Accelerating the development of in vitro models of chemoresistance

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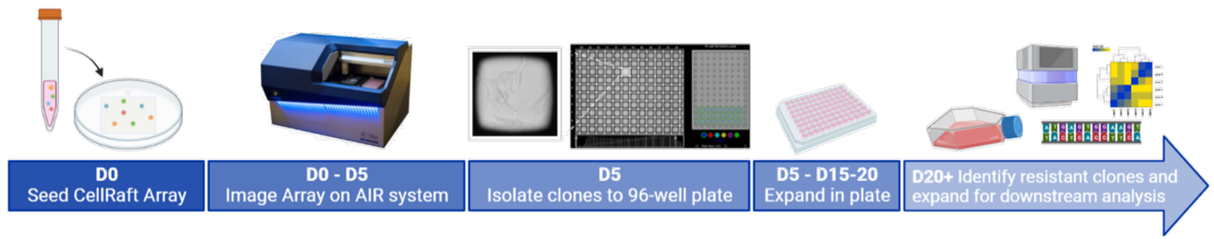
Background

- Ewing's sarcoma (ES) affects more than 200 children and adolescents per year in the United States[1]. ES tumors are highly resistant to current therapeutics, and over 70% of patients with metastatic ES tumors will die of the disease[2]. Even in localized ES, 30-40% of patients experience relapse, and the five-year survival rate after recurrence is only 15-25%[3].
- A lab at the University of North Carolina needed monoclonal populations of two Ewing's sarcoma cell lines, MHH-ES-1 and TC-71, resistant to two common chemotherapeutics, SN-38 and etoposide, in order to study chemoresistance mechanisms. Cells failed to proliferate using traditional methods of cloning.
- Traditional cloning methods such as flow cytometers, droplet dispensers, and limiting dilution are often time-consuming, labor-intensive, and leave a single cell alone in a well lacking growth factors and mitogens from neighboring cells.
- CellRaft® technology provides flask-like culture conditions with spatial segregation, software-guided identification of CellRafts containing monoclonal colonies, and automated isolation of monoclonal colonies into collection plates for further expansion.

Methods



Use of CellRaft Cytometry to identify rafts containing monoclonal colonies

Single cell, day 0 — Monoclonal colony, day 3

Figure 1: Representative example of 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.

Hundreds of monoclonal colonies grew for each cell line

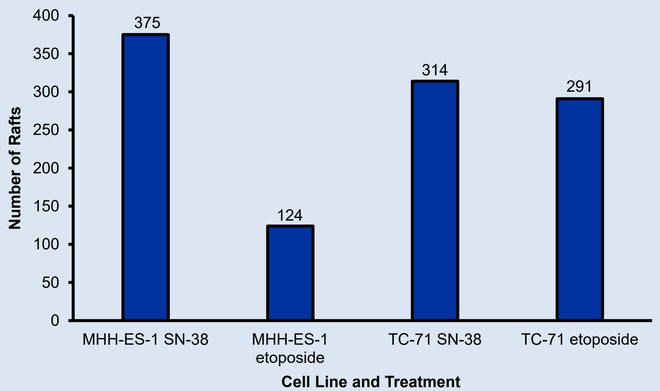


Figure 3: Number of rafts containing monoclonal colonies at final scan identified using CellRaft Cytometry. Over 100 monoclonal colonies for each cell line were available for isolation.

