CELL Microsystems®

RAFTNOTE

Unlock the potential to develop cell lines from a menagerie of species

Introduction

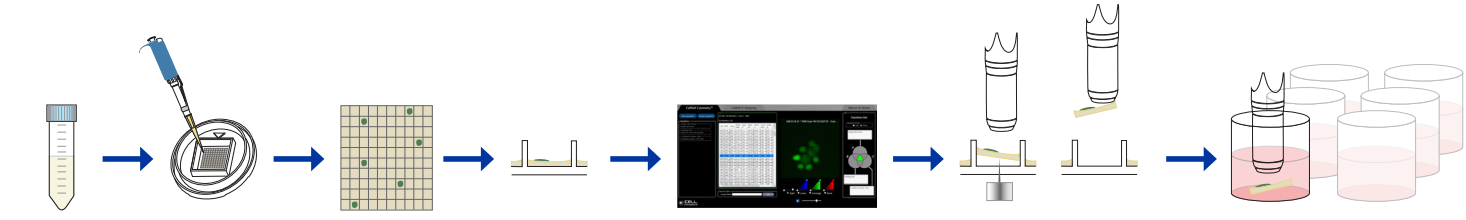
As biotechnology advances and research areas become more granular, there is an increased demand for a broad suite of cell lines and cell models for use in applications like biologics production, disease modeling, drug testing, and general cell biology studies. These applications reach into nearly every life science sector, including veterinary medicine, agriculture, and public health.

However, the potential of genetically and species-diverse cell lines is hampered by the tools available to produce them. Traditional methods of cell sorting, such as flow cytometry and limiting dilution, can dramatically reduce the viability of cells, restricting these methods to cell lines that can survive these stressors, such as HEK293 and CHO-K1 cells. While cell sorters have been used with limited success with these common cell lines, there is a need for automated technology that can reliably support a broad range of cell types.

CellRaft® Technology offers a better solution for the culture and isolation of cells that goes beyond the traditional production cell lines. The CellRaft AIR® System, accompanied by the CellRaft Array and CellRaft Cytometry™ software integrates flask-like culture conditions, single cell separation, and AI-driven software for the identification of monoclonal colonies, and gentle, automated retrieval. This integration enables intelligent isolation of CellRafts, facilitating efficient single-cell workflows that reach beyond human and mouse.

"At Colossal Biosciences, we only work with non-model organisms and primary cells and cell lines that haven't been studied before. These cells often fail to grow out after monoclonal isolation using flow sorting. In the few months since adopting the CellRaft, we have isolated thousands of validated monoclonals from elephants, canids and deer with consistent outgrowth efficiencies."

Dr. Sven Bocklandt, Species Director at Colossal where he is leading a team of genome engineers and cell biologists focused on bringing back extinct animal species.



CellRaft Technology Combines the Power of Flask-like Culture Conditions and Single-Cell Separation to Produce High Viability Cells, Colonies, and Organoids

Questions this RaftNote answers

1. How do I successfully culture diverse cell types?
2. Is there a better alternative to limiting dilution for generating monoclonal cell lines?
3. What is the best way to isolate cells?
4. How can I increase the viability of monoclonal cells?

Experimental Design

Seven different cell lines (Vero, COS-7, PC-12, C6, BHK-21, PK15, and MDCK) were maintained in standard culture conditions according to manufacturer instructions. If a suspension line was being tested, the array was first coated overnight with poly-D-lysine, poly-L-lysine, or poly-L-ornithine to facilitate cell adhesion to the CellRaft. Cells were seeded on both sizes of quad-reservoir CellRaft Array (100 x 100 and 200 x 200 µm microwells) at varying seeding densities ranging from 0.3-2.5 cells per raft to determine the optimal raft size and seeding density for each cell line. An initial scan to image and identify single cells was performed 4 hours post-seeding, then every 24 hours (or more frequently as needed) to capture clonal growth. Monoclonal colonies were identified using CellRaft Cytometry, and rafts containing monoclonal colonies were isolated into 96-well plates and monitored for up to 10 days for growth off-raft.

