Self-Seeding Microwells to Isolate and Expand Single Cells

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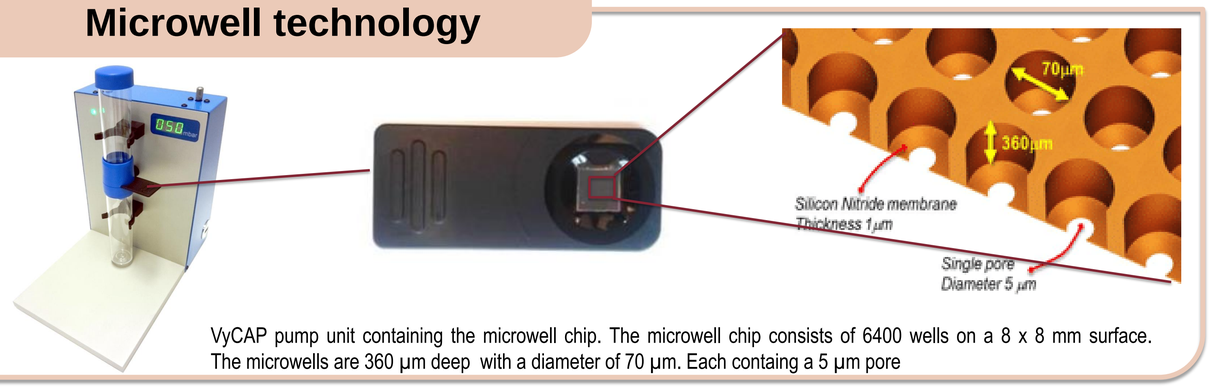
Introduction

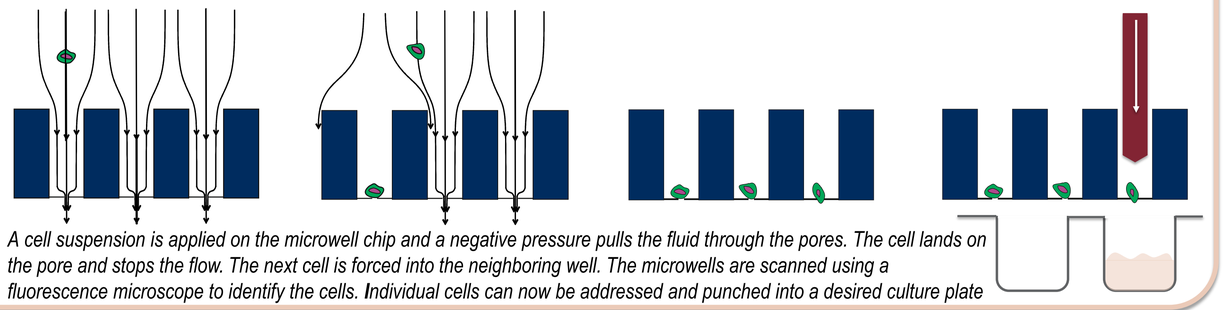
Circulating tumor cells (CTCs) can be isolated from blood and serve as a source of tumor material. Expansions of CTCs may permit functional treatment-efficacy tests in combination with genetics, epigenetics and proteomics screening. We present **a fast workflow to isolate, capture, sort, image and culture 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



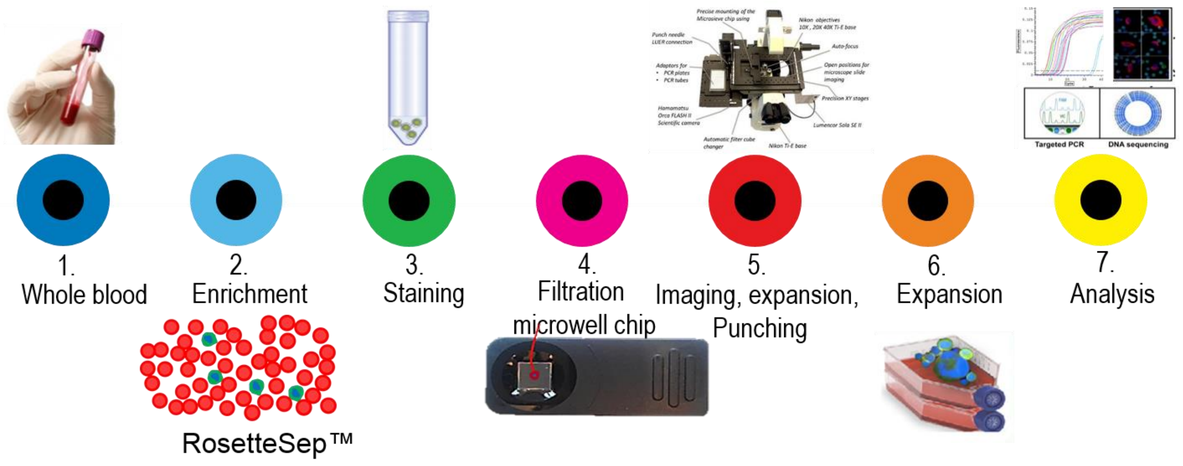
VyCAP pump unit containing the microwell chip. The microwell chip consists of 6400 wells on a 8 x 8 mm surface. The microwells are 360 µm deep with a diameter of 70 µm. Each containg a 5 µm pore

