CELL Microsystems®

CASE STUDY

Accelerating the Development of In Vitro Models of Chemoresistance

UNC Liu Lab Case Study

Introduction

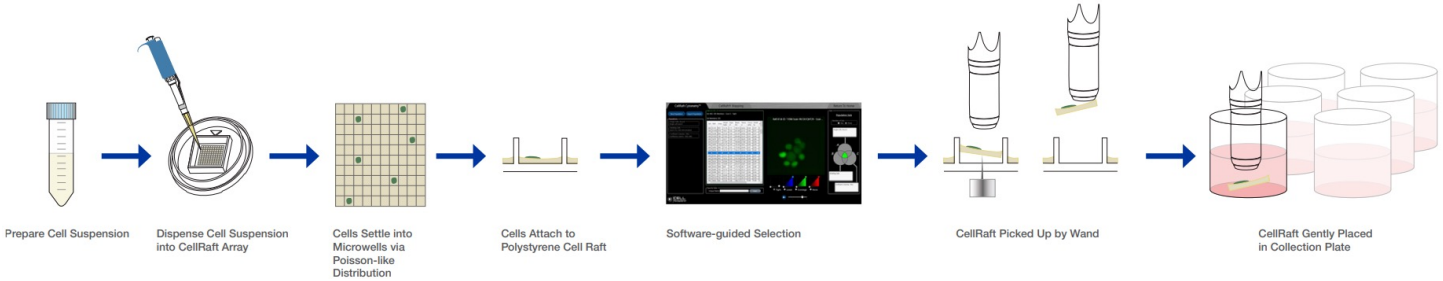
Cancer is the second leading cause of mortality in the United States, contributing to over 600,000 deaths per year[1]. While cancer treatments have improved considerably in recent decades, resistance of cancer cells to chemotherapeutic drugs (chemoresistance) remains a significant problem. Chemoresistance can occur through a diverse range of mechanisms, many of which are not fully understood. The ability to effectively model chemoresistance in vitro is essential for elucidating resistance mechanisms and improving patient outcomes.

In vitro chemoresistance studies often begin by exposing a cell line to a chemotherapeutic agent to generate a chemoresistant population of cells. Due to the genetic heterogeneity of cancer cell lines, single cell cloning is subsequently performed to obtain a genetically identical population of resistant cells. Downstream analysis can then be performed on the monoclonal, chemoresistant cell line to elucidate innate and acquired resistance mechanisms.

Conventional methods of single cell cloning include flow cytometry, single cell dispensing, limiting dilution, or use of semi-solid media. However, in addition to being time-consuming and labor-intensive, these methods often fail due to dispensing a single cell alone in a well, lacking growth factors and mitogens from neighboring cells. This case study demonstrates how CellRaft® Technology, a novel cell line development platform, was used to generate monoclonal chemoselected Ewing's sarcoma cell lines after failed attempts at cloning by limiting dilution.

Key Highlights:

1. Limiting dilution failed to generate viable clones of Ewing's sarcoma cells as single cells did not proliferate.
2. Clones were successfully propagated and isolated on the CellRaft AIR® System and CellRaft Cytometry™ identified hundreds of monoclonal colonies on a single CellRaft® Array per cell line.
3. Clonal colonies were isolated into 96-well plates and were successfully expanded for downstream analysis. While only 5 clones per cell line were promised, up to 43 clones were generated with a portion of those clones exhibiting chemoresistance.

