Newsletter August 2012

MIRACLE



EU-Project 257743

Magnetic **I**solation and molecula**R** **A**nalysis of single **C**ircu**L**ating and disseminated tumor c**E**lls on chip (**MIRACLE**)

(01.09.10-31.08.14)

The project objective

The overall strategy of MIRACLE is the capture and multigene analysis of circulating tumor cells (CTCs) from clinical samples. The CTCs are immunologically captured and then characterized by a transistor-embedded active sieve. After cell lysis, multiple genes are amplified by RT-PCR and multiplex ligand-dependent probe amplification (MLPA) and then quantitatively detected by an electrochemical sensor array. The CTC counts and single-cell genotype will be used for cancer prognosis.

The consortium

- Interuniversitair Micro-Electronica Centrum VZW, Belgium
- Universitat Rovira i Virgili, Spain
- Institut für Mikrotechnik Mainz GmbH, Germany
- MRC Holland B.V., Netherland
- Oslo Universitetssykehus HK, Norway
- THINXXS Microtechnology AG, Germany
- ConsulTech GmbH, Germany
- Kungliga Tekniska Hoegskolan, Sweden
- MultiD Analyses AB, Sweden
- Fujirebio Diagnostics AB, Sweden
- European CanCer Organisation, Belgium
- Labman Automation Ltd., United Kingdom
- ICsense, Belgium

AIM

The MIRACLE project aims at the realization of a miniaturized system for immuno-magnetic isolation of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs), as well as genotyping by molecular analysis. The technical platform is supported by microelectronic, microfluidic, surface chemistry and bioinformatic technologies as well as (pre-) industrial manufacturing practice.

The technology

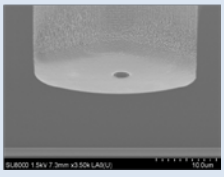
Complete integrated platform

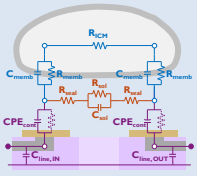
- Direct processing of clinical samples (e.g. blood)
- Microelectronic chip enables high-throughput single cell manipulation, characterization and lysis (10,000 cells analysis per chip)
- Simultaneous gene amplification by MLPA (31 genes for breast cancer)
- Proved electrochemical DNA quantification
- Microfluidics allows single-cell sensitivity and highly parallel multi-gene detection

Year 1&2 of MIRACLE mainly focused on feasibility tests & optimizations of major scientific & technical challenges, in order to verify the scientific principles and assess the technical hurdles and solutions. The progress in year 1 is summarized by functional modules.

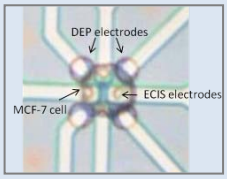
1.1 Cell isolation and analysis (WP 1, 4, 5, 6, 7)

Because of the low abundance of CTCs (down to 1 cell/mL peripheral blood), antibody-coated magnetic nanoparticles were used to capture CTCs. A special two-reservoir mixing device was designed for the sake of efficient cell-bead mixing and capture. We have been able to capture 10 MCF-7 cells in 5 mL blood sample with intact viability. The cell capture efficiency will be further improved toward single cell level by the employment of multiple antibodies and system optimizations.


(a)



(b)



(c)

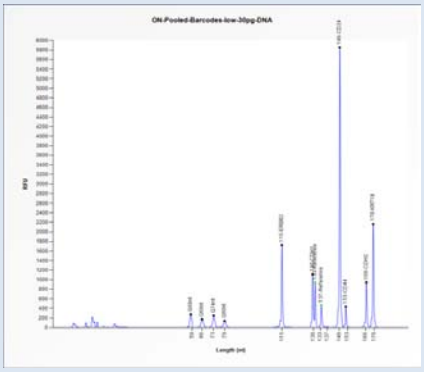
Figure 1 (a) An (inverted) passive sieve fabricated by deep silicon etch, (b) a test multi-electrode device for electrical manipulation and measurement on MCF-7 cells, (c) the equivalent circuit model for cell impedance measurement.

In spite of the state-of-the-art antibody-antigen recognition, the captured cells still inevitably include a few thousands of leukocytes in addition to only a few CTCs. The isolated cells will be transferred to an active sieve device for CTC identification and counting. The active sieve is a microchip composed of 10,000 through-silicon pores, where every pore can be individually addressed for dielectrophoretic cell positioning, cell impedance measurement and cell lysis by integrated transistor circuit. The identified CTCs were then lyzed for downstream gene amplification. The first version of active sieve was designed in year 1 and will be taped out for fabrication in year 2. In the mean time, the sieve package was also being tested aiming at a wafer-level microfluidic & electrical packaging approach to comply with pilot production.

