FULLY AUTOMATED IMMUNOMAGNETIC LAB-ON-CHIP FOR RARE CANCER CELLS SORTING, ENUMERATION AND IN-SITU ANALYSIS

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ABSTRACT

We report on the development of a fully automated microfluidic platform that can sort rare cancer cells with a capture rate above 90%, while maintaining a very low (<0.04%) contamination rate. This system relies on the use of antibody-coated magnetic microspheres self-assembled into columns, thus creating a dense sieve in which target cells are captured based on their surface antibodies expression. Additionally, complete automation of the capture and analysis processes, along with a preliminary clinical study on patients sample showed that this tool is suitable for high throughput (>3ml/h) capture of very rare cells (under 1 per ml of blood) along with a use in a more fundamental research context.

KEYWORDS : Metastatic disease, cancer diagnosis, circulating tumor cells, cell sorting, lab-on-chip

INTRODUCTION

Metastases, responsible for 90% of the deaths of cancer patients, are generated by cells escaped from the primary cancer that circulates in blood. Clinical studies report that the detection of those Circulating Tumor Cells (CTCs) is associated with the onset of later metastatic relapse [1,2], and that CTC levels in blood are also correlated with the survival of patients with metastases [3]. Thus, CTCs are of great interest for follow-up, cancer relapse prediction and treatment orientation, but CTCs sorting and studying is challenging due to their rareness in blood (0.1 to hundreds of CTC per ml of blood as compared to billions of erythrocytes and millions of leucocytes). Commercial technologies such as Veridex (CellSearch®) are very efficient but show limitations when using multiple capture and/or staining biomarkers. Microfluidic systems used for CTC capture [4] such as the "CTC-chip" [5] use either mechanical properties of cells (e.g. size and deformability) or biomarkers expression (e.g. specific membrane antigens) to sort them specifically. The EpCAM (Epithelial Cell Adhesion Molecule) membrane antigen is commonly used as a target; its antibody being grafted on the chips walls, which requires long chemical preparation of the surface to be coated. We have overcome this cumbersome chip fabrication process by implementing the Ephesia technology, based on columns of antibody-coated beads, in a high (>3ml/h) throughput microfluidic device and shown its ability to sort circulating tumor cells from blood of metastatic cancer patients.

EXPERIMENTAL

The capture device is based on the Ephesia technology [6], inside a 5 cm diameter circular PDMS chip. In a microfluidic chamber, antibody-grafted (anti-EpCAM) 4.5µm magnetic beads are introduced at 30 µl/min. Using a coil to create a magnetic field perpendicular to the flow, the beads are assembled into columns above the magnetic pattern chosen (see Figure 1.a, 1.b and 2), thus creating a dense sieve in which cells have a very high probability of colliding into the columns. As a sample is injected in the columns array, target cells are captured specifically through antibody/antigen interaction while non-target cells pass through the capture device. Once immobilized, multiple stainings such as Nucleus, CD45 and Cytokeratin can be easily achieved. Because the transparent chip lies on an inverted microscope, it is possible to achieve high resolution microscopy in-situ. The chip design was optimized [7] (see Figure 1.a) in order to achieve the high throughput (>3ml/h) necessary

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for the treatment of a blood tube in less than 2 hours, while ensuring an homogeneous flow velocity inside the capture chambers. Complete optimization and automation of the different staining and optical analysis steps were implemented, reducing operating time and reagent consumption while providing highly reproducible results.

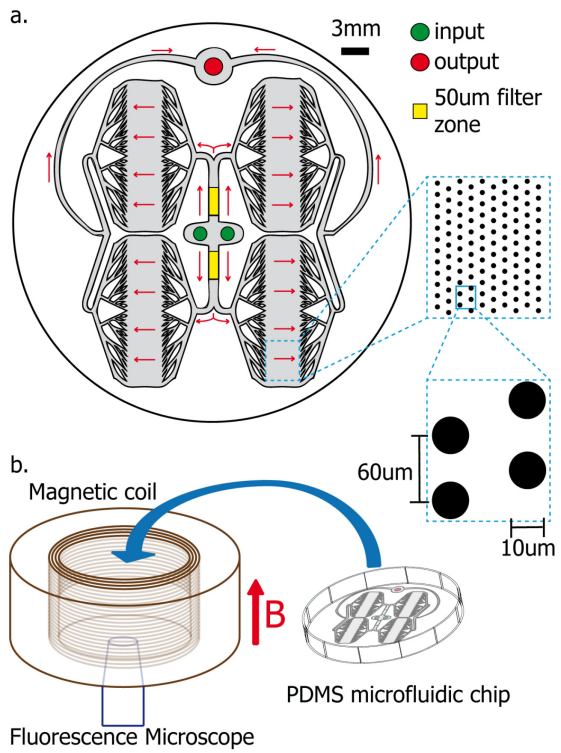

Figure 1: a. Chip design, with the magnetic patterning. b. The chip is inserted in a magnetic coil and installed on a fluorescence microscope.

