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OMNI Life Science

RAFTNOTE

Taking the fear out of iPS cell line development

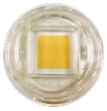
How to dramatically improve the efficiency and success of iPSC cloning, while decreasing time, expense, and effort

Since their discovery in 2006 by Takahashi and Yamanaka, induced pluripotent stem cells (iPSCs) have seen a meteoric rise in their use throughout academic and industrial research. Generated by reprogramming terminally differentiated somatic cells with four key transcription factors (Oct4, Sox2, KLF4, and c-myc), human iPSCs have the potential to unlimitedly proliferate and differentiate into all of the key cell types in the human body. These cells hold promise for a myriad of therapeutic research areas, including cancer and regenerative and personalized medicine. In addition, they alleviate many of the ethical concerns and sourcing issues associated with pluripotent cells such as human embryonic stem cells. Despite significant strides in technology and increased access for researchers, iPSC culture remains a challenge, thereby limiting its widespread utility for therapeutic and research use. Common pain points include:

1) iPSC lines are sensitive and easily perturbed, requiring constant maintenance.
2) Poor culture conditions and cell line instability can lead to spontaneous differentiation and loss of pluripotency.
3) Generating monoclonal cell lines is incredibly challenging, with low efficiency and lack of proven clonality.
4) The labor, cost, and reagent burden associated with iPSC maintenance and workflows is incredibly high and often prohibitive for the end user.

Key Highlights:

1) Viability during single-cell iPSC workflows is improved by using the CellRaft Array that provides **flask-like culture conditions while maintaining spatial segregation.**
2) iPSCs can be imaged at the single cell stage and monitored for clonal growth and pluripotency using software-guided analytical tools.
3) CellRaft Technology provides a fully automated, single-instrument iPSC workflow that decreases cost and time per clone, while **screening 500X more cells per consumable** than a standard 96-well plate.

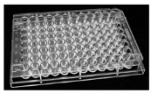


Seed Cells
- 200 um CellRaft Array
- Seed iPS cells on coated Array

Image
- CellRaft AIR System
- Perform serial scans to monitor iPSCs

Identify
- CellRaft Cytometry
- Use brightfield and fluorecence analysis to select population of interest based on phenotypic and morphologic parameters

Isolate
- CellRaft AIR System
- Automated isolation of CellRafts containing iPSC colonies of interest into coated 96-well collection plates



Expand
- 96-well collection plates containing iPSC colonies can be used for downstream analysis or expanded

Hypothesis

Inefficient iPSC cloning is a common bottleneck in stem cell research. We hypothesized that the favorable culture environment of the CellRaft Array would improve single cell iPSC cloning significantly, not only by increasing the number of clones generated, but also by decreasing the time to develop a validated cell line.

Materials and Methods

A stable line of iPSCs was adapted to single cell passaging from aggregate passaging. Once adapted, cells were seeded at a 5:1 ratio of cells:rafts on the CellRaft Array in 3mL of iPSC culture media. Using this same population of cells, 3 plates were also seeded at 10 cells/mL (Countess II, Thermo Fisher), in 100uL iPSC culture media, to compare limiting dilution to CellRaft Technology. ROCK inhibitor was added to the media, and to feed the cells, ½ changes were performed in the Array until isolation. At the day of isolation, plates were pre-coated with extracellular matrix, pre-loaded with media, and the CellRaft Array was filled to 5mL with media. Tra-1-60 (Thermo Fisher) was used at the manufacturer's recommended concentration and left to stain on the CellRaft Array for 1 hour. The CellRaft Array was rinsed and imaged, and positive clonal rafts were selected and isolated into the 96-well collection plate.

Results/Data

Four different iPSC cell lines were seeded on 200µm CellRaft Arrays coated with either iMatrix-511 (Matrixome), hESC-qualified Matrigel (Corning), or Laminin-521 (STEMCELL Technologies). As shown in Figure 1, single iPSCs can easily be identified on individual CellRafts, allowing for visual confirmation of monoclonality. Serial scans on the AIR System enable precise monitoring of cell viability, phenotype, and colony formation. The live iPSC colonies were stained with Tra-1-60, demonstrating that the cells remain pluripotent on the CellRafts and ensuring that there is no spontaneous differentiation (Figure 2). Once sizeable colonies formed, CellRafts containing monoclonal iPSC colonies of interest were isolated into iMatrix-511 or hESC-qualified Matrigel pre-coated 96 well plates, and colony outgrowth off raft was monitored (Figure 3). The iPSCs were allowed to continue growing in the collection plate, with over 70% efficiency (Figure 3). **Over 200 viable, verified monoclonal iPSC colonies were identified** per consumable, compared to only 10 colonies per 96-well plate in limiting dilution that display poor morphology and are of unknown clonality (Figure 4).

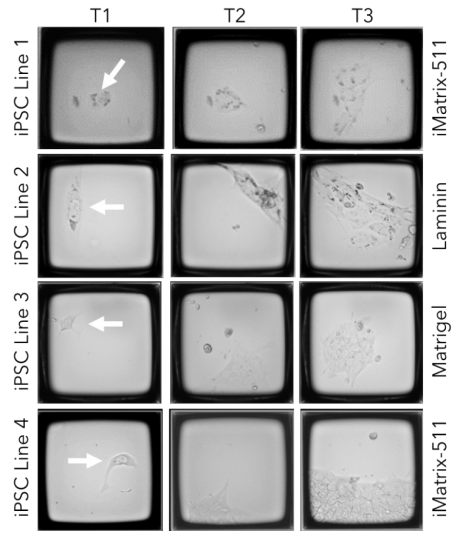


Figure 1: Track and trace of iPSC clone formation on the CellRaft AIR. Four different iPSC cell lines were seeded on CellRaft arrays on one of three coatings (iMatrix-511, h-ESC Matrigel, or Laminin). The arrays were serially scanned starting 4 hours post-cell seeding and every 24 hours after to monitor clone formation.

