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Microfluidic capture of endothelial progenitor cells in human blood samples

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ABSTRACT

We present a method to efficiently trap circulating endothelial progenitor cells (EPCs) in human blood samples by using a microfluidic device with a short cell traveling length. This device consists of a wide channel separated into three parallel parts by two linear arrays of micro-columns. Flow-derivation microstructures are patterned on both up- and down-side walls of the center part of the channel, forcing the cells to encounter the narrowly spaced micro-columns and then be trapped with the help of specified immunological interaction. Both theoretical and experimental data confirmed the reliability of this concept. By using human umbilical cord blood-derived EPCs spiked into a buffer solution or a blood sample, we demonstrate a capture rate of more than 50% for a traveling length of only 1 cm, which is clearly advantageous in terms of cell trapping efficiency and image processing speed.

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1. Introduction

Circulating endothelial progenitor cells (EPCs) have been proposed as an important indicator for cardiovascular diseases risk assessment, disease progression, and response to therapy. They can differentiate into endothelial cells then participate to the repair of blood vessels and to angiogenesis. The detection of circulating EPCs is a challenge since they are rare, i.e., in 1 mL of blood just a few tens of EPCs among 10 million leukocytes and 5 billion erythrocytes. Similarly, capture and analyses of circulating tumor cells (CTCs) in blood samples of cancer patients are also challenging so that the developed methods should be applicable to both cases. Previously, different methods have been developed for trapping rare cells, including magnetic activated cell sorting [1], identification by size [2], etc. Microfluidic devices are now studied as alternative solutions. By integrating micro-post array into microfluidic channels, CTCs could be trapped and enumerated [3]. By adding chaotic mixing structures on the upside wall of the channel, the trajectory of the cells could be changed to increase the probability of encountering the downside wall coated with anti-epithelial cell adhesion molecule (EpCAM) with [4] or without [5] additional dense nano-features. Previous study also reported trapping of human-circulating EPCs for diagnosis of cardiovascular disease [6]. More recently, alginate hydrogels functionalized with four-arm PEG molecules and antibodies were used for capture and release of EPCs from whole blood in microfluidic devices [7]. These examples showed the feasibility of using microfluidic devices in rare cell trapping but the demonstrated trapping efficiency were still low, which obliged a long traveling distance of cell samples.

In the present work, we propose a novel device configuration which allowed us to dramatically improve the efficiency of rