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Self-sorting microwells to isolate and expand single circulating tumor cells

A fast workflow to isolate, capture, sort, image and culture cells

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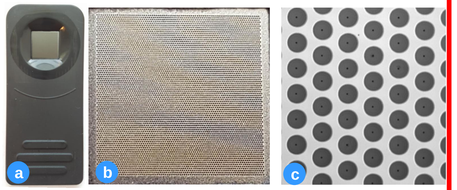
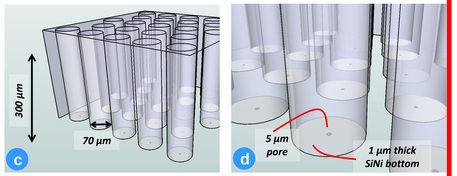
Introduction

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 a VyCAP self-sorting microwell chip. After seeding single cells in a microwell chip, cells can be cultured inside the chip, or can be transferred to a tissue culture plate for clonal expansion or downstream applications.

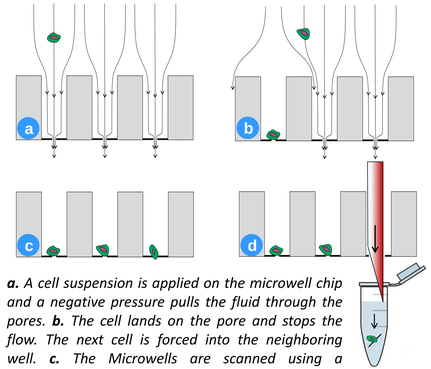
Microwell Principles



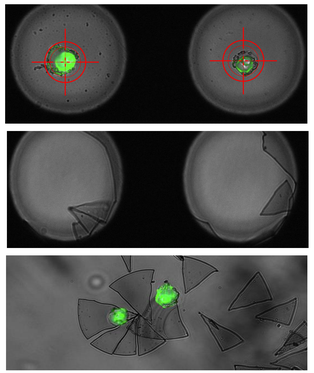


a. *The microwell chip fits in a microscope slide sized plastic holder.* ***b.*** *The filter consists of 6400 wells on a 8 x 8 mm surface.* ***c.*** *The microwells are 300 µm deep with a diameter of 70 µm.* ***d.*** *A single 5 µm pore is positioned in the middle of bottom of each well. The SiNi bottom of the well has a thickness of 1 µm.*

Single Cell Isolation



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

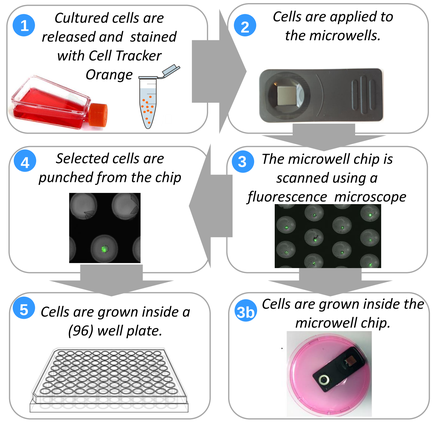


1. microwells with cells are selected for punching.

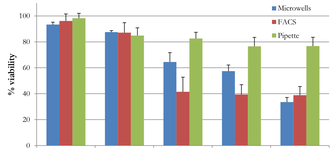
2. After the microwells are punched out the system will re-image the wells to confirm that cells are punched.

3. Cells and shards can be found back underneath the microwells.

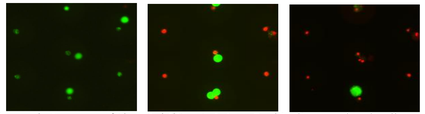
Workflow single cell isolation and expansion



Single cell viability inside a microwell chip

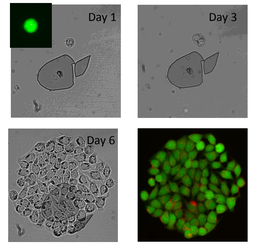


MCF-7 cells are seeded in the microwell chip and their viability is compared to other methods of single cell sorting in culture plates. Cells are stained at different intervals to determine the viability.



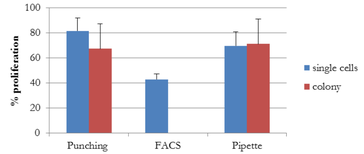
Example images of the viability staining. Red indicates dead cells (EthD1) and green live cells (Calc AM)

Single cell growth after punching into TC plate



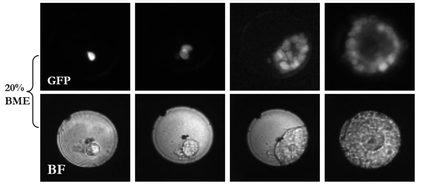
Series of images showing the growth of a single MCF-7 cell after it has been punched out of the microwell chip. Calcein AM green (alive) and EthD1 red (dead) fluorescence.

Growth efficiency after sorting



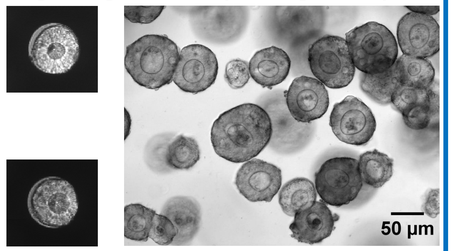
Growth of single cell and colonies after isolation. Number of cells forming colonies (3-5 cells) after 14 days culture is quantified. For FACS only growth from single cells is shown because sorting of colonies is not possible.

CRC organoid culture in a microwell chip



Typical images of CRC organoids cultured in microwells in the presence of EGF + noggin + R-spondin1. Round-shape organoids with concentric cell arrangement were generated.

Single cell growth after punching into TC plate



Organoid morphology in microwells and tissue culture plate. CRC organoids cultured in microwells (Left) show similar morphology to organoids cultured in tissue culture plate (right). After 3 days culturing, the formation of crypt-like domains appear. The lumen is filled with dead cell material (dark gray) creating cyst pressure.

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. We showed that cells from different origin could be cultured in microwells. In addition, 3D tumor organoids from CRCs were generated in the microwells. Furthermore we showed that the microwell chip could be used as a cell sorter. Single cells or colonies isolated in the microwells could be easily punched out of the microwell chip for further culture. The presented experiments give a good indication of the characteristics and potential of the microwell chip.

Outlook

The tools presented here can be used for research involving single cell isolation to study cell populations grown from individual cells. Research is ongoing to isolate, sort and culture patient circulating tumor cells.

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