Accelerate Cell Line Development by Isolating Monoclonal Colonies, Not Single Cells

The benefits of flask-like culture for improving single cell colony formation

Breakthroughs in drug discovery, vaccine development, and personalized medicine hinge on the development of cell-based models, which often depends on reliably generating single cell derived colonies. Despite this critical starting point, obtaining monoclonal colonies has historically been a frustrating roadblock in the lab and a persistent struggle for the field. Adding to the challenge of cell line development, oftentimes the cells being used are fragile, rare, or gene-edited, making the barrier to success even higher. Well-established options for generating research cell lines include single cell dispensing technologies such as flow cytometers, droplet dispensers, and manual pipetting with limiting dilution. However, because proliferation and cell viability are largely dependent on cell-cell interactions and paracrine signaling, depositing a single cell in a well often results in an inhospitable microenvironment, leading to poor cell survival and phenotypic selection.

In contrast to these single cell technologies, the hallmark of CellRaft® Technology is the ability to maintain the benefits of bulk cell culture, while also providing single cell track and trace resolution. Using the CellRaft Array, thousands of cells can be cultured in a shared media reservoir, while growing spatially segregated from one another in microwells containing a microscale polystyrene growth surface. To demonstrate the benefits of this flask-like culture conditions, which provides a shared culture environment for growing colonies, we compared the single cell colony forming efficiency and colony outgrowth of five different cell lines using either CellRaft Technology or manual limiting dilution. On the CellRaft AIR® System, cells were isolated at either the one cell-, two cell-, four cell-, or colony-stage of cell expansion, and the data clearly demonstrated that isolating colonies, not single cells, is transformative for cell line development success.

Key Highlights:

1) The main failure mode for cell line development is the inability of an isolated single cell to generate a viable colony.
2) Subjecting cells to manipulations such as fluidics or transfection contributes to the poor efficiency of single cell cloning.
3) Isolating colonies rather than single cells leads to successful cell line development, even for the most challenging or fragile cell lines.

Questions this RaftNote Answers

1. Do different cell lines have different monoclonal colony forming abilities?
2. How do I increase the viability of monoclonal colonies?
3. What is the best way to get monoclonal colonies?
4. How can I prove monoclonality?

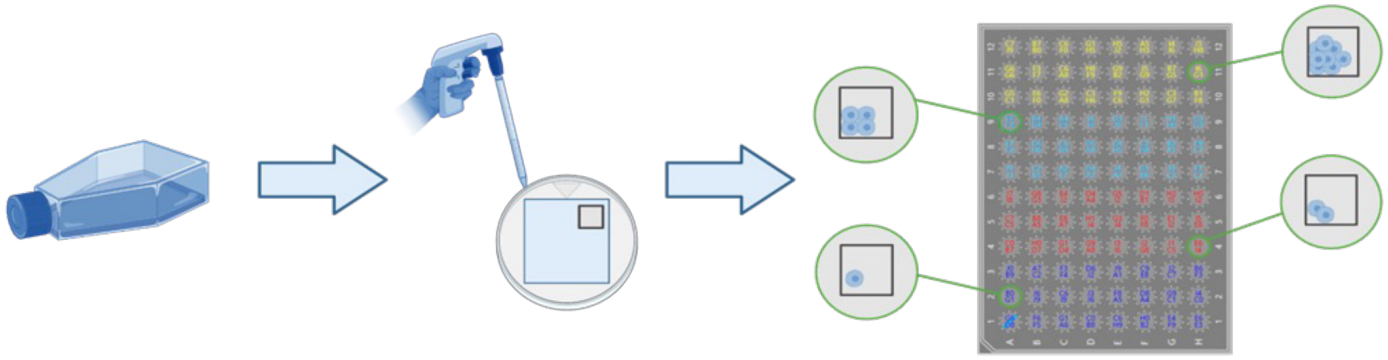

Figure 1. Isolation of CellRafts Containing Exponential Cell Division States. Five different cell lines were seeded on CellRaft Arrays at the recommended cell density for each line. The arrays were scanned 4 hours post-cell seeding to identify CellRafts containing single cells. CellRafts containing a single cell were isolated on day 1 (dark blue wells). The remaining single-cell rafts were monitored over the course of 5 days, and a subset of these rafts was isolated at the 2-cell stage on day 2 (red wells), at the 4-cell stage on day 3 (light blue wells), or after a monoclonal colony had formed on day 4-5 (yellow wells). Colony growth after isolation was monitored in the collection plate over 7-10 days to determine outgrowth efficiency.