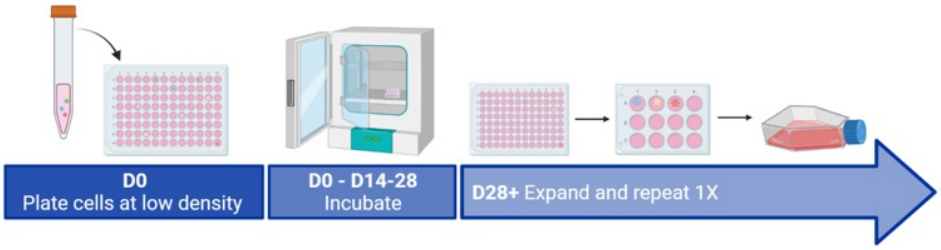


Scientific Problem

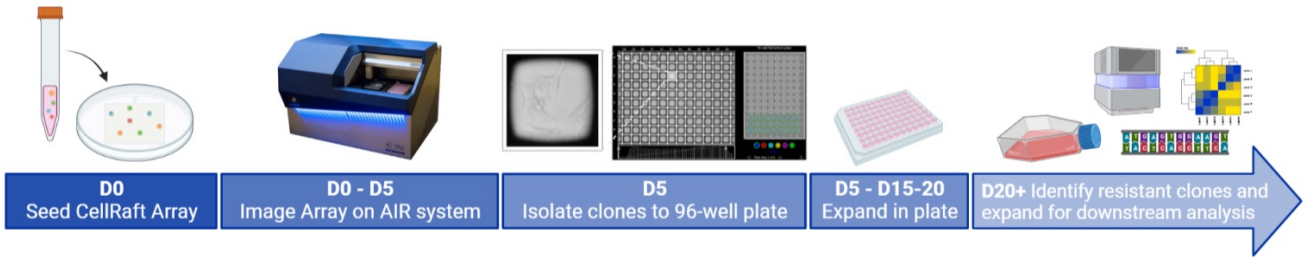
To elucidate the mechanisms driving chemoresistance in Ewing's sarcoma patients, researchers at the University of North Carolina at Chapel Hill exposed Ewing's sarcoma cell lines, MHH-ES-1 and TC-71, to common chemotherapeutics SN-38 and etoposide to select for a chemoresistant population of cells. These populations were then plated using limiting dilution to generate a monoclonal population, but single cells failed to expand. The researchers turned to Cell Microsystems to leverage the CellRaft Technology, a novel single cell cloning and cell line development platform.

Experimental Design

Polyclonal populations of MHH-ES-1 and TC-71 cells selected for resistance to SN-38 and etoposide were seeded on 100 µm single-reservoir CellRaft Arrays. Cells were imaged on the CellRaft AIR System 4 hours post-seeding to identify single cells and every subsequent 24 hours over several days to monitor viability and clonality until monoclonal colonies reached ≥50% confluency. CellRaft Cytometry was used to identify monoclonal colonies as CellRafts that contained a single cell at the first scan and a small colony at the final scan. CellRafts containing monoclonal colonies were isolated into 96-well plates using the CellRaft AIR System and cultured for 10-15 days before the clones were assessed for maintenance of chemoresistance.



Conventional Workflow for Cloning by Limiting Dilution: On day 0, a single cell suspension is plated at low density (0.5-5 cells/100 µL) in 96-well plates. Cells are cultured for 7-14 days before identification of wells containing putative monoclonal colonies, then cultured for an additional 2-4 weeks before expansion out of the 96-well plate. Because clonal assurance is low, the protocol is repeated at least once to increase the probability of monoclonality, resulting in a workflow that takes at least 6 weeks and consumes large amounts of media and cultureware.



CellRaft AIR Workflow: On day 0, a chemoselected polyclonal cell population is seeded onto a 100 µm single-reservoir CellRaft Array. The CellRaft Array is scanned on the CellRaft AIR System 4 hours post-seeding and every subsequent 24 hours until isolation. CellRafts containing monoclonal colonies are identified using CellRaft Cytometry and automatically deposited into a 96-well plate using the CellRaft AIR System. Clones are expanded in the 96-well plate for 10-15 days before the identification of clones that have retained chemoresistance and further expansion of resistant clones for downstream analysis. The entire workflow from seeding the CellRaft Array to expansion of clones out of the 96-well plate can be completed in under 3 weeks with less than 2 hours of hands-on time per cell line.

Results

CellRaft Technology allowed for successful single cell cloning of chemoselected Ewing's sarcoma cell lines (Figure 1). CellRafts containing monoclonal colonies were identified using CellRaft Cytometry (Figure 2). Over 100 monoclonal colonies were identified on each CellRaft Array for each cell line. Outgrowth efficiency after isolation to 96-well plates was greater than 20% after 7 days, providing up to 43 clones per cell line available for downstream analysis. Following chemotherapeutic treatment of clones with SN-38 or etoposide, between 5 and 15 clones for each cell line were found to have retained chemoresistance (Table 1). The workflow for each cell line, from seeding the CellRaft Array to expansion of clones out of the 96-well plate, was completed in less than 3 weeks with less than 2 hours of hands-on time per cell line.

