DEVELPOEMENT OF NOVEL CIRCULATING TUMOR CELLS SEPARATION AND NON-LABELING DETECTION BY CIRCULATING TUMOR CELLS' SPECIFIC PROPERTIES

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ABSTRACT

We developed non-labeling detection and easy separation method of circulating tumor cells (CTCs) based on CTCs' specific properties. Our developed method requires no special apparatus and tedious pretreatment before separation and detection of CTCs. In addition, our methods can also separate and detect epithelial cell adhesion molecule (EpCAM) negative CTCs, which cannot be attained by present methods. Therefore, our method would become accurate CTCs' analysis method and useful for point-of-care testing of CTCs.

KEYWORDS

Cell separation, biomimetic microspace, circulating tumor cells

INTRODUCTION

An analysis of CTC has been expected as less invasive cancer diagnosis after surgery because the number of CTC is related to the size of tumor and CTC collection from blood is less invasive compared to biopsy [1]. Despite of these advantages over biopsy, the CTC analysis in a clinic has been hampered because the counting and separation of CTC from blood require troublesome procedures, high sensitivity and high separation efficiency techniques because number of CTC is only a few in the blood [2]. In addition, most of the present CTCs' separation methods including Cellsearch system employ antibody-antigen (EpCAM) specific interaction for CTC separation from blood cells and cannot collect EpCAM negative CTCs. Therefore, the development of CTC analysis method based on a new principle has been desired for collection of EpCAM negative and positive CTCs and accurate analysis. In this paper, we focused on the specific property of CTC, and propose a novel detection and separation method of CTCs based on a new concept. Tumor cells secrete protease to destroy intercellular molecules and can slip out from primary tumor to vessel as shown in Figure 1. We employ this invasive property in detection and separation of CTCs, and mimic this environment in the microfluidic device.

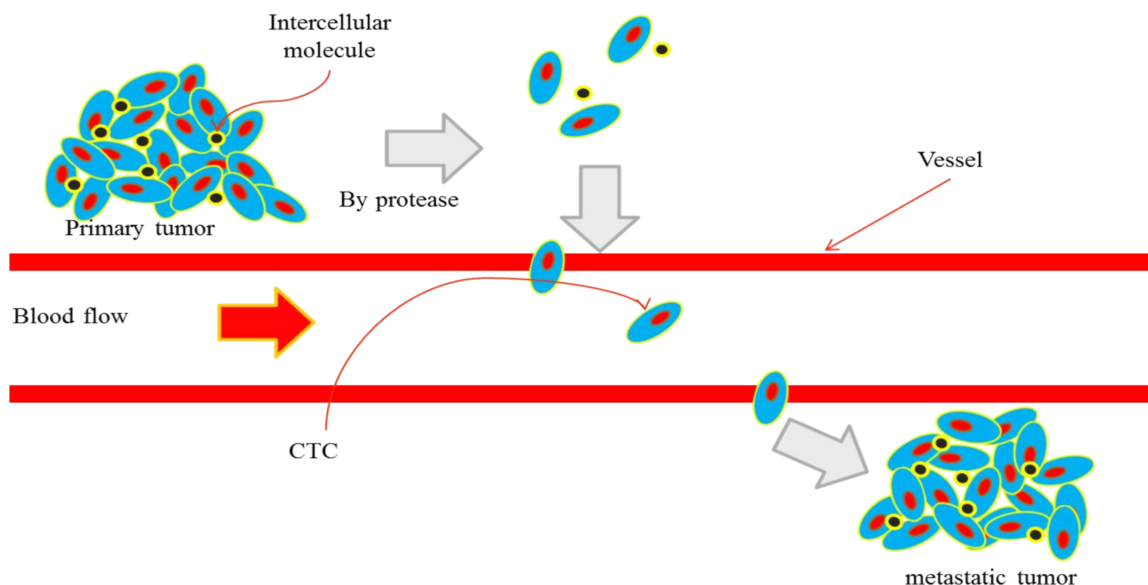

Figure 1. Mechanism of CTCs generation

EXPERIMENT

Procedures for detection and separation of CTCs are shown in Figure 2. For detection and separation of EpCAM positive and negative CTCs, we prepared fibrin gel coated PDMS microchips. This microchip permits CTCs to rapidly attach on fibrin gel surfaces, to be trapped by specific interaction between fibrin gel and CTCs, and to be detected as fluorescence points by CTC's secreted enzyme reaction. Thus, detection and separation of EpCAM positive and negative CTC can be attained in easy manner.

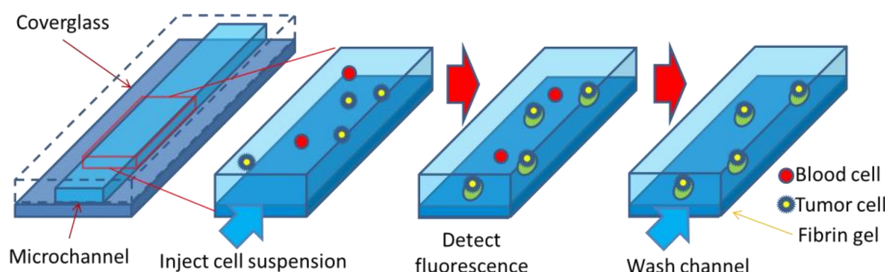

Figure 2. Principle of our developed detection and separation methods for CTCs

Fibrin gel coated PDMS microchip was prepared by following procedures: PDMS microchannel was prepared by soft lithography method, irradiated by air plasma, and then bonded with a cover glass. After chip fabrication, the cover glass was modified with 3-aminopropyltriethoxysilane to strongly immobilize fibrin gel. To this microchannel, mixtures of fibrinogen, thrombin and specific peptide to CTCs' secreted enzyme were introduced, incubated for two minutes and washed out by PBS. Stepwise modification of the cover glass was confirmed with contact angle measurements and was successfully attained as shown in Table 1. After modification of microchannel, we performed cell separation and detection using CTCs model cells as shown in Figure 2, and assessed the performance of our developed method.

