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Selective isolation of magnetic nanoparticle-mediated heterogeneity subpopulation of circulating tumor cells using magnetic gradient based microfluidic system

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ABSTRACT

Relocation mechanisms of the circulating tumor cells (CTCs) from the primary site to the secondary site through the blood vessel network cause tumor metastasis. 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. In this study we have developed magnetic force gradient based microfluidic chip (Mag-Gradient Chip) 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 CTCs. The Mag-Gradient Chip 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 Mag-Gradient Chip. 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. The Mag-Gradient Chip can isolate the 3 ml of heterogeneous CTCs sample in 1 h with high isolating yield. The EpCAM expression level dose not means essential condition of the metastatic CTCs, but the Mag-Gradient Chip can shorten the date to diagnose the cancer metastasis in clinic.

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. This tumor cells are well known as the important clinical cue for diagnosis or prognosis of tumor metastasis. 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 1–100 in 5×10^9 blood cells, 1 ml volume of peripheral blood from human cancer patient) (Hyun and Jung, 2014) in cancer patient's blood sample. Many researchers have developed their own microfluidic system to isolate the CTCs using biological (Nagrath et al., 2007; Stott et al., 2010), magnetical (Kang et al., 2012), mechanical (Park et al., 2014; Tang et al., 2014), and hydro-dynamical (Sollier et al., 2014; Park and Jung, 2009; Choi et al., 2011; Murlidhar et al., 2014) methods as well as combined method (Ozkumur et al., 2013; Moon et al., 2011) with activated sorting mechanisms.

Recently, not only for the rarity of CTCs, but also the characterization of heterogeneity of CTCs are raising as main challengeable problem on microfluidic research field. The metastatic CTCs in the blood vessel required certain transforming step for extravasating known as de-differentiation for enabling invasion and dissemination into other organs. (Prang et al., 2005) Up to now, most of CTCs researches has focused on the direct cell culture of CTCs on their own microfluidic system for the further downstream analysis such as DNA sequencing to characterize the

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heterogeneity of CTCs. (Kang et al., 2012) Although few researchers have tried to isolate and specify the heterogeneity of CTCs simultaneously using the microfluidic system, it is still a formidable challenge due to the low isolating yield, purity of heterogeneous CTCs and complex fluidic system (Mohamadi et al., 2015).

To solve the problem with simple microfluidic system, we have developed the magnetic force gradient-based microfluidic system (Mag-Gradient Chip) which can specify the heterogeneity subpopulation of CTCs based on the EpCAM expression differences while the CTCs is being isolated by magnetic nanoparticles (MNPs). We have synthesized and surface modified the MNPs for the specific binding with the EpCAM on the CTCs. The different number of EpCAM on the EpCAM positive or negative CTCs is influenced by different amount of magnetic driven force. The magnetic force difference let the CTCs isolates at the different location on the Mag-Gradient Chip. Our experimental results show that the Mag-Gradient Chip promises to isolate and specify the heterogenic CTCs at the same time with high yield.

2. Materials and methods

2.1. Microchannel design

The microchannel for the Mag-Gradient Chip is shown in Fig. 1. The microchannel has inlets, sample preparation segments, CTCs trapping segments, and a broadened outlet. Multiple micro-post arrays in the sample preparation segments designed to break down the aggregated cells or filtering the debris before CTCs reached the trapping segments. Five serpentine microfluidic trapping segments were fabricated for the CTCs trapping as shown in Fig. 1. Each trapping segment has 30 rectangle sub-segments which are located on perpendicular direction in the fluid flow (Kang et al., 2012). From the serpentine trapping segment no.5 to 1, the magnetic flux density is square inverse proportion to the distance from the permanent magnet bar and it can generate the magnetic force gradient to isolate the EpCAM-positive or negative CTCs at different location of the segment channel. The distance between magnetic bar and each trapping segment channel no. 1, 2, 3, 4, and 5 is positioned in 500 μm, 1550 μm, 2600 μm, 3650 μm, and 4700 μm gap from the magnet bar, respectively. The designed depth of Mag-Gradient Chip is 80 μm. The detailed Mag-Gradient Chip fabrication process is described in Supplementary materials.

