Selective Isolation of Heterogenic Circulating Tumor Cells(CTCs) using Magnetic Gradient based Microfluidic System

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ABSTRACT

Circulating tumor cells(CTCs) is one of key marker of cancer metastasis in human body. Despite of the importance to diagnose the cancer metastasis by CTCs, still it is formidable challenge to use in the clinical purpose because of the rarity and the heterogeneity of CTCs in the cancer patient's peripheral blood sample. To solve those addressed limitations, we have developed magnetic force gradient based microfluidic system for isolating the total number of CTCs in the sample and characterizing the state of CTCs simultaneously with respect to the epithelial cell adhesion molecule (EpCAM) expression level. We have synthesized magnetic nanoparticles (MNPs) using hydrothermal method and functionalized anti-EpCAM on their surface for the specific binding with EpCAM on the tumor cell membrane. The microfluidic system designed to isolate and classify the CTCs by isolating at the different location in the chip using magnetic force differences depending on the EpCAM expression level. We observed 95.7% of EpCAM positive and 79.3% of EpCAM negative CTCs isolated in the microfluidic system. At the same time, the 71.3% of isolated EpCAM positive CTCs were isolated at the first half area whereas the 76.9% of EpCAM negative CTCs were collected at the latter half area.

1. INTRODUCTION

Circulating tumor cells(CTCs) is the single cancer cell which circulated human body through the blood vessel network from the primary tumor site after lack of nutrient and oxygen due to the overpopulation. The CTCs in the blood vessel keep trying to find the secondary site where they can survive with abundant nutrient and oxygen supplement. This relocation mechanism is called metastasis of tumor. The single cancer cells escaped from the primary tumor site is mostly filtered at lymph node where they met the first immune system of our body. Because of this primary filtration process, the detection of CTCs is extremely hard because of its rarity (typically 1–1,000 in 5x10^9 blood cells in 1ml volume of peripheral blood from human cancer patient[1]. Recently, not only for the rarity of CTCs, but also the characterization of heterogeneity of CTCs are rising as main challengeable problem on microfluidic research field. Few researchers have tried to isolate and specify the heterogeneity of CTCs simultaneously using the microfluidic system. But it is still a formidable challenge due to the low isolating yield, purity of heterogeneous CTCs and complex fluidic system[2]. To solve the problem with simple microfluidic system, we have developed the magnetic force gradient-based microfluidic system which can specify the heterogeneity subpopulation of CTCs based on the EpCAM expression differences while the CTCs is being isolated by magnetic nanoparticles (MNPs).

2. MATERIALS AND METHODS

2.1. Microchannel design

The microchannel for the Magnetic gradient chip is shown in figure 1. The microchannel has inlet, sample preparation segments, CTCs trapping segments, and outlet. The microfluidic system has 342 sorting segments (100μm×400μm×160μm, W×D×H). Theoretically, 1.46x10^6 cells can be captured. Mag-Gradient chip was fabricated using standard soft-lithography which well known to fabricate microfluidic channel, i.e., an SU-8 negative photo-resist polymer master template on silicon dioxide wafer, PDMS (poly(dimethylsiloxane) soft polymer molding, and oxygen plasma bonding on glass surface.
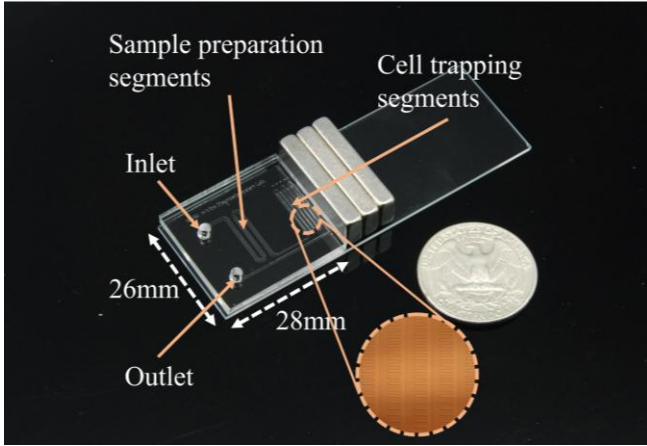
Figure 1. Optical and microscopic images of the Mag-gradient chip.