Single cell isolation

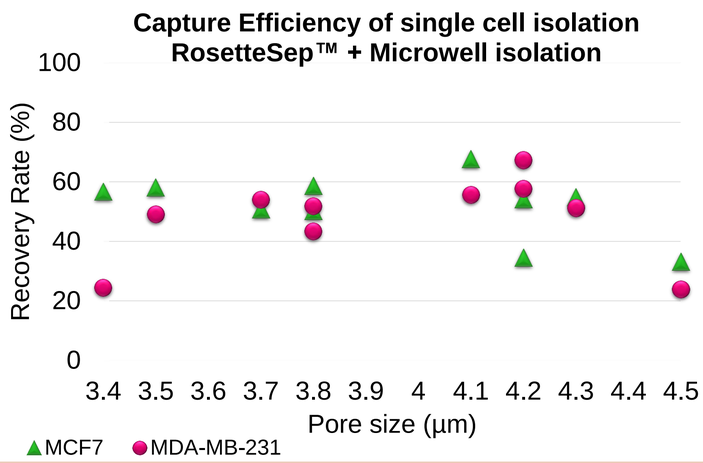


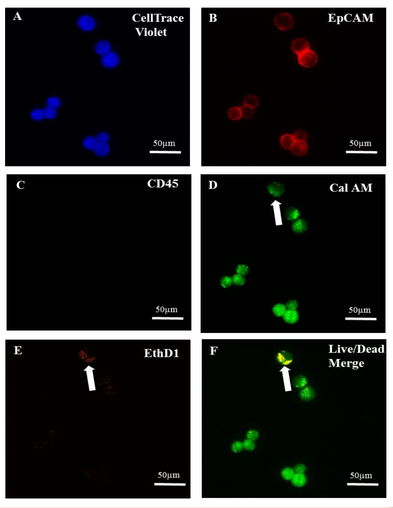
A cell suspension is applied on the microwell chip and a negative pressure pulls the fluid through the pores. The cell lands on the pore and stops the flow. The next cell is forced into the neighboring well. The microwells are scanned using a fluorescence microscope to identify the cells. Individual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