Materials and Methods

CellRaft AIR Cloning

Five different cell lines were used to determine if isolation at discrete stages of cell expansion impacted monoclonal colony formation: CHO-K1 (ATCC, CCL-61), HEK293T (ATCC, CRL-3216), HT-1080 (ATCC, CCL-121), aHer2+ (ATCC, 10463), and iPSCs (KYOU-DXR019B; ATCC, ACS-1023). All cell lines were handled according to the manufacturer's instruction and passaged at least twice before cell seeding onto CellRaft Arrays. Cells were seeded according to previous cell characterizations. Cells seeded on the CellRaft Arrays were scanned daily starting four hours post-seeding. CellRafts with cells at the single cell-, two cell-, four cell-, and colony-stage of single cell expansion were isolated into 96-well collection plates on consecutive days. After isolation, cells were observed for colony formation in the 96-well collection plates using a Nikon microscope.

Limiting Dilution Cloning

Cells were diluted to 5-10 cells/mL depending on the cell line, and 100uL of the cell suspension was added to each well of three 96-well plates to achieve approximately 0.5 - 1.0 cell per well. Each plate was visually examined using brightfield microscopy at least three times a week to monitor single cells and monoclonal colony formation.

Results

CHO-K1

Chinese hamster ovarian (CHO) cells are one of the most widely used cells lines for industrial production of recombinant therapeutic proteins. Despite the ease of culture and robust nature, in traditional limiting dilution experiments, only 9% of single CHO-K1 cells formed viable colonies. In contrast with the CellRaft AIR System, isolation at the single cell- and two cell-stage of single CHO-K1 cell expansion, clonal outgrowth was 70% and 87%, respectively. Furthermore, at the four cell- and colony-stage, CHO-K1 clonal outgrowth increased to 98% and 99%, with nearly all of the isolated clones forming viable colonies downstream (Figure 2-3, Table 1).

HEK293T

Similar to CHO cells, human embryonic kidney 293 (HEK293) cells are commonly used in cell biology research for protein expression, production, and vaccine development. In experiments with HEK293T cells in limiting dilution, only 12% of single cells formed viable monoclonal colonies (Table 1). However, the colony outgrowth for HEK293T cells isolated at the single cell-stage of cell expansion from the CellRaft Array was 44%. As the single HEK293T cells continued to grow on the CellRafts prior to isolation, the efficiency of viable colonies improved to 64%, 79%, and 98% for the two cell-, four cell-, and colony-stages, respectively (Figure 2, Table 1).

HT-1080

Although less common than the production cell lines above, the HT-1080 cell line has been used extensively for cancer research and in vitro transfection. A limiting dilution comparison was also performed for the HT-1080s, and only 30% of the wells in the 96-well plates contained viable colonies. For the one cell- and two-cell stage of single HT-1080 cell expansion, clonal outgrowth was 22% and 47%, respectively. However, at the four cell- and colony-stage, the CellRaft AIR System improved the HT-1080s and viable colony outgrowth to 96% (Figure 2, Table 1).

AHER2+

The AHer2 cell line is a AHer2+ clone that produces HER2 antibody. Obtaining clonal hybridoma cell lines is a challenging but necessary step in therapeutic antibody production. The colony outgrowth for the single cell- and two-cell stage of single AHer2+ cell expansion was 12% and 18%, respectively. However, by allowing the cells to continue to divide on the CellRaft array, monoclonal colony growth improved nearly 3-fold to 34% by isolating colonies rather than single cells (Figure 2, Table 1).

iPSCs

Induced pluripotent stem cells (iPSCs) have the unique ability to differentiate into any of the three germ layers of the body, making them a powerful tool in disease modeling and drug screening. However, the culture of iPSCs is tedious, and iPS cell line development has become a frustrating bottleneck for scientists. With traditional limiting dilution, only 10% of wells contained viable iPSC clones. Similarly, at the single cell- and two cell-stage of iPS cell expansion, only 10% and 28% of wells in the 96-well plates contained viable clones, demonstrating the enormous challenge of cloning these sensitive cells. However, by the four cell- and colony-stage, the iPSC colony formation dramatically increased to 87% and 98%, respectively (Figure 2, 4, Table 1).

Across the five cell lines tested, the average formation of colonies from single cells on CellRafts was 31%. However, when CellRafts with single cell derived colonies were isolated, the average colony outgrowth increased up to 96%, demonstrating that by simply allowing the single cells to continue to grow in a shared microenvironment prior to colony isolation, clonal cell line development is possible for even the most challenging cell types.