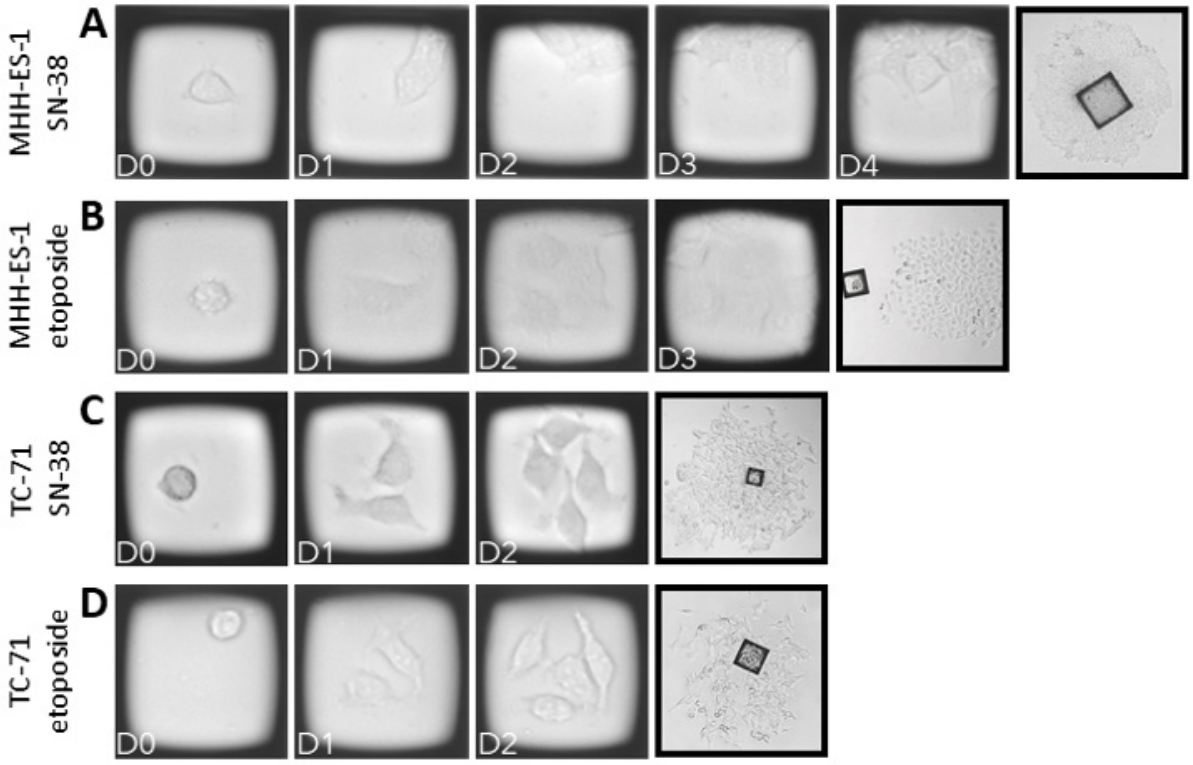
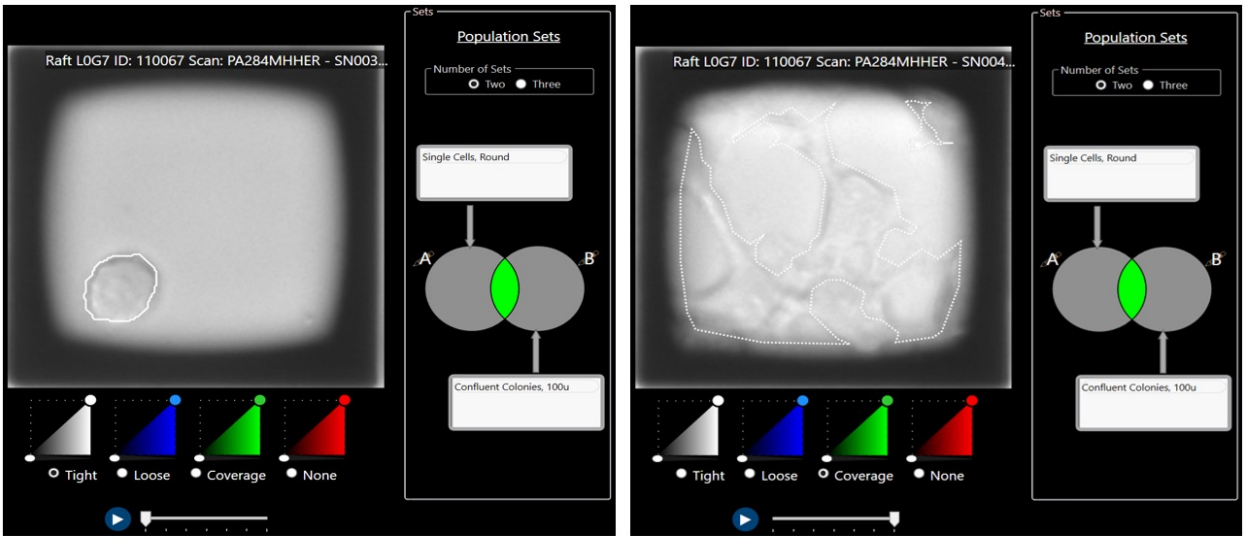


