Single Cell Isolation and DNA Analysis from Circulating Tumor Cells using Self Sorting Microwell Filters

Joost Swennenhuis[1], Arjan GJ Tibbe[2], Michiel Stevens[2], Nikolas H. Stoecklein[3], Rui Neves[3], Hien Duy Tong[4], Cees van Rijn[5], Leon WMM Terstappen[1]

j.f.swennenhuis@utwente.nl

1. Department of Medical Cell BioPhysics, MIRA institute, University of Twente, Enschede, The Netherlands; **2.** VyCAP BV, Hallenweg 23, 7522 NH, Enschede, The Netherlands
3. Heinrich Heine University, Dusseldorf, Germany. **4.**Laboratory for Nanotechnology, Vietnam National University, HoChiMinh, Vietnam; **5.** Laboratory of Organic Chemistry, Dreijenplein 8, University of Wageningen, Wageningen, the Netherlands

Abstract 1606
AACR 2015

Introduction

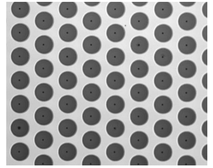
The heterogeneity of tumor cells dictates the need for analysis at the single cell level. Gene expression can be altered during the course of the disease and is accelerated under the influence of therapy. This imposes the need for a tumor biopsy each time therapeutic intervention is required. Circulating Tumor Cells (CTC) represent a unique opportunity for a "real time liquid biopsy". Current available CTC counting technologies are hampered by inefficiency to isolate individual CTC for further molecular characterization to unveil the best treatment strategy. Here we introduce a simple method to obtain single and pure CTC. The presented method is compatible with the routine laboratory workflow.

The method uses a specially designed Microwell filter to isolate all single cells from a cell suspension. This is followed by punching the CTC out of this filter into a PCR tube.

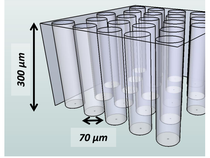
Microwell Principles

Microwell Filter Design

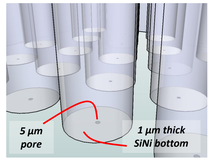
The Microwell filter fits in a microscope slide sized plastic holder.



The filter consists of 6400 wells on a 8 x 8 mm surface.

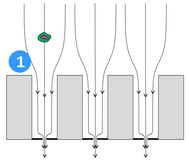


The Microwells are 300 µm deep with a diameter of 70 µm.

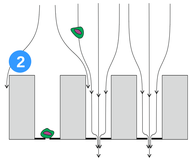


A single 5 µm pore is positioned in the middle of bottom of each well. The bottom of the well is made of 1 µm thick silicon nitride.

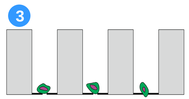
Single Cell Isolation



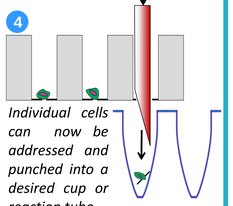
A cell suspension is applied on the Microwell filter. Vacuum pulls the fluid through the pores.



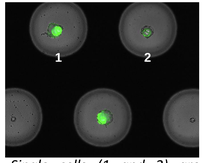
The cell lands on the pore and stops the flow. The next cell is forced into the neighboring well.



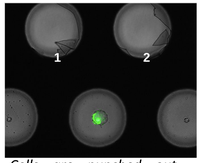
The Microwells are scanned using a fluorescence microscope to identify the cells and are next dried and stored.



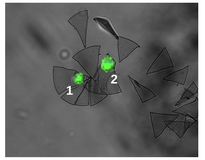
Individual cells can now be addressed and punched into a desired cup or reaction tube.



Single cells (1 and 2) are selected in the Microwell filter.



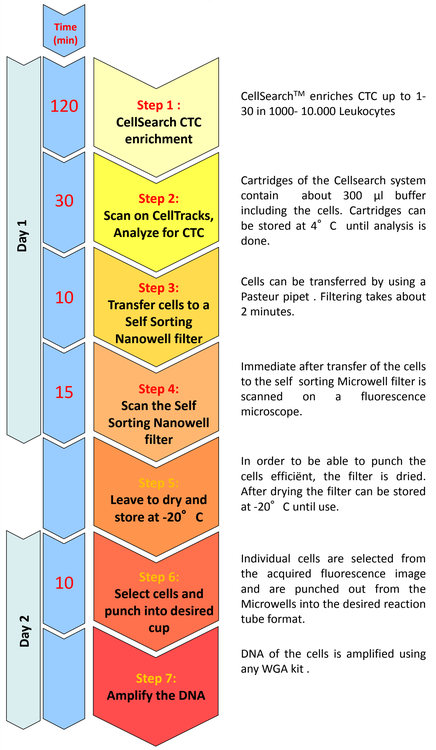
Cells are punched out with a needle.