Workflow

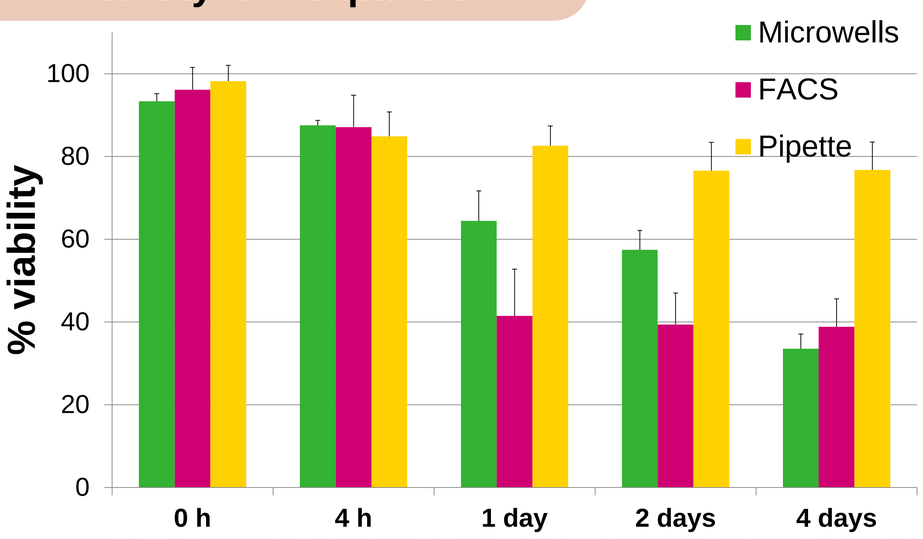


Enrichment and staining

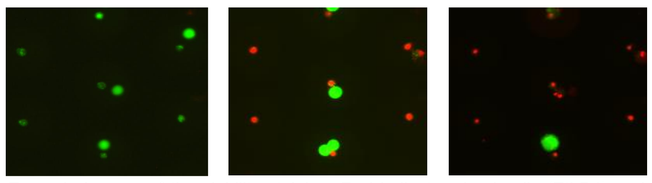




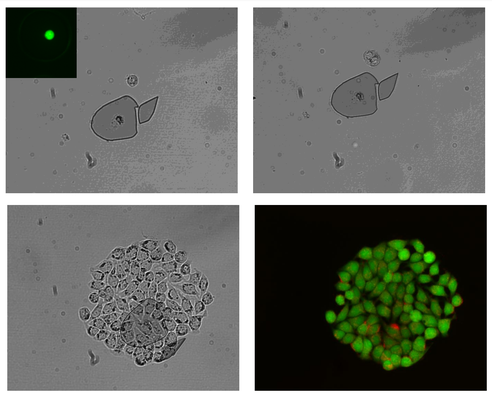
Viability and expansion



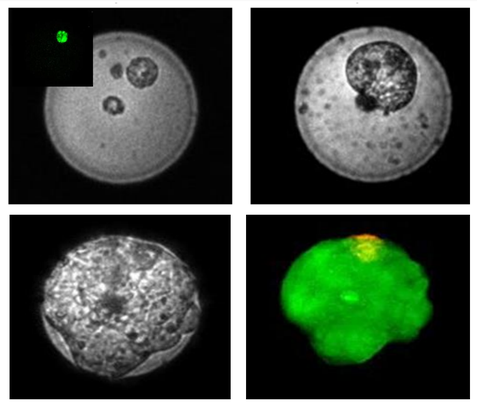
MCF-7 cells are seeded in the microwell chip and their viability is compared to other methods of single cell sorting in culture plates. Cells are stained at different intervals to determine the viability.

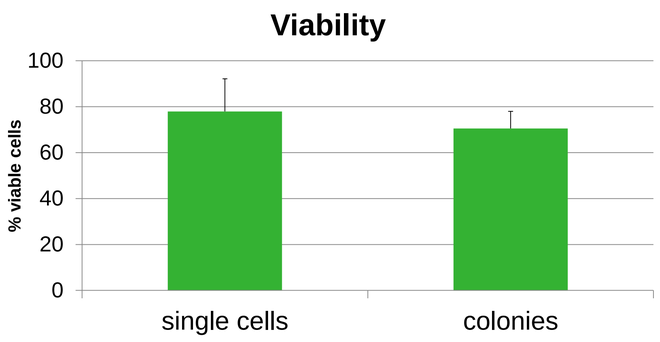


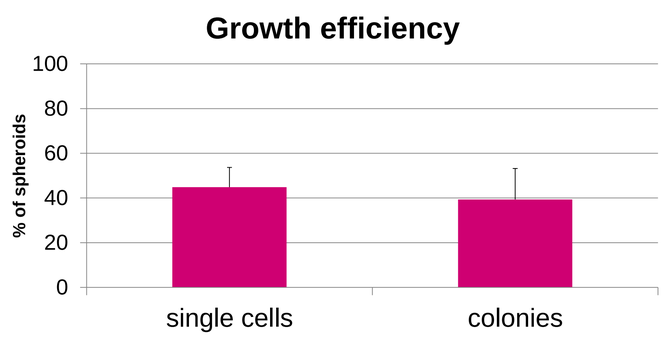
Example images of the viability staining. Red indicates dead cells (EthD1) and green live cells (Calc AM)



Series of images showing the growth of a single MCF-7 cell after it has been punched out of the microwell chip (left) and directly cultured inside the microwell chip (right)
Calcein AM green (alive) and EthD1 red (dead) fluorescence.

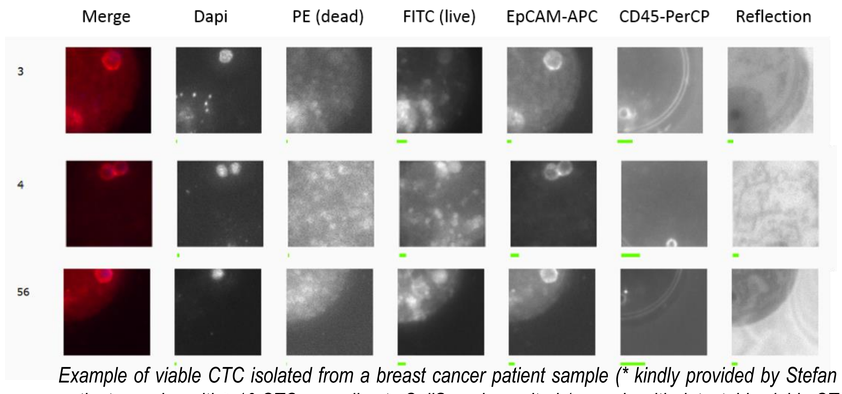


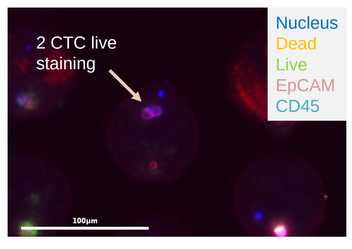




EDTA blood was spiked with MCF-7 cells. The sample was enriched and next distributed across the microwells. Left) Cell viability after punching: MCF-7 punched as a single cell or as a colony of cells in a culture plate. Single cells were punched out directly after seeding (0 hours); colonies were punched out after 2 days of culturing inside the microfluidic wells. Cells were stained after 4 hours and the number of viable cells was determined. Right) Next, cells were tracked for 14 days and growth efficiency was determined. Single cells and colonies established inside microfluidic wells (from single cells) were punched and cultured.

Patient samples





Example of viable CTC isolated from a breast cancer patient sample (kindly provided by Stefan Sleijfer group, Erasmus MC) processing 5 patient samples with ≥10 CTC according to CellSearch resulted 1 sample with detectable viable CTC in microwell chip*

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.

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