-10). On day 10, single organoids were phenotypically selected by size using software tools and isolated into 96-well plates into maturation media. Cerebral organoids maturation was performed in 96-well plates to day 42.

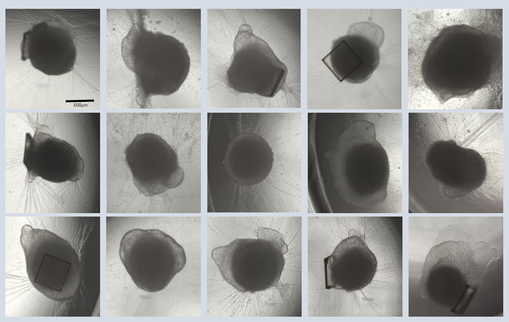


Figure 10. Size-selected organoids maintain well-to-well reproducibility for downstream assays. On day 10, CellRaft Cytometry is used to select organoids for isolation based on diameter. Single organoids were isolated into 96-well plates into maturation media and maintained in the 96-well plates to day 42. Brightfield images are representative of replicate wells within an assay plate and demonstrate the ability to maintain a high-degree of consistency for downstream drug screening.

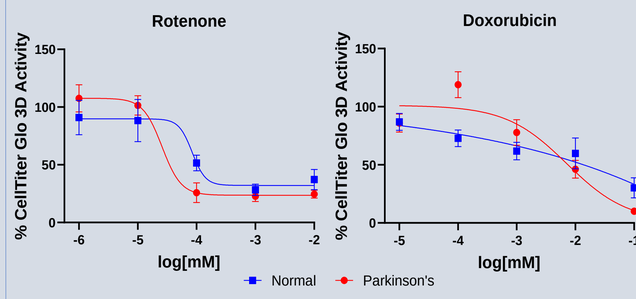


Figure 11. Disease model organoids have increased sensitivity to Neurotoxic Compounds. Day 43 mature cerebral organoids, derived from hiPSCs derived from normal (KYOU-DXR0109B), or Parkinson's (DYS-0530) donors were exposed to a 5-point dose curve of Rotenone (insecticide) or Doxorubicin (anti-cancer drug) to evaluate disease-related neurotoxicity sensitivity. EC50 values were 2-fold higher in organoids derived from normal donors for both Rotenone (Normal EC50 = 0.105µM, Parkinson's EC50 = 0.034µM) and Doxorubicin (Normal EC50 = 14.1µM, Parkinson's EC50 = 7.4µM

CONCLUSIONS

- CellRaft Technology automates laborious manual reprogramming and differentiation workflows for efficient and simplified generation of hiPSC-derived cell types.
- CellRaft Cytometry software provides quantitative analysis with customizable algorithms to detect and filter for monoclonality, fluorescence, and morphological features.
- The automated workflows for NPC differentiation increased cell yields, improved homogeneity of differentiation, and shortened experimental timelines compared to the traditional manual workflows.
- Using automated workflows for creating customized organoid assay plates enabled inter- and intra-assay reproducibility across normal and diseased cell lines.

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