Punched cells and bottoms of the SiNi membrane.

Workflow

From Patient Blood to Single Cell DNA



CellSearch™ enriches CTC up to 1-30 in 1000- 10.000 Leukocytes

Cartridges of the Cellsearch system contain about 300 µl buffer including the cells. Cartridges can be stored at 4° C until analysis is done.

Cells can be transferred by using a Pasteur pipet . Filtering takes about 2 minutes.

Immediate after transfer of the cells to the self sorting Microwell filter is scanned on a fluorescence microscope.

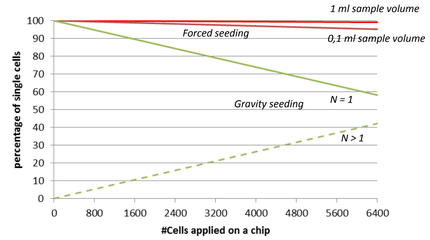
In order to be able to punch the cells efficiënt, the filter is dried. After drying the filter can be stored at -20° C until use.

Individual cells are selected from the acquired fluorescence image and are punched out from the Microwells into the desired reaction tube format.

DNA of the cells is amplified using any WGA kit .

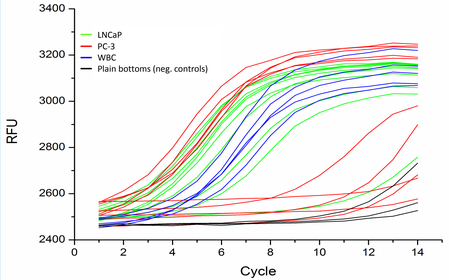
The workflow for punching single CTC into cups takes only a limited amount of time. Since the cups are dried it is possible to store the slides for longer periods before punching and processing the cells. This way more patient samples can be processed in parallel.

Single Cell Seeding Efficiency



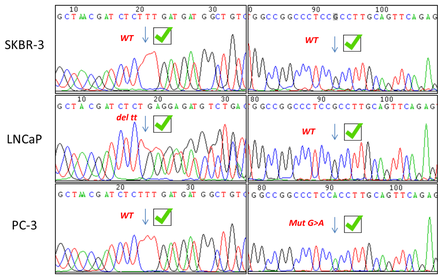
The X-axis displays the number of cells applied on a Microwell filter with 6400 wells. The Y-axis displays the percentage of cell containing wells, containing 1 cell. Red lines represent the probability for forced seeding on the Microwell filter for a sample volume of 1 and 0.1 mL. The green line presents the percentage for seeding by sedimentation. Green dotted line gives the percentage of wells containing more than 1 cell.

Single cell Whole Genome Amplification



Graph presents 25 WGA DNA amplification curves of single cells that were punched from a Microwell filter. The 25 cells were punched from a single Microwell filter 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.

Mutations in Cells Punched from one Filter



Sanger sequence data of the ROBO2 and PTEN gene of punched cells are presented. Cells are punched from the same filter. SKBR cells are WT for both tested genes. LNCaP cells have a tt deletion in PTEN and are WT for ROBO2. PC-3 cells have a G>A mutation in ROBO2 and are WT for PTEN.

Conclusions

We introduced a self-sorting Microwell filter in combination with an efficient method for the isolation of single cells from a CTC enriched cell suspension. The single isolated cells can be amplified with different WGA kits and cells isolated with CellSearch can subsequently be used for downstream applications such as PCR and Sanger sequencing more than 95% of all the Microwells hold single cells.

Outlook

- Isolating single CTC from patient samples
- Analyzing the cells using CGH or NGS
- Increasing the number of wells on the filter

MIRA
INSTITUTE FOR BIOMEDICAL TECHNOLOGY AND TECHNICAL MEDICINE

UNIVERSITEIT TWENTE.