Table 1. Water contact angle of each surfaces

	glass	APTS treated glass	Fibrin coated glass
Contact angle	<10	63.8	109.4

RESULTS AND DISCUSSION

At first, we attempted non-labeling detection of CTCs with the mixture of EpCAM negative cells and diluted blood. Figure 3 shows that blue fluorescence from specific peptide to CTCs' secreted enzyme can be observed only around CTC, and indicates that we can successfully detect only CTCs in a non-labeling manner. In addition, EpCAM positive cells were also detected in the same manner (data is not shown here). From these results, we can easily distinguish CTCs from blood cells, and count the number of EpCAM positive and negatice CTCs. Subsequently, separation of CTCs from blood cells was attempted. Figure 4 shows that almost only CTCs (EpCAM negative) can be retained on the fibrin gel coated microchannel after washing out the microchannel. In addition, trapping efficiency of CTCs after incubation on fibrin gel coated microchannel was estimated by varying flow rates. Figure 5 indicates that more than 80% CTCs can be trapped under 20 μl /min flow rate after 5h incubation. Thus, detection and separation of CTCS can be easily attained with our developed method.

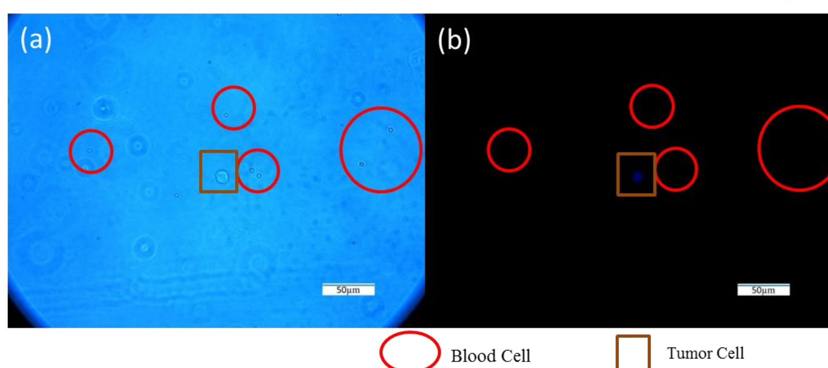

Figure 3. Micrscope images of EpCAM negative CTCs and blood cells mixtures.
(a) bright field and (b) fluorescence images (Scale bar = 50 μm)

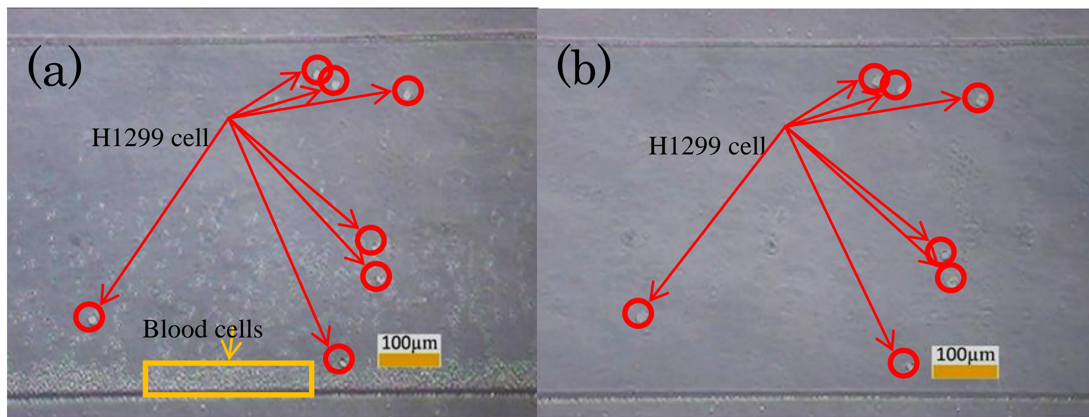

Figure 4. Microscope images of the fibrin gel coated microchannel (a) before washing and (b) after washing

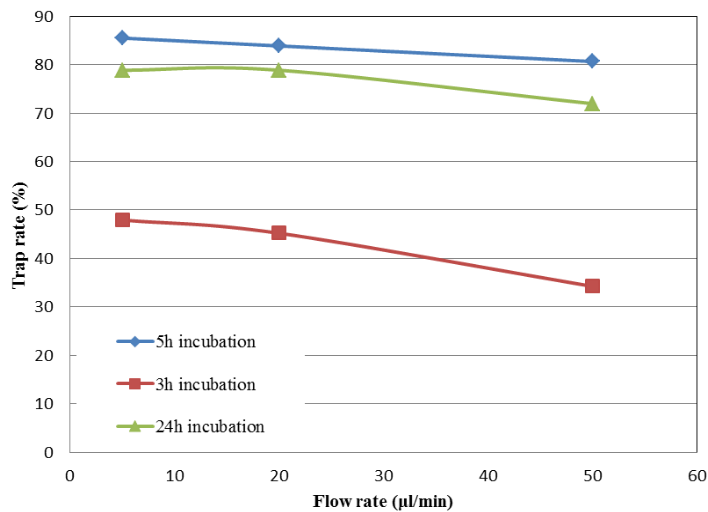

Figure 5. Trapping efficiency of CTCs under various flow rates

CONCLUSIONS

Our developed method based on CTCs' specific property enables easy detection and separation of CTCs. Therefore, our method would be applicable for clinical assay of cancer diagnosis.

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