ACCELERATING THE USE OF iPSCs IN DRUG DEVELOPMENT AND PERSONALIZED MEDICINE

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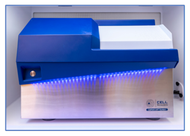
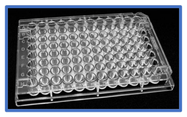
BACKGROUND

- Flow cytometers, droplet dispensers, and limiting dilution, which aim to deposit a **single iPSC in a well**, often result in a **strenuous** and **unnatural** environment that leads to low efficiency of clonal survival.
- The **CellRaft® Array** offers a more biological culture environment by providing **flask-like culture** conditions combined with single-cell segregation for tens of thousands of cells per consumable.
- Given the unique ability of the **CellRaft AIR® System** to identify CellRafts containing discrete numbers of cells at different timepoints, we sought to compare the clonal formation efficiency of cells isolated at various stages along the exponential growth curve to demonstrate whether isolating a colony rather than a single cell **improved** the efficiency and success rates of single cell cloning **workflows**.

METHODS





Seed Cells
- Coat 200µm CellRaft® Array with desired matrix
- Seed iPSCs

Image
- CellRaft AIR® System
- Perform serial 2D scans to monitor iPSCs

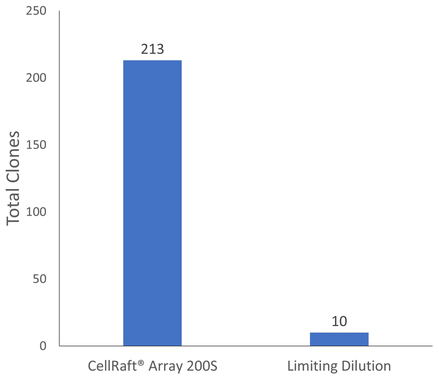
Identify
- CellRaft Cytometry™
- Brightfield or fluorescent analysis

Isolate
- CellRaft AIR System
- Isolate CellRafts® with iPSC colonies into 96-well plates

Expand
- 96-well collection plates containing viable iPSC colonies for downstream

RESULTS

- In limiting dilution, only 10% of single iPS cells form viable monoclonal colonies. Similarly, at the single cell-stage of iPSC cell expansion, using the CellRaft technology, only 10% of wells in the 96 well plates contained viable clones.
- By isolating **CellRafts** at the four cell- and colony-stage, iPSC clonal outgrowth **increased** significantly to **87%** and **98%,** respectively.
- Allowing the single cells to continue to grow undisturbed in a shared microenvironment prior to colony isolation, **optimizes** outgrowth efficiency such that it is no longer a rate limiting factor in cell line development **workflows**.
- CellRaft technology supports a variety of **iPSC workflows** including **expansion** of single cells, **differentiation** into various cell types or tissue-specific organoids, **characterization** of fluorescent expression or stained cells, and **reprogramming** human fibroblasts into iPSCs.



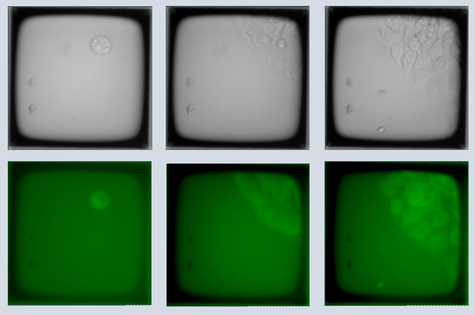
Single human-iPSCs were seeded directly onto an uncoated 200µM Single CellRaft Array in dilute iMatrix-511 (Matrixome) at a density of 5:1 (cells:rafts). In parallel, limiting dilution comparison plates were seeded at a density of 1 cell/well in n=5 96-well plates pre-coated with h-ESC Matrigel.

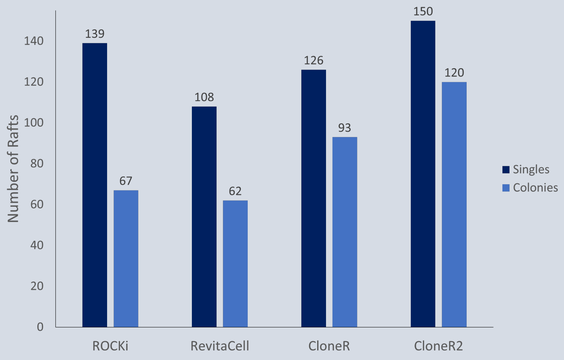
CELL Microsystems

- **Generate 100s of monoclonal iPSC lines in under two weeks**
- **Achieve >90% clonal outgrowth by isolating monoclonal colonies**
- **Enable all iPSC workflows from reprogramming to differentiation on one consumable**
- **Optimize your iPSCs for 2D and 3D culture**