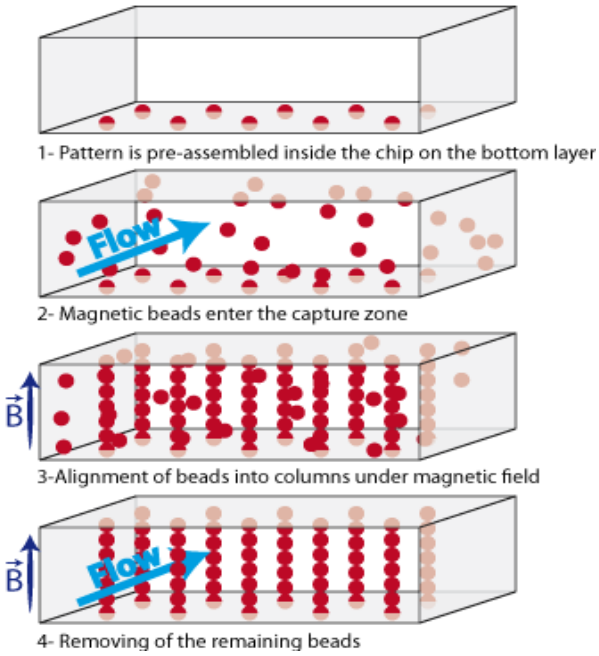

Figure 2: Magnetic columns formation process.. A 30mT magnetic field oriented perpendicular to the flow is generated in a 40µm high channel.

RESULTS

Using cell lines (SKBR3, MCF7 for breast cancer, PC3 for prostate cancer, Raji and Jurkat as lymphoid cells), we managed to increase capture efficiency up to 90.6% (+/- 5.8%, N=9) for cell quantity as low as 50 cells in 1ml (Figure 2). Spiking MCF7 into Raji (1:10000 ratio) showed similar capture efficiency while specificity remains high (i.e. non-specific capture below 0.4%). The clinical potential of our microfluidic device has been further investigated by analyzing real patient samples (N=10) from breast and prostate cancer. It showed performances similar to the commercial Cellsearch system (N=8), and better results in the case of specific cancers where additional stainings could be used (such as PSA in prostate cancer, N=2). This preliminary study shows that the system is suitable for a clinical use on real sample, up to 48 hours after blood collection. A complete experiment could be achieved in 6 hours, including the sample preparation process. Finally, we demonstrate the flexibility of the system by using different antibodies for capture, cultivating cancer cells directly on chip or retrieving their genomic content for further analysis such as mutations detection.

CONCLUSION

This paper reports on the development of a fully-automated, highly reproducible microfluidic lab on chip that meets the clinical needs of very rare cells sorting, while allowing for a highly versatile use in a more fundamental research context. It opens the route towards a better understanding of the metastatic disease via clinical and biological studies.

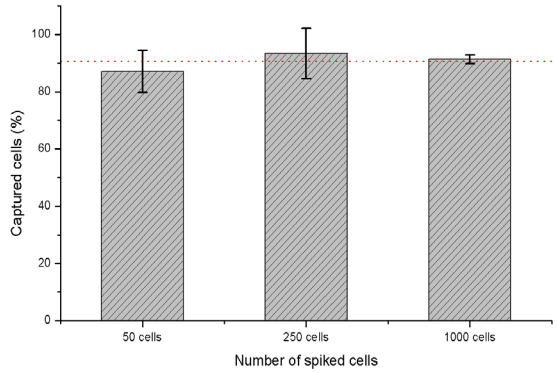

Figure 3: Spiking of MCF7 epithelial cells into buffer showed capture efficiency around 90%, even for low-number of cells.(up to 1ml processed)

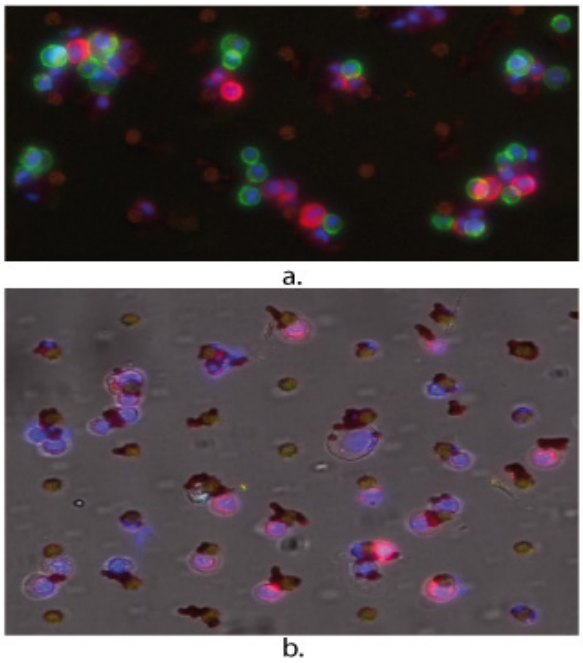

Figure 4. a. Spiked cell lines captured on beads. b. Breast cancer cells from patient sample. (nucleus (blue), CD45 (green), Cytokeratin (red) and transmission (grey levels))

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