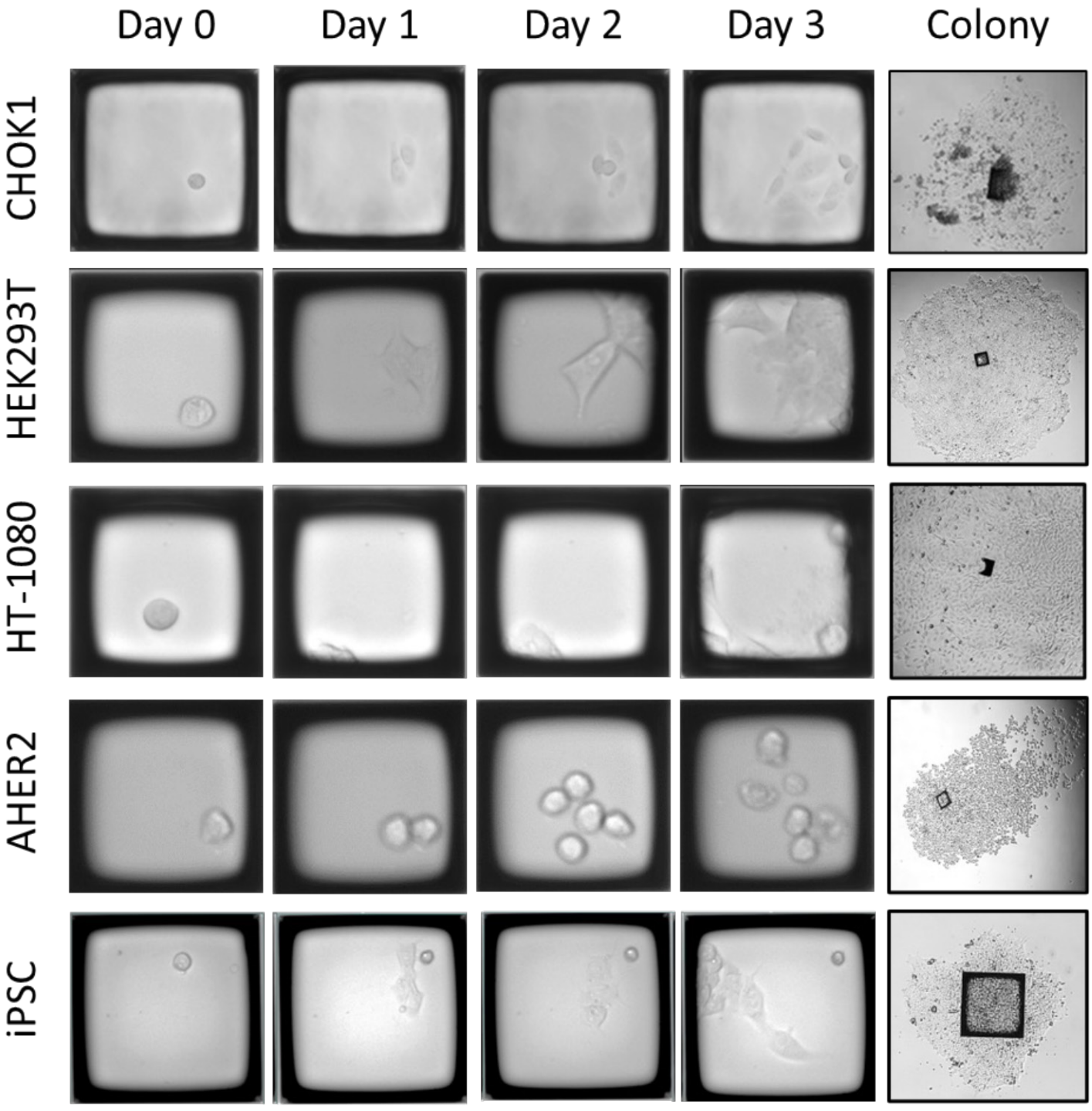

Figure 2. Track and Trace Single Cell Colony Formation on the CellRaft Array. CellRaft arrays seeded with five different cell types were scanned on the CellRaft AIR system to monitor single cell growth and colony formation on array. After array, colony growth off raft was observed on a benchtop microscope.