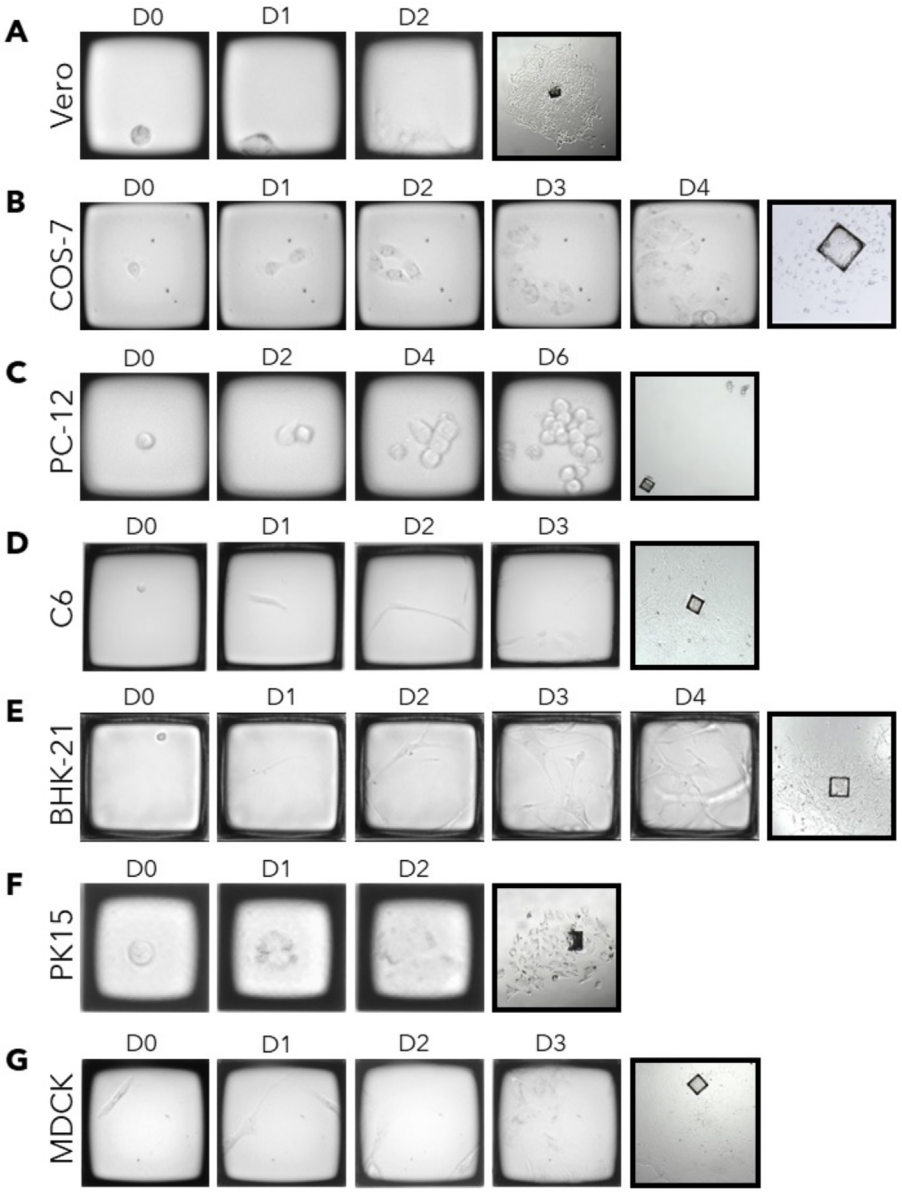


Figure 1. Various cell lines tested on the CellRaft AIR System tracking growth from a single cell to a colony and growth off-raft post-isolation in a 96-well plate. (A) Vero (B) COS-7 (C) PC-12 (D) C6 (E) BHK-21 (F) PK15 (G) MDCK

Results

Each cell line was imaged, propagated, and isolated using the CellRaft AIR System (Figure 1). CellRaft Cytometry identified hundreds of monoclonal colonies for each cell line, and rafts containing monoclonal colonies were isolated into 96-well collection plates. After 10 days, over 70% of tested lines exhibited growth off-raft in the 96-well collection plate. The percentage of single cells that proliferated into monoclonal colonies for each cell line is shown in Figure 2, with key findings summarized in Table 1.

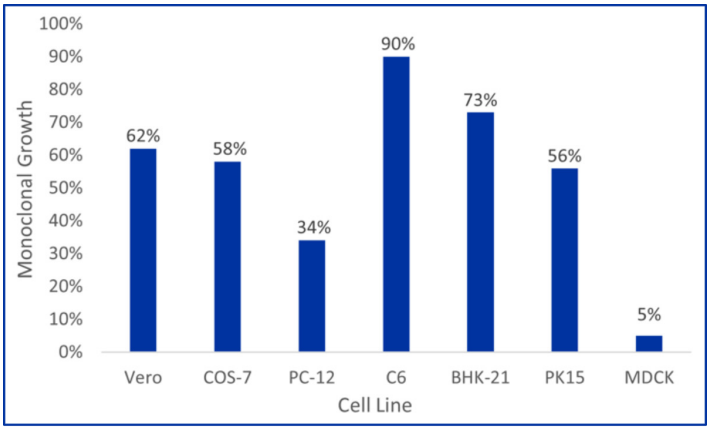


Figure 2. Percentage of single cells that proliferated into monoclonal colonies for various cell lines tested on the CellRaft AIR System.