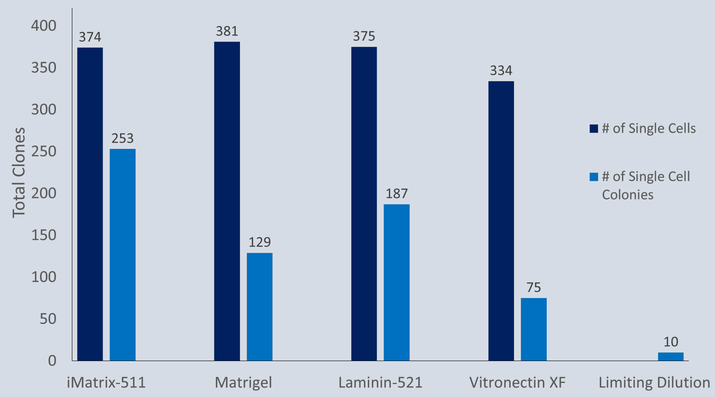
Take a picture to learn more

iPSCs on CellRaft® Arrays



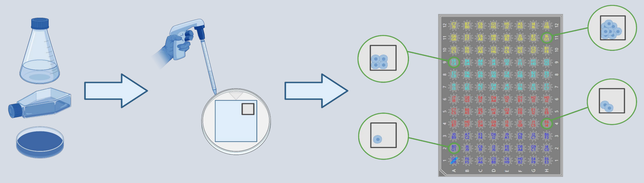


200µm Single CellRaft Array was coated with iMatrix-511 overnight in the incubator. GFP iPSCs were seeded at a ratio of 5 cells per CellRaft in mTesR Plus, dilute iMatrix-511, and various additives. Serial scans of the array were performed daily and CellRafts containing monoclonal colonies were isolated using the CellRaft AIR® System

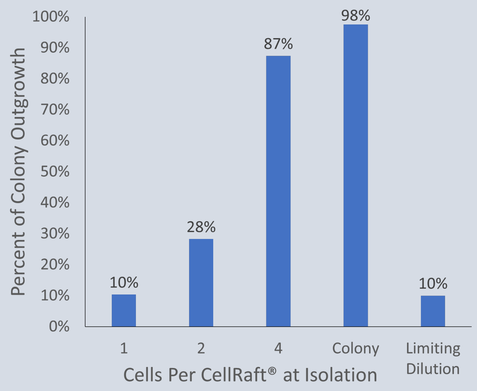


iPSCs were seeded onto a 200µm Quad CellRaft Array in various dilute concentrations of common matrices. Single cell-derived iPSC colonies were identified and isolated on the CellRaft AIR System.

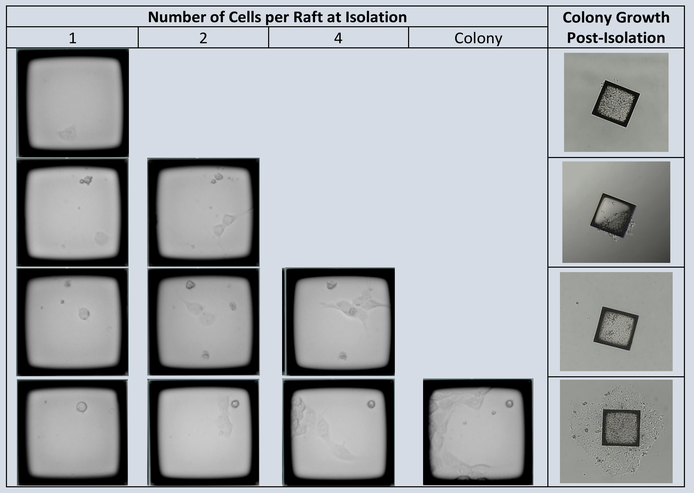
iPSC Single Cell Expansion



iPSCs were seeded onto a 200µm Quad CellRaft Array in dilute iMatrix-511. The Array was serially scanned every day for 3 days to compare the clonal formation efficiency of iPSCs isolated at various stages along the exponential growth curve to determine the optimal stage for reliable colony growth.

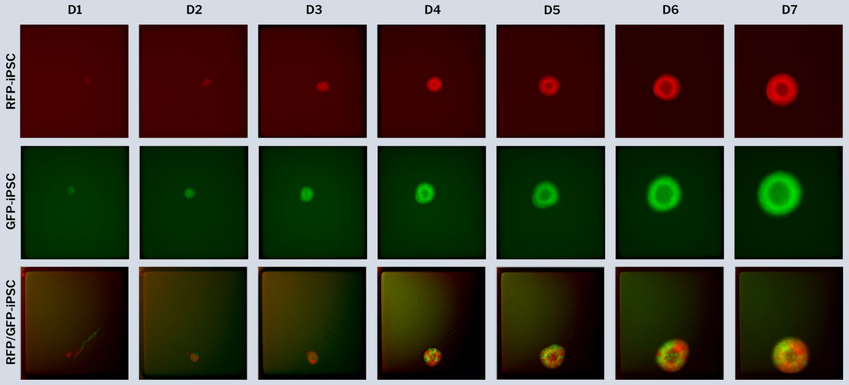


Outgrowth efficiency of isolated CellRafts containing single iPSC-expanded cells was observed, in comparison with limiting dilution plates.



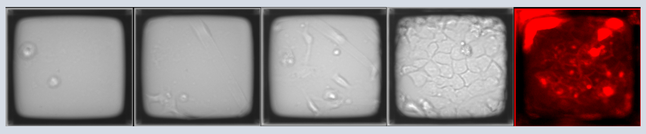
Four hours post-seeding, the CellRaft Array was imaged to identify CellRafts containing iPSCs. Over the ensuing 3 days, CellRafts at various stages along the growth curve were isolated. Following isolation, colony outgrowth was monitored for one week.

iPSC Differentiated Organoids



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.

Reprogram iPSCs



A 200µm Single CellRaft Array and a 6-well plate were coated with dilute Geltrex. Human fibroblasts were transfected then seeded onto either the array or 6-well plate. Array and 6-well plate were imaged daily. After 15 days, cells were live stained for Tra-1-60 prior to isolation, to confirm pluripotency of reprogrammed iPSC clones.