Self-Sorting Nanowell Plate for the Isolation and Characterization of Single Circulating Tumor Cells

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Abstract 3065
AACR 2014

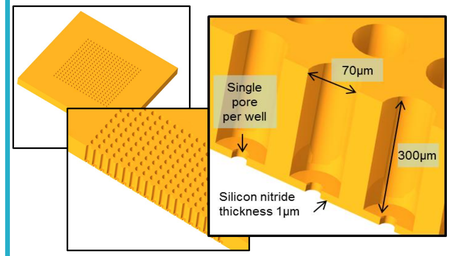
Introduction

Next breakthrough in the promise of CTC is their characterization to uncover treatment targets enabling the administration of a therapy with a high likelihood of being effective. Tumor cells are however heterogeneous, which dictates the need for analysis at the single cell level. In addition, gene expression can be altered during the course of the disease and is accelerated under the influence of therapy. This imposes the need for a tumor biopsy each time therapeutic intervention is required. As biopsies are difficult if not impossible to obtain, CTC represent a unique opportunity for a liquid biopsy through a blood sampling procedure. CTC are however extremely rare and only few are available for a detailed analysis. A variety of technologies have been introduced to enrich and count CTC from blood, but all are hampered by inefficiency to isolate individual CTC for further molecular characterization to unveil the best treatment strategy. Here we introduce a simple solution to obtain and analyze the genetic make-up of individual CTC and demonstrate this with cultured prostate cancer cells.

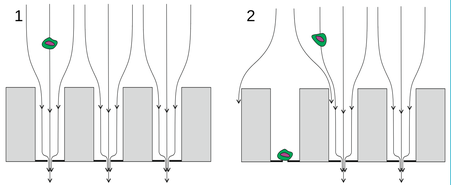
We present a self-sorting micro-fabricated well plate for the capture and subsequent genetic make-up of individual Circulating Tumor Cells (CTC). A cell suspension is filtered through a microwell plate containing wells with a diameter of 70µm having a 5µm hole in the bottom of the well. After a cell has plugged the hole, the flow within the well is halted, enabling a perfect distribution of individual cells over the microwells. Typically more than 90% of all the wells hold single cells.

Microwell principles

Microwell plate design

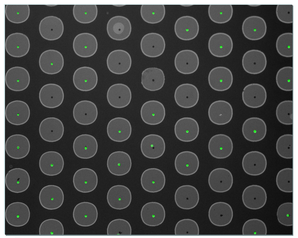


Single cell capture



A cell suspension is applied on the microwell plate. Vacuum 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.

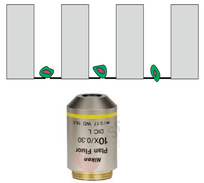


Fluorescence image of cells in microwells. Each well contains a single cell.

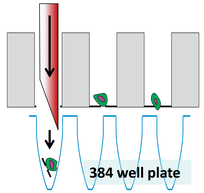
Single cell isolation

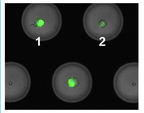
Single cell isolation procedure

Step 1 : Scan the individual wells

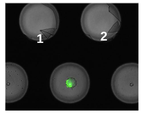


Step 2: Transfer the cell of interest by punching

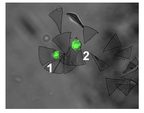




***1:** Select the cells by fluorescence*



***2:** Punch the cells out of the microwells*



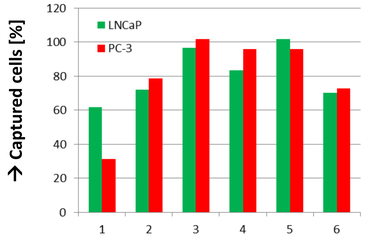
***3:** Example of punched cells*

Capture efficiency and DNA amplification

Experimental procedure:

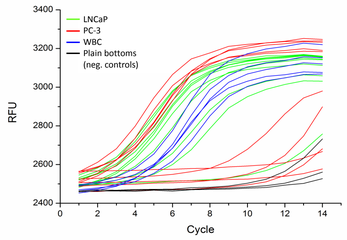
1. PC3 and LNCaP cells are spiked and **fixed** in blood in **CellSave™** tubes
2. Cells are **enriched** and **quantified** with CELLSEARCH™
3. Cells from the CELLSEARCH™ are filtered into **microwells**
4. Cells are **located** and **quantified** in the microwells
5. 30 PC3, 30 LNCaP, 15 leukocytes and 9 empty wells are **punched** into 384 well plates
6. DNA in all punched wells is **amplified** using the *Single Cell Whole Genome Amplification kit* from New England Biolabs.
7. **Quality** of the amplified DNA is tested by a 12-gene qPCR

Capture efficiency



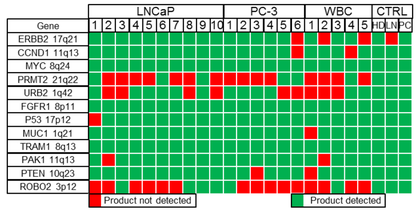
In 6 experiments LNCAP and PC3 cells were spikes in 7,5 ml blood of a healthy donor. Next, the sample was processed with CELLSEARCH™ and the number of cells was determined. Next, the sample was transferred to the microwell plate. On average 80% of the cells were recovered in the microwell plate. The cells were selected and punched.

Whole Genome Amplification



Graph presents 25 WGA DNA amplification curves of single cells that were punched from a microwell plate. The 25 cells were punched from a single microwell plate and cells were selected on basis of their fluorescence image. Reactions are regarded as positive when amplification has started before the 8th cycle. In total 79% of 75 punched cells generated WGA product. None of the negative controls (n=9) amplified before the 8th cycle.

Quality of WGA products



Quality of the WGA samples was tested by performing a qPCR on 12 genes. The samples are regarded positive when the Ct was below 40 cycles. The three control samples (CTRL) are qPCR's on isolated genomic DNA from Healthy Donor (HD), LNCaP (LN) and PC-3 (PC).

Conclusions

We present a fast, simple and controlled single cell sorting method for isolation and analysis of CTC's with a:

- Single cell capture efficiency in the microwells of 84%.
- Single cell amplification efficiency of 79% .
- 80% of the tested amplicons can be found in the single cell WGA products

Outlook

- Isolating single CTC from Patient samples
- Analyzing the cells using CGH or NGS
- Enlarging the number of wells on a chip

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