2.2. Magnetic nanoparticle conjugation

The MCF-7 and MDA-MB-231 cells from same origin carcinoma (i.e. human breast cancer cells), which have different EpCAM expression level, were selected. (Hyun and Jung, 2014) The MCF-7 and MDA-MB-231 have 222.7×10^3 binding sites/cell and 1.7×10^3 binding sites/cell on the membrane surface, respectively. (Prang et al., 2005) The immuno-fluorescence stained cell images were shown in supplementary materials Fig. S1(a, b) To conjugate the magnetic particles with CTCs, Fe_3O_4 MNPs have been synthesized by a simple hydrothermal treatment of $FeCl_3$, citrate, polyacrylamide, and urea. (Cheng et al., 2010). The characteristics of synthesized MNPs were shown in supplementary materials Fig. S2. Hydroxylated MNPs were functionalized with (3-Glycidyloxypropyl)tri-methoxysilane (Sigma-Aldrich, Missouri) for immobilization of EpCAM antibody (Polyclonal, Rabbit/IgG, Thermo Scientific, Massachusetts). After the washing process with ethanol and Dulbecco's phosphate buffered saline (DPBS, Thermo Scientific, Massachusetts), the surface modified MNPs were conjugate with the EpCAM antibody. 2 mg/ml of this EpCAM antibody-

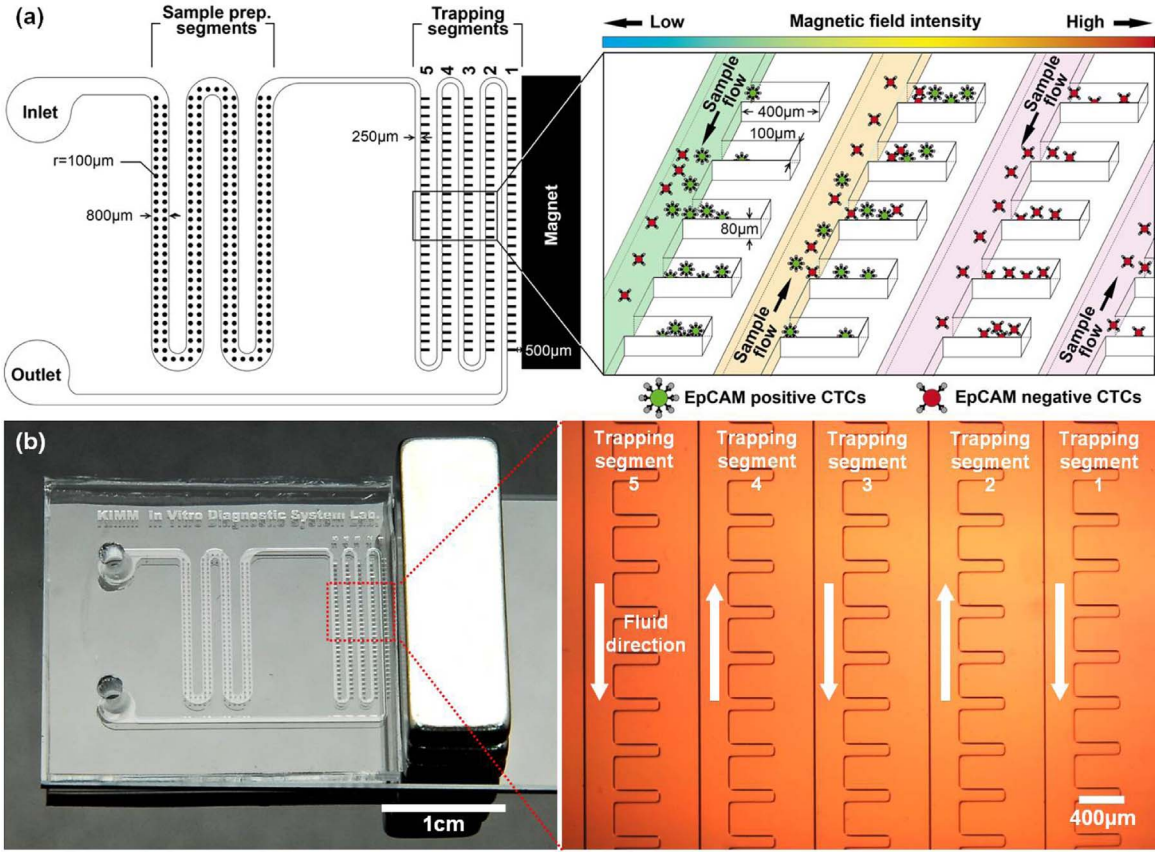

Fig. 1. (a) Schematic diagram, and (b) Optical and microscopic images of the Mag-Gradient Chip.

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