LAB-ON-A-CHIP FOR THE MAGNETIC ISOLATION AND ANALYSIS OF CIRCULATING TUMOR CELLS

C. Liu[1,*], T. Stakenborg[1], O. Henry[2], C. O'Sullivan[2], E. Borgen[3], C. Schirmer[3], N. Laddach[4], T. Roeser[5], D. Latta[5], M. Ritzi-Lehnert[5], C. Fermer[6], J. van de Flierdt[7], S. Hauch[7] and L. Lagae[1]

[1]Imec, Belgium. [2]Universitat Rovira I Virgili, Spain. [3]Oslo University Hospital, Norway. [4]MRC-Holland, the Netherlands. [5]Institut für Mikrotechnik Mainz GmbH, Germany. [6]Fujirebio Diagnostics AB, Sweden. [7]AdnaGen, Germany.

ABSTRACT

The isolation and analysis of circulating tumor cells is very challenging because of the low abundance of CTCs in the peripheral blood and the physiological and morphological similarity to other blood cells. We report a miniaturized system which comprises immuomagnetic CTC isolation, multiplex ligation-dependent probe amplification (MLPA) and electrochemical gene detection.

KEYWORDS: circulating tumor cell, lab-on-a-chip, immunomagnetic isolation, cell sorting, DNA amplification, electrochemical sensor

INTRODUCTION

Circulating tumor cells (CTC) have been attracting increasing attention due to its important role in cancer metastasis. The isolation of CTCs, however, is very challenging because of the low abundance of CTCs in the peripheral blood (< 5 CTCs/mL), in comparison with other cells, e.g. 5×10^6 white blood cells (WBC) and 5×10^9 red blood cells (RBC) per mL. Moreover, the uncertainty of cell size and of surface marker specificity further increases the difficulty for CTC identification and analyses. Recently, there have been products and reports to overcome the technological hurdle based on novel micro- and nanotechnology, such as CTC isolation by antibody-coated nanoparticles (CellSearch®, Veridex), or by microfluidic filtration using micro structures [1]. However, they are suffering from either the non-specificity of single surface marker or cumbersome instrumentation and operations. Here we report a miniaturized system which integrates several sequential modules for CTC analysis: immunomagnetic cell isolation, RT-MLPA amplification and electrochemical gene detection[2] (Figure 1).

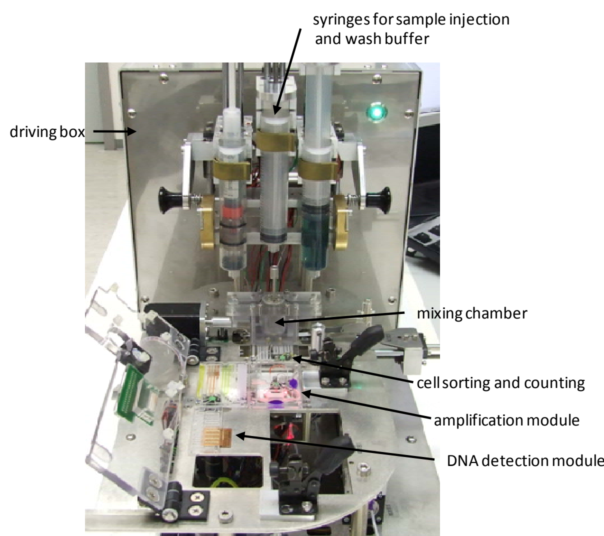


Figure 1 System overview

CTC ISOLATION

Magnetic particles coated with antibodies against EpCAM and MUC-1 markers (BreastSelect®, AdnaGen) were mixed with spiked MCF-7 cells in 7.5 mL blood by a paddle wheel mixer in an incubation chamber (Figure 2). After mixing for 30 minutes, a positive magnetic isolation was applied by an external magnet, followed by PBS buffer washing of the magnetically labeled cells. Afterward, the magnetic content, including cell-bead complexes and excessive beads, was resuspended in 100 µL buffer and transferred to the cell sorter chip.

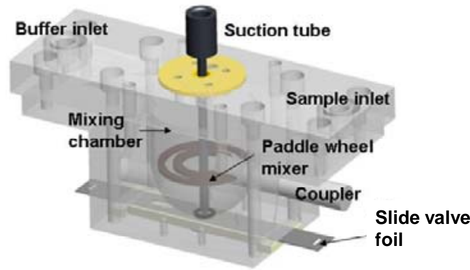
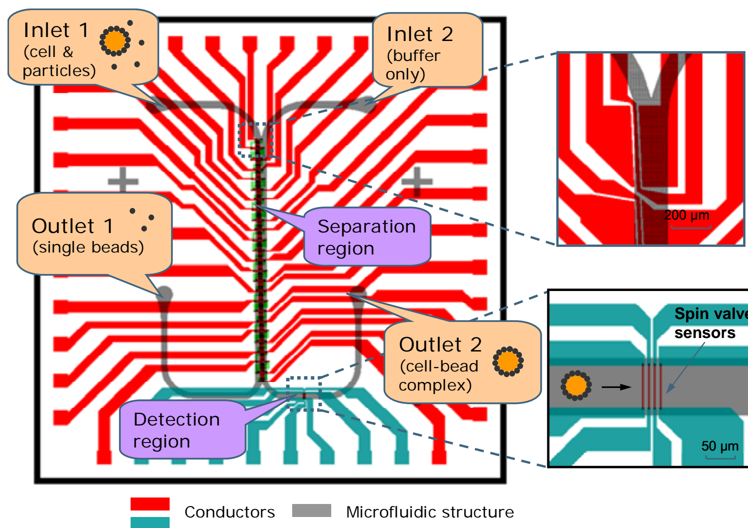
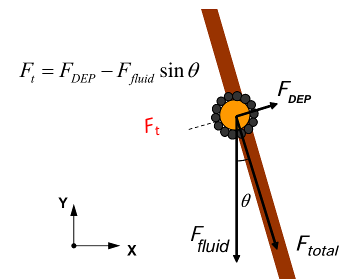


Figure 2 The cell isolation module. The fluid is injected from the multiple inlets and mixed by the paddle wheel mixer, and hence the cells are bound with magnetic particles. The magnetic content can be attracted by the magnet when it moved to proximity, which allows buffer replacement and cell washing. Finally, the mixture of cell-bead complexes and excessive beads are moved to the microfluidic cell sorter underneath.

