Biosensors and Bioelectronics 102 (2018) 157–163

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

High-purity capture of CTCs based on micro-beads enhanced isolation by size of epithelial tumor cells (ISET) method

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ARTICLE INFO

Keywords:
Microbeads
CTC detection
ISET
Cancer patient blood
High purity

ABSTRACT

In this paper, we develop a low-cost size-based microfluidic chip using conventional polycarbonate membrane to isolate CTCs from blood, and propose a strategy to increase the capture efficiency before cell filtration by a size enlargement method utilizing modified microbeads specifically binding to CTCs. Up to 91% of target cells were isolated from whole blood samples using our microfluidic capture system at a flow rate of 1 mL/min. Moreover, a WBC depletion process is introduced which greatly decreases the WBC retaining on the filter membrane. The tests of immunofluorescence analysis of cells captured on the membrane were performed, which demonstrates that the device could provide a dependable CTC identification and CTC count in whole blood samples. Finally, the device was further validated in the detection of CTCs from blood samples of cancer patients, and it indicates a promising capability to detect CTC response to treatment.

1. Introduction

Due to the growing threat from cancer, the need for CTC (circulating tumor cell) detection has increasingly become urgency (Alix-Panabieres et al., 2014; Paterlini-Brechot et al., 2007). Many approaches have been used to isolate CTCs from whole blood, including immunoaffinity separation using immunomagnetic beads (Wen et al., 2014; Tang et al., 2016; Xiong et al., 2016), microfluidic platforms (Hajba et al., 2014; Karabacak et al., 2014; Sajay et al., 2014) and nanostructured substrates conjugated with antibodies against surface markers of cancer cells (Sun et al., 2016, 2015; Wang et al., 2009), while few of them have been successfully applied in clinic. Size-based isolation of CTCs, in which epithelial-derived tumor cells are considered to be larger and more rigid than most peripheral blood cells, is a classical technique being widely used owe to its standardized operation and noticeable recovery rate (Desitter et al., 2011). Even so, there are two main challenges in this traditional isolation by size of epithelial tumor cells (ISET). Size-based filtration has an obvious limitation with unsatisfactory isolation purity of CTCs, resulting from a trade-off with recovery rate, which will make troubles in the following CTC identification. It has been reported that around 0.1% of white blood cells (WBCs) are retained on the membranes with 8 μm pores (Ma et al., 2013). Unfortunately, the seemingly low retaining efficiency results in a very poor purity with tens of thousands of WBCs in the pre-identified CTC samples as there are millions of WBCs in the whole blood. On the other hand, cellular size of CTCs varies over a wide range from 4 μm to 30 μm (Allard et al., 2004), even among CTCs from the same patient, which will lead to the loss of smaller CTCs passing through filters with a given pore size.

As one of the most promising techniques to realize clinical application and commercialization, size-based platforms, including membrane-based (Coumans et al., 2013a, 2013b; Lee et al., 2013; Li et al., 2015; Zheng et al., 2007) and microfluidic filters systems (Riahi et al., 2014), have drawn various innovative ideas to overcome these challenges in recent years. The depletion of WBCs from whole blood prior to size filtration attracts much attention to improve the recovery purity of CTCs, and many effective approaches have been proposed, such as immunomagnetic negative separation (Bhuvanendran Nair Gourikutty et al., 2016; Sajay et al., 2014), inertial-based microfluidic separation (Wang et al., 2015) and reform of filter pores in a conical shape (Hosokawa et al., 2010; Kim et al., 2016; Tang et al., 2014). Recently, affinity capture was combined into size-based filtration, including antibody functionalized microfilters (Meunier et al., 2016) and immunomagnetic isolation (Chang et al., 2015; Chung et al., 2011; Kim

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https://doi.org/10.1016/j.bios.2017.11.026
Received 22 August 2017; Received in revised form 18 October 2017; Accepted 6 November 2017
Available online 07 November 2017
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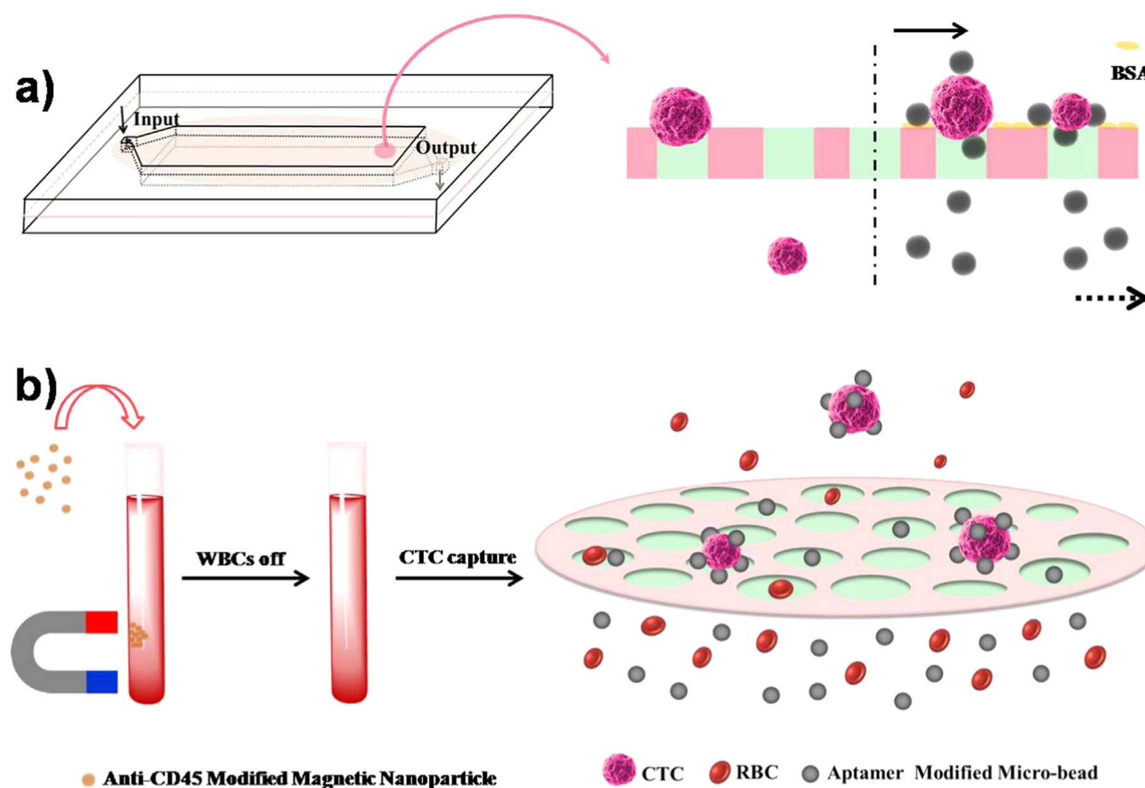