Table 1. Data summary of cell lines tested on the CellRaft AIR System.

Cell line	Species	Raft size preference	Optimal seeding density (cells:rafts)	Number of monoclonal colonies on preferred array	Percent outgrowth off-raft from preferred array
Vero	Monkey	100 µm	0.625:1	383	86%
COS-7	Monkey	200 µm	0.625:1	157	65%
PC-12	Rat	100 µm	2.5:1	510	91%
C6	Rat	No preference	1.25:1on 100 µm 2.5:1 on 200 µm	513 on 100 µm 109 on 200 µm	99% on 100 µm 97% on 200 µm
BHK-21	Hamster	200 µm	1.25:1	286	84%
PK15	Pig	100 µm	0.625:1	723	73%
MDCK	Dog	200 µm	1.25:1	206	90%

Discussion

The CellRaft AIR System was able to successfully isolate monoclonal colonies from cell lines derived from a variety of species including monkey, rat, hamster, pig, and dog. Importantly, even cell lines that had low frequency for monoclonal colony formation (e.g. MDCK) were able to be isolated and expanded with a high degree of efficiency. Each of these lines has important implications for nearly every life science application.

Vero and COS-7 cells, isolated from African green monkey kidneys, are used for toxicity screening, parasitology and virology research, and vaccine production. Vero cells are one of the most widely used lines for cell-based vaccine production and have been utilized for vaccines against COVID-19, poliomyelitis, rabies, smallpox, influenza, and Ebola [1].

PC-12 cells, isolated from the rat adrenal medulla, are widely used in neurobiology studies, while C6 cells, isolated from the brain of a rat with glioma, provide a valuable research model for human glioma. BHK-21 cells are used for vaccine and recombinant protein

production including Coagulation Factor VIIa, a lifesaving therapeutic for some hemophilia patients [2]. PK15 cells, isolated from the kidney of a pig, are used to gain a better understanding of swine viruses and prevent costly outbreaks within herds [3]. MDCK cells are used for a variety of cell biology studies including cell-cell adhesions, toxicity studies, growth factor responses, and branching morphogenesis [4].

The importance of these cell lines is underscored by their extensive presence in the scientific literature, with thousands of cumulative citations in PubMed over the past two decades. (Figure 3).

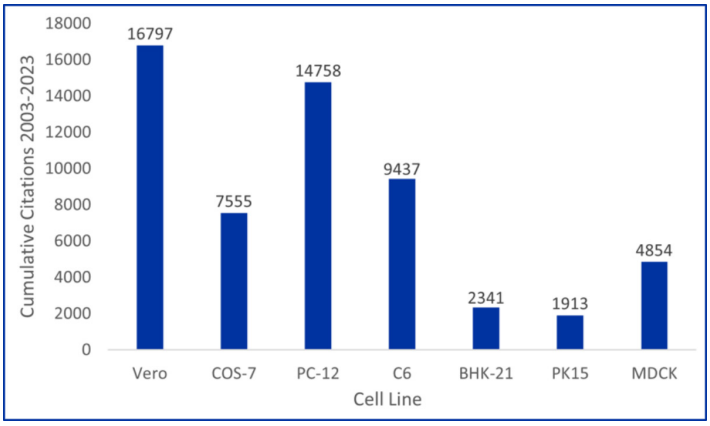


Figure 3. Cumulative PubMed citations from all article types for each cell line from 2003-2023

Conclusion

The ability to generate monoclonal cell lines from a diverse range of cells is essential for nearly every life science industry, including veterinary medicine, pharmaceuticals, and agriculture. This demand will continue to increase as biotechnology advances and research and development become more granular and specialized. The CellRaft AIR System meets this demand by providing a flexible platform for the propagation, automated identification, and gentle isolation of a diverse range of cell types, facilitating the use of monoclonal colonies in a variety of downstream applications.

Literature Cited

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2. Sha, M. Vero Cell-Based Vaccine Production: Cell lines, Media, and Bioreactor Options. *Eppendorf*, Inc. (2021).
3. Yin M, Hu D, Li P, et al. Cloning and Identification of PK15 Cells for Enhanced Replication of Classical Swine Fever Virus. *J Vet Res*. **64(1)**, 9-14 (2020).
4. O'Brien, L.E., Zegers, M.M.P., Mostov, K.E. Building epithelial architecture: insights from three-dimensional culture models. *Nat Rev Mol Cell Biol.* **3**, 531-537 (2002).

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

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