This Newsletter describes work undertaken in the context of EC FP7-ICT project 257743. It is funded by the European Commission. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

SEVENTH FRAMEWORK PROGRAMME

1.2 Multiplex gene amplification (WP 2, 5, 6, 7)



CTCs are not only extremely rare, but also very heterogeneous in their phenotype and genotype. Thus, single cell analysis is significantly important. For this reason, we aim at amplification and detection for multiple genes at single cell level. After cell lysis, the mRNA of CTCs are collected and amplified in order to study the expression profile. Multiplex Ligation-dependent probe Amplification (MLPA) was selected due to the multiplexing capabilities. For breast cancer and prostate cancer, 31 and 23 genes were selected, respectively. In Year 1, the probemix for breast cancer was tested for three cell lines and spiked tumor cells off-chip and the probemix for prostate cancer was developed. For the breast cancer probe mix, the gene specificity was verified, with detection limit of single cell RNA level.

Meanwhile, an injection-moulded amplification chip was fabricated and optimized for thermal cycling . The chip performance was verified by on-chip RT-MLPA tests with fluorescent detection. Future efforts will be focusing on single-cell sensitivity with clinical samples for on-chip amplifications.

1.3 DNA detection (WP 3, 4, 5, 6, 7)

After MLPA amplifications, the DNA amplicons are quantified by an electrochemicalsensor array. The sensor chip comprises of 64 electrodes on a print circuit board for parallel detection of multiple genes (Figure 3b). For every gene, the target fragment hybridizes to immobilized probes on the sensor surface with a redox label. Electrical current catalyzed by the redox label is sensed in order to quantify the labels and hence the amplicons [Figure 3(a)].

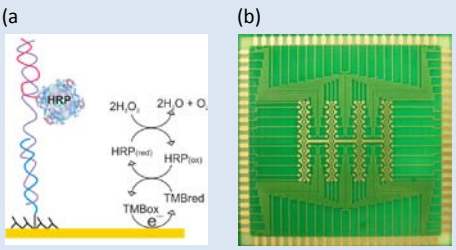


Figure 3 (a) A principle of the DNA sensor; (b) the DNA sensor array.

Two approaches were tested to optimize the surface chemistry: co-immobilization of short thiolated DNA probes and DNA polymer conjugates. A detection limit of 50 pM was achieved. For higher detection sensitivity and efficiency, several approaches were tested toward a nanoelectrode array instead of normal flat electrodes, where templated DNA deposition and colloidal lithography showed promising results.

1.4 System integration & benchmarking (WP 4, 5, 6, 7)

The MIRACLE system and components were designed based on requirements from clinical cancer diagnosis as well as to meet the instrumentation regulations such as CE mark. The cell isolation, gene amplification and detection modules are all supported by microfluidics technology, in particular, by injection moulding to improve component reliability. These modules will be evaluated separately in clinical conditions and then be integrated. Several critical issues were addressed in the first year, such as reagent storage, thermal control method, pneumatic interfacing, twin film valve, etc.

Publications:

Stakenborg et al. (2010) *Automated genotyping of circulating tumor cells*; Expert Review of Molecular Diagnostics; 10(6), 723-729

Henry, OYF et al. (2011) *Three-dimensional Arrangement of Short DNA Oligonucleotides at Surfaces via the Synthesis of DNA-branched Polyacrylamide Brushes by SI-ATRP*; accepted in Macromol Rapid Commun.

Henry, OYF et al. (2010) "*Bipodal PEGylated alkanethiol for the enhanced electrochemical detection of genetic markers involved in breast cancer*." Biosens Bioelectron 26(4): 1500-1506.

Henry, O. Y. F., S. Kirwan, et al. (2011) "*Electrochemical genosensor based on three-dimensional DNA polymer brushes monolayers*". Accepted in Electrochem Com. Minor Revision.

Henry, O. Y. F., O'Sullivan, K. (2011) "*Rapid DNA hybridisation in microfluidics*". Accepted in TrAC. Minor revision.

P. Salvo, R. Verplancke, F. Bossuyt, D. Latta, B. Vandecasteele, C. Liu and J. Vanfleteren (2012) *"Adhesive bonding by SU-8 transfer for assembling microfluidic devices",* Microfluidics and Nanofluidics, published online, June 23, 2012, (IF-2010 : 3.507)