Self-Seeding Microwells to Isolate and Expand Single Cells

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Introduction

Circulating tumor cells (CTCs) can be isolated from blood and serve as a source of tumor material. Expansions of CTCs may permit functional treatment-elicacy tests in combination with genetics, epigenetics and proteomics screening. We present a fast workflow to isolate, capture, sort, image and culture cells inside the VyCAP self-sorting microwell chip. After seeding single cells in the microwell chip, cells can be cultured inside the microwells, or can be transferred from the microwells towards a tissue culture plate for clonal expansion or downstream applications.

Microwell technology

Single cell isolation

A cell suspension is applied on the microwell chip and a negative pressure pulls the fluid through the pores. The cell lands on the pore and stops the flow. The next cell is forced into the neighboring well. The microwells are stained using a fluorescence-microscope to identify the cells. Individual cells can now be addressed and punched into a detection culture plate.

Workflow

Enrichment and staining

Capture Efficiency of single cell isolation RosetteSep™ + Microwell isolation

Viability and expansion

Examples of viability staining. Red indicates dead cells (EDTA) and green live cells (Calcein).

Viability

Growth efficiency

Patient samples

Conclusions

- This poster shows a preliminary study performed to characterize microwells for isolation and culturing of single cells.
- From several experiments we determined that the microwell chip enables the seeding and sorting of viable single cells.
- Single cells or colonies isolated in the microwells could easily be isolated by punching from the microwell chip for further culture.
- Our workflow allows for the isolation and identification of viable cells from patient samples.

Example of viable CTC isolated from a breast cancer patient sample. **70 cells isolated** by Fikri Abali group, Erasmus MC processing 5 patient samples with VyCAP cell isolation.

CellSearch resulted 1 sample with detectable viable CTC in microwell chip

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