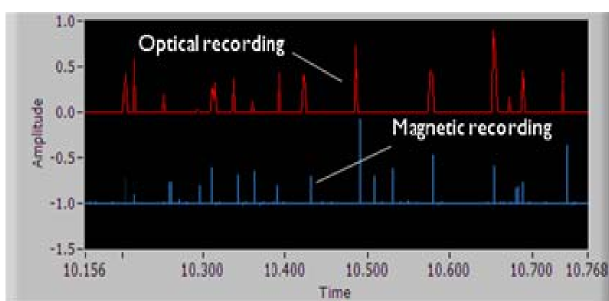
CELL SORTING AND COUNTING

Because of the clinical relevance of the CTC concentration, we are also testing an microelectronic cell sorter chip for to be integrated into the system. The cell sorter first isolated the magnetically labeled target cells by positive dielectrophoresis (DEP). After the isolation, the cells were counted by embedded giant magnetoresistive (GMR) sensors. An overview of the chip is shown in Figure 3a. The cell sorting took place in an X-shape microfluidic channel composed of two inlets and two outlets. The mixture of cell-MP complexes and single MPs was injected through Inlet 1 (upper-left inlet in Figure 3a) and clear PBS buffer through Inlet 2 (upper-right inlet in Figure 3a). The two flows met at the first junction of the long separation region. The sorting device was buried under the X-shape microfluidic channel. There were eight sorting segments in the separation region, not placed parallel to the flow direction but tilted with a small angle θ (the 8th element is shown in Figure 3a). With positive DEP in 8.5% sucrose buffer, the cells were attracted to the tilted electrode, because the DEP force, F_{DEP}, was larger than the fluidic drag force F_{fluid}, in the transport plane, i.e. the attractive force $F_t > 0$. Thus, the cell-particle complexes were carried by the total in-plane force to the detection region and finally to Outlet 2 (bottom-right outlet in Figure 3a). By contrast, MPs experienced negative DEP, i.e. $F_t < 0$, thus ending up at Outlet 1 (bottom-left outlet in Figure 3a). In addition to the cell sorting structure, an array of magnetic sensors comprising four giant magnetoresistive (GMR) spin valve sensors were embedded in the Outlet 2 branch channel in order to count the cells. The stray magnetic field of MPs was consecutively detected by the sensors, hence cell-MP complexes were counted. The cell sorter was benchmarked using simultaneous optical & magnetic counting (Figure 3c). A Zeiss confocal microscope was used for optical in-flow cell counting. Cell isolation efficiency of 80% was obtained for MCF-7 cells, from which 79% cells were magnetically counted. The undetected cells were probably with low MP coverage and/or the conjugated MPs were too far from the sensors when the cells flowed over.





(a) (b)



(c)

Figure 3 The cell sorter chip.

MULTIPLEX GENE AMPLIFICATION AND DETECTION

Aside from cell quantification, 20 genetic markers were selected for genotyping (BIRC5, CEACAM3, EGFR, ERBB2, ESR1, ESR2, GRP, KRT19, KRT20, MUC1, MYC, PGR, PLAUR, PTPRC, S100A4, SCGB2A1, SCGB2A2, TACSTD1, TERT, PTPRC, ACTB and CD45). For this purpose, the isolated cells were lyzed and RNA were extracted. The RNA's were amplified by RT-PCR and MLPA. The MLPA kit was evaluated using standard cell lines including MCF-7.

After amplification, the amplicons were detected by an electrochemical sensor array. The sensors were gold micro electrodes with self-assembled monolayer with immobilized DNA probes. The target DNA fragments were hybridized onto the sensor surface and finally conjugated with Horseradish peroxidise (HRP) labels. Electrons were released from the redox reactions mediated by HRP, hence the electrical current was measured for amplicon quantification. The DNA sensor was evaluated by measuring five amplicons at different concentration in a sensor array of 16 electrodes (Figure 4).

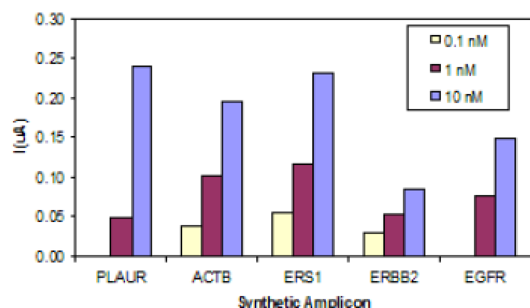


Figure 4. The electrochemical detection of five markers.

CONCLUSION

We demonstrated the integration of cell isolation, gene amplification and detection in a same miniaturized system for CTC analysis. The use of magnetic particles minimized cell loss during isolation. In comparison to prior products and report, the inclusion of 20 markers improved the capability and flexibility for genetic characterization. The reduced sample consumption (due to the nature of microfluidics), multiplex PCR and genosensor array featured the system as suitable for fast, versatile and low-cost characterization tool for CTC diagnostics.

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CONTACT

*C. Liu, tel: +32-16-288953; chengxun.liu@imec.be