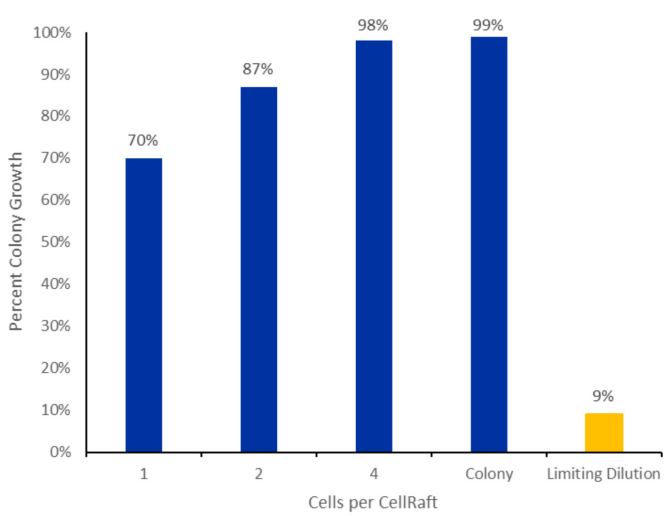

Figure 3. CHOK1 cells demonstrate clonal outgrowth even from a single cell. After isolating CellRafts containing different numbers of CHO cells, the percentage of wells with viable colonies was calculated compared to the number of wells with a single colony in limiting dilution (yellow bar).

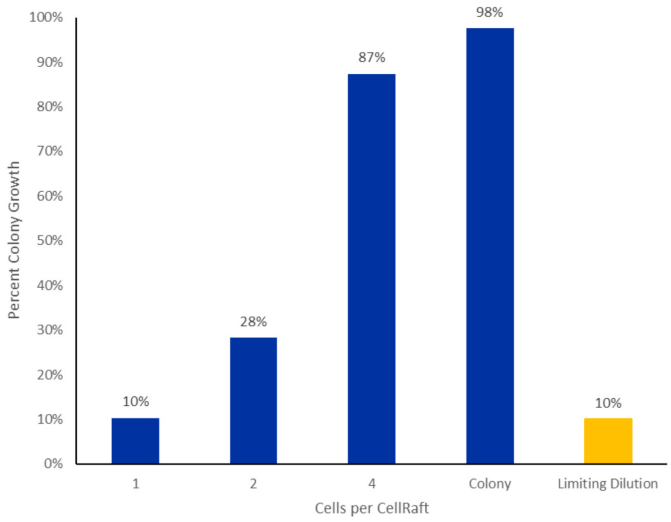

Figure 4. iPSCs have poor colony formation efficiency from single cells. After isolating CellRafts containing different numbers of iPSCs, the percentage of wells with viable colonies was calculated compared to the number of wells with a single colony in limiting dilution (yellow bar).

Table 1. Comparison of Clonal Outgrowth

		Cells Per CellRaft at Isolation			
Cell Line	**Limiting Dilution**	**1**	**2**	**4**	**>4**
iPSCs	10%	10%	20%	87%	98%
AHER2	ND	12%	18%	21%	34%
HT-1080	30%	22%	47%	96%	96%
HEK293T	12%	44%	64%	79%	98%
CHO-K1	9%	70%	87%	98%	99%
AVERAGE	17%	32%	47%	76%	85%

Discussion

Efficient and successful monoclonal colony formation from single cells has been an ongoing battle that has inhibited the ability of researchers to defeat maladies that impact human health. Despite new instruments and advanced culture methods, many cell lines are incapable of adapting to single cell culture to generate monoclonal colonies. Robust cell lines, such as the CHO cells and HEK293s, are frequently leveraged in these types of workflows because they are amenable to single cell cloning. By limiting cell line development to these two cell models, the breadth of research across a diversity of tissue types and species is restricted. Using CellRaft Technology, we are able to open up the possibilities for clonal cell line development using an expansive range of cell types, including many that have been challenging for single cell culture with other methodologies. Importantly, these results clearly demonstrate the benefits of cell-cell interactions and the surrounding environment for generating single cell derived colonies with a high degree of efficiency.

Conclusion

Accelerating single cell workflows is essential for improving drug development and personalized medicine. However, the strenuous conditions of single cell culture often result in infrequent monoclonal colonies and cell death. Although newer technologies such as low-pressure droplet dispensers purport to improve single cell viability, these instruments are often also focused on CHOK1 and HEK293T workflows. As demonstrated above, these cells are capable of forming colonies from single cells fairly robustly, while other more useful cell lines such as cancer cell models or primary cells are not. The formation of monoclonal colonies from these more challenging cell types is significantly improved when cells are cultured within a flask-like culture environment that supports cell viability, such as the CellRaft Array. This permissive environment enables the generation and characterization of hundreds of monoclonal colonies, providing an alternative for cell line development that is more effective, as well as time, labor, and cost-efficient.

For more information on the presented data or CellRaft Technology, visit cellmicrosystems.com

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