Visual confirmation of monoclonality and rapid colony growth

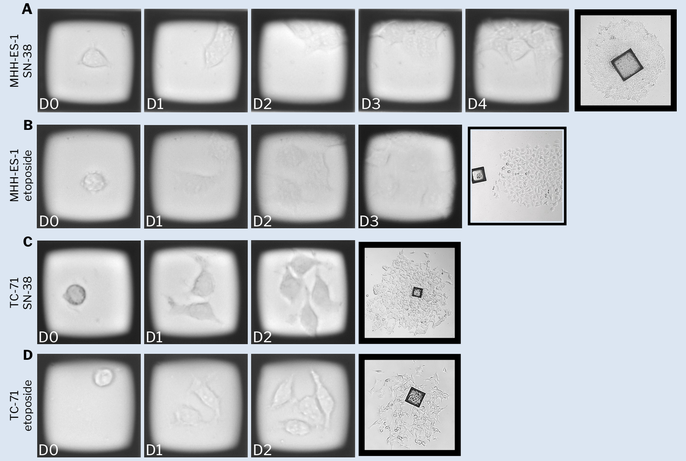


Figure 5: Representative time-course images of Ewing's sarcoma cells on CellRafts. CellRaft arrays were imaged on the CellRaft AIR® system 4 hours post-seeding to identify single cells and every subsequent 24 hours to capture clonal growth. Monoclonal colonies were identified using CellRaft Cytometry. Growth off-raft was imaged on a bench microscope 5-10 days post-isolation.

Over 1000 single cells were identified for each cell line

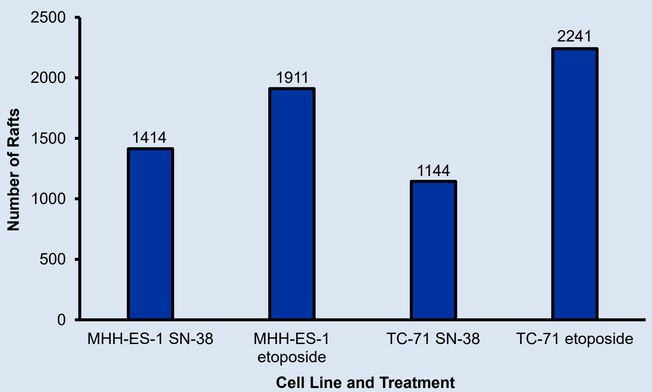


Figure 2: Number of rafts containing single cells at scan 1 identified using CellRaft Cytometry. Over 1000 single cells were identified for each cell line.

Successful expansion of monoclonal colonies for downstream testing

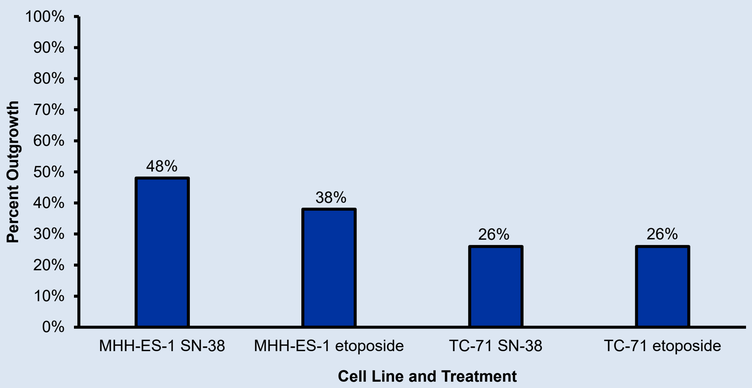


Figure 4: Percent of isolated rafts with growth off-raft after 7 days of incubation in collection plates. Colonies expanding off-raft can be incubated further until ready for expansion out of the collection plate.

Summary

- Traditional limiting dilution failed to generate clones as cells could not proliferate in isolation. By leveraging CellRaft technology, over one hundred clones were generated on just one consumable per cell line.
- Allowing single cells to proliferate in flask-like culture with shared media significantly improved cloning efficiency compared to previous attempts at limiting dilution. CellRafts are imaged serially providing an image record of clonal growth as well as viability and doubling time.
- The entire workflow, from seeding the CellRaft Array to expansion out of the collection plate, can be completed in less than three weeks with less than one hour of hands-on time, compared to over a year of failed attempts at limiting dilution.

"CellRaft not only speeds up this process by providing many more clones, but more importantly, the unique monitoring process provided by CellRaft supplies additional information for each single clone with higher quality of clones." – Associate Professor, University of North Carolina

References

1. Esiashvili et al. Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance Epidemiology and End Results data. *J Pediatr Hematol Oncol.* (6):425-30 (2008).
2. Balamuth & Womer. Ewing's sarcoma. *Lancet Oncol.* 11: 184–192 (2010).
3. Bosma et al. Prognostic factors for survival in Ewing sarcoma: A systematic review. *Surg Oncol.* 27:603-610 (2018).