Fig. 1. Diagram of CTC capture based on Micro-beads Enhanced Isolation by Size of Epithelial Tumor Cells (ISET) Method. a) Employing modified microbeads to enlarge the size of CTCs specifically increases the capture efficiency of smaller CTCs. b) A WBC depletion is introduced which greatly decreases the WBC retaining on the filter membrane.

et al., 2012), to decrease the loss of smaller CTCs. Moreover, most attentions have been focused on microfabricated microfluidic devices for the superiority in standardized sample processing. Meanwhile it suffers from limited throughput, frequent sample clogging and complicated fabrication processes leading to a high cost.

In this paper, we fabricated a low-cost microfluidic chip with high flux capacity using conventional polycarbonate membrane, on which bovine serum albumin (BSA) was modified to decrease nonspecific adhesion of blood cells, and microbeads grafted with aptamers against epithelial cell adhesion molecule (EpCAM) (Song et al., 2013) were employed to target and bind onto CTCs specifically, enlarging the size of target tumor cells, to increase the capture efficiency of smaller CTCs in patient blood, as shown in Fig. 1a. Moreover, to improve the purity of target CTCs, WBCs were depleted from blood by an immunomagnetic separation process before the size filtration, as shown in Fig. 1b. The tests on this chip can be performed reproducibly under fully standardized conditions, and the isolation of CTCs can be accomplished within 70–90 min, including the treatment of WBCs depletion from whole blood.

2. Experimental details

2.1. Materials

Membrane filters (Isopore™, 8 µm TETP) were purchased from Merck Millipore Co. Ltd. Amine-functionalized microbeads (5 µm) were purchased from VDOBIOTECH Co. Ltd. (Suzhou, China). Streptavidin modified magnetic nanoparticles (500 nm) were purchased from the EmerTher Company. Biotinylated antibodies against CD45, anti-pan Cytokeratin monoclonal antibodies conjugated with FITC (anti-pan-CK-FITC) and anti-CD45 monoclonal antibodies conjugated with phycoerythrin (anti-CD45-PE) were purchased from Univ-bio Co. Ltd. (Shanghai, China).

All oligonucleotides were purchased from Sangon Biotech Co. Ltd. (Shanghai, China) and HPLC-purified by manufacturer. DNA aptamers used here for microbeads modification: 5′-biotin-C6-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG-3′.

2.2. Fabrication of microfluidic chips

The chip consists of two layers of PDMS devices, and the chamber of PDMS device (4 mm*17 mm) was obtained by a mold of 3 M tape with a thickness of 300 µm. After preparation of the upper and bottom PDMS layers, holes of inlet and outlet were punched, respectively. Both of PDMS layers and the filter membrane were treated with plasma and then integrated into a whole.

2.3. Modification of bio-interfaces for CTC isolation

Modification of microfluidic chips: The chips were filled with 1 mg/mL BSA solution for 2 h just after their integration, and then washed by PBS.

Modification of microbeads: The amine-functionalized microbeads were treated in 2.5% glutaraldehyde solution at room temperature for 1 h and then washed with PBS three times. Next, the microbeads were incubated in 10 µg/mL of streptavidin (SA) solution for 1 h, leading to the immobilization of SA onto aldehydized microbeads. After washing with PBS to remove excess SA, the microbeads were treated with 1 µM bio-aptamer solution and incubated for 2 h to obtain aptamer modified microbeads.

2.4. CTC isolation

Prior to filtration tests, the target cells (10^4 cells/mL) were incubated with prepared microbeads (0.1 mg/mL) for 30 min at 37 °C, 5% CO_2. The cells were prestained by DIO (or DII) fluorescent dye. The microbeads bound cells were then injected to the filter at a flow rate of 1 mL/min via a peristaltic pump. Cell spiked samples were prepared by

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