3. RESULT

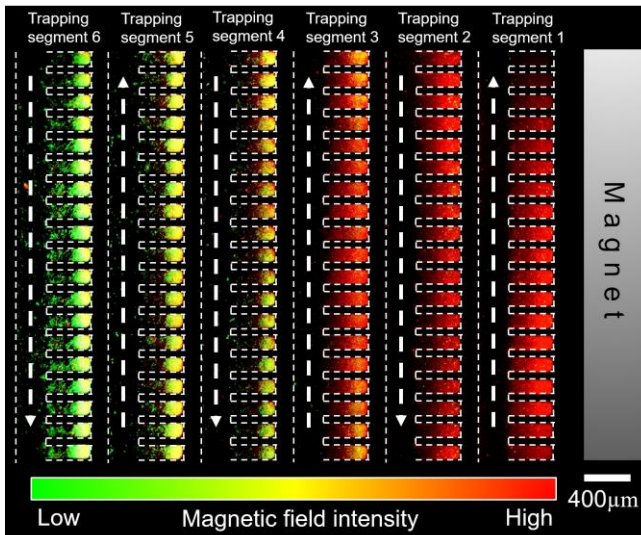### 3.1. Feasibility test of magnetic microparticles

Figure 2. Feasibility test result using 1 and 5µm diameter fluorescence magnetic micro-particle (Deep red: 1µm, Green: 5µm)

1µm and 5µm diameter magnetic particles have same magnetic force with MNPs labeled MCF-7 and MDA-MB-231, respectively. Magnetic particle data is tested at 50µl/min fluid flow velocity under 4.75kG neodymium magnet. The 79.9% of 5µm particles isolated in segment 6-4, whereas 74.6% of 1µm particles isolated in segment 4-1 shown in figure 2.

3.2. Cell line result

To validate the Mag-gradient chip, we have tested the chip with MCF-7 and MDA-MB-231 cell line from human breast carcinoma.
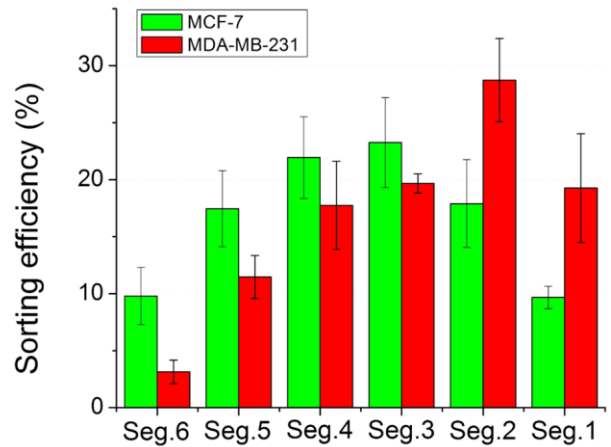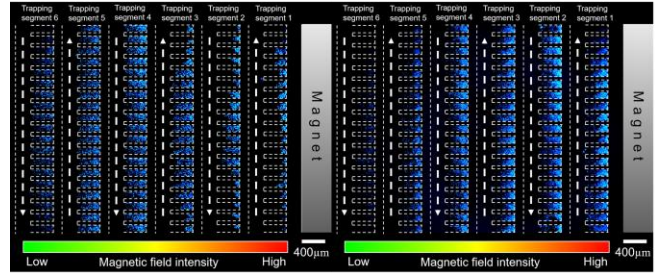

Figure 3. The selective isolation result of MCF-7 and MDA-MB-231.

The major difference between MCF-7 and MDA-MB-231 is EpCAM expression level on their membrane. The capturing efficiencies of MCF-7 and MDA-MB-231 are 95.28 ± 1.85% and 74.85 ± 3.44%.(n=3, 10^5cells/ml) The 72.43% of MCF-7 is located between segment 6-3 whereas 85.4% of MDA-MB-231 is located between segment 4-1. This result shows Mag-gradient chip can capture the heterogenic CTC and selective isolate at the same time.

4. CONCLUSION

The EpCAM expression level difference is one of the key markers to distinguish the metastatic or non-metastatic cancer cells. The Mag-gradient chip can isolate the heterogenic CTCs at the different location using magnetic intensity gradient. The Mag-gradient chip is not only can isolate the cells but also selectively isolate the heterogenic CTC with high efficiency. This simple and multi-functional Mag-gradient chip can be suitable system for cancer metastasis diagnosis.

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