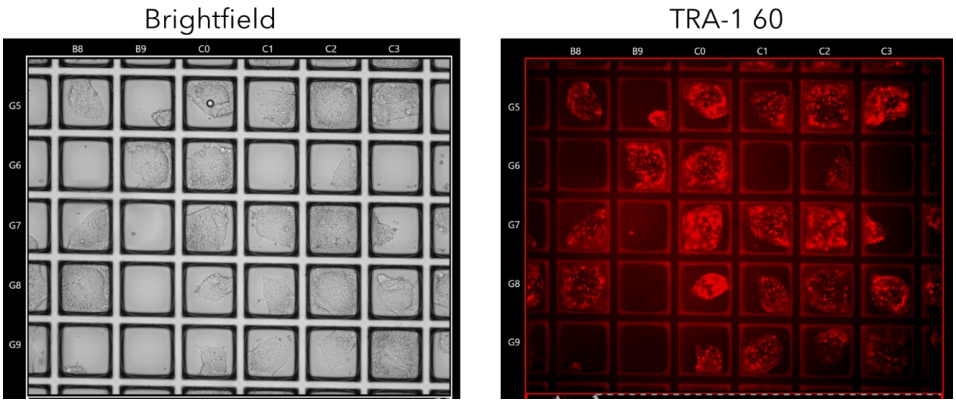


Figure 2: Live Tra-1-60 Staining for Pluripotency. iPSC colonies were stained with live Tra-1-60 antibody to identify pluripotent colonies prior to isolation.

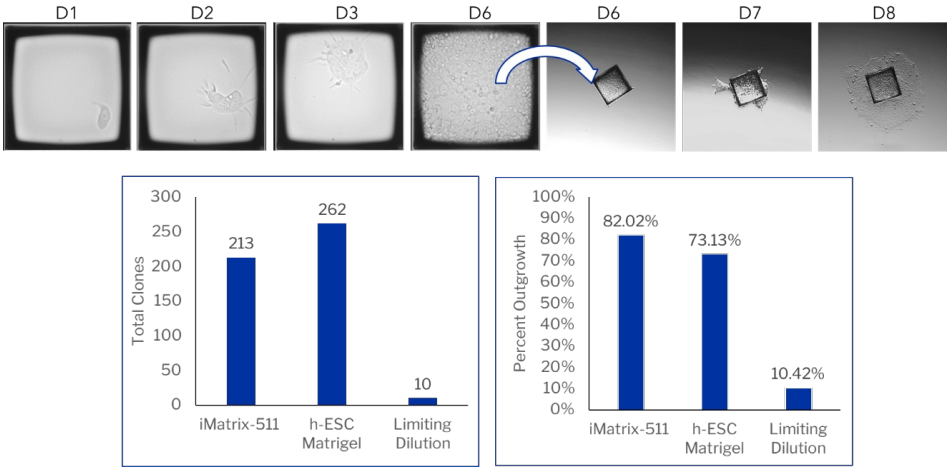


Figure 3: CellRaft AIR vs limiting dilution for monoclonal iPSC development. iPSCs were seeded on either a CellRaft Array coated with iMatrix-511 (Matrixome) or h-ESC Matrigel (Corning) or on iMatrix-511 coated 96 well plates for limiting dilution. Colony formation was monitored using the CellRaft AIR or manual observation.

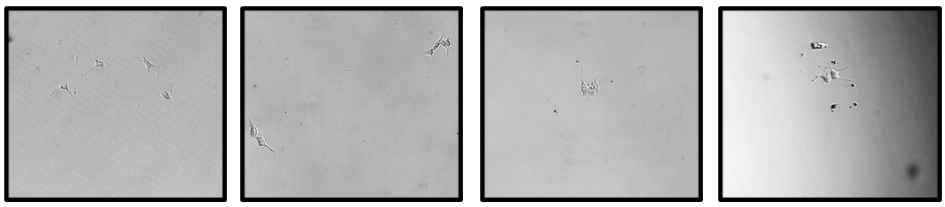


Figure 4: Limiting Dilution images from a 96-well plate with unknown cell clonality. iPSCs were seeded on a pre-coated plate with iMatrix-511 (Matrixome).

Discussion

To determine whether these key features could alleviate bottlenecks hindering iPSC workflows, we compared clonal iPSC development using CellRaft Technology to traditional limiting dilution. Using the CellRaft Array, we were able to generate over 200 single cell derived iPSC clones on a single array, compared to 10 clones in a single 96-well plate with limiting dilution. This workflow required **1000X less iPSC coating and 2000X less media per cell screened** (Table 1). In addition, the image acquisition over time allows for the detailed assessment of monoclonality, ensuring that successful clones are not heterogeneous and eliminating the need for downstream clonal characterization and shortening the time to use in downstream applications.

Table 1: Consumables per cells screened

	iPSCs screened/plate	uL Coating/cell	uL Media/cell
CellRaft Array	60,000	0.05	0.05
96 well plate	96	52.1	104.2

Conclusion

The ability of the CellRaft Array to provide flask-like culture conditions while maintaining spatial separation of single cells greatly improved iPSC viability and monoclonal colony formation. In addition, a single consumable can screen thousands of iPSCs, decreasing the time required for cell line generation and therapeutic discovery. Together, CellRaft Array technology and the automated imagining and isolation capabilities of the CellRaft AIR System can increase the utility of iPSCs by collapsing complicated iPSC maintenance and cell line development workflows.

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