Figure 1. Representative time-course images of Ewing's sarcoma cells on CellRafts (20X magnification) and outgrowth off-Raft (10X magnification). CellRaft Arrays were imaged 4 hours post-seeding to identify single cells and every subsequent 24 hours to monitor clonal proliferation. Monoclonal colonies were identified using CellRaft Cytometry. Growth off-Raft was imaged in brightfield on an inverted microscope between 5-10 days post-isolation of the CellRafts. (A) MHH-ES-1 selected with SN-38, (B) MHH-ES-1 selected with etoposide, (C) TC-71 selected with SN-38, and (D) TC-71 selected with etoposide.



Single cell, day 0

Monoclonal colony, day 3

Figure 2. Representative example of the identification of monoclonal colonies using CellRaft Cytometry. Two population sets, single cells on day 0 and confluent colonies on day 3, are overlayed to identify CellRafts containing monoclonal colonies.

Cell Line and Treatment	Single cells	Monoclonal colonies	Percent outgrowth efficiency of clones	Expanded clones	Chemoresistant clones
MHH-ES-1 SN-38	1414	375	48%	42	15
MHH-ES-1 etoposide	1911	124	38%	35	5
TC-71 SN-38	1144	314	26%	23	12
TC-71 etoposide	2241	291	26%	19	12

Table 1: Data summary for each chemoselected cell line. CellRafts containing single cells at scan 1 and monoclonal colonies at the final scan were identified using CellRaft Cytometry. CellRafts containing monoclonal colonies were isolated using the CellRaft AIR System and deposited into 96-well collection plates. Outgrowth was manually observed and recorded as cells growing off-Raft in the collection plate at 7 days post-isolation. Outgrowth efficiency was calculated as wells with growth off-Raft as a percentage of total wells. Expanded clones are clones that successfully expanded off of the isolated CellRafts, while chemoresistant clones are the portion of expanded clones that survived chemotherapeutic treatment.

Discussion

The generation of monoclonal, homogeneous populations of cells is an essential step in many cell biology workflows. While there are several conventional methods for single cell cloning, many leave a single cell alone in a well, lacking growth factors and mitogens from neighboring cells. Many cells are unable to proliferate in this state, especially if the cells have been exposed to upstream stressors like drug treatment or gene editing. This struggle was exemplified in the case study presented here, where attempts at single cell cloning of chemoresistant populations of Ewing's sarcoma cells by limiting dilution failed as cells were unable to proliferate past the single cell stage.

The low efficiency of cloning by limiting dilution is exacerbated by the need for a monoclonal population that has maintained chemoresistance throughout the single cell cloning workflow. After single cell cloning of the cells in this case study, only 14-63% of the monoclonal populations isolated from the CellRaft Array retained chemoresistance. The unique ability of the CellRaft Array to generate and screen hundreds of monoclonal colonies increases the likelihood that monoclonal populations which have retained their chemoresistance can be isolated and expanded.

CellRaft Technology was successfully utilized to generate a significant number of fully expanded clones in under three weeks with less than two hours of hands-on time per cell line. Downstream analyses like whole genome sequencing, RNA sequencing, and murine xenograft models can be performed on these expanded clones to elucidate mechanisms of innate and acquired resistance, allowing for the development of cancer treatments that evade chemoresistance. While only 5 clones were promised, up to 43 clones were provided for each cell line, allowing for more data to be gathered across a wider range of phenotypes.

Conclusions

As biomedical research continues to advance, there is increasing demand for an efficient method of developing monoclonal cell lines. In this case study, CellRaft Technology was used to generate hundreds of clones in less than three weeks after failed attempts at cloning by limiting dilution, allowing researchers to continue discovering life-saving treatment targets for cancer patients.

"We are amazed by the high efficiency of the unique pipeline Cell Microsystems utilized. We were offered hundreds of single clones for two cancer cell lines treated with two distinct compounds – more than 100 clones for each condition. More importantly, specific clone formation analyses including the morphology and speed for each single clone are provided. This is of great help for us to further select suitable clones for downstream sequencing analyses." – Pengda Liu, Associate Professor, University of North Carolina

References

1. Mortality in the United States, 2022. NCHS data brief, no. 492. Hyattsville, MD: National Center for Health Statistics. 2022.
2. *Biorender.* BioRender. (n.d.). Retrieved March 28, 2023, from https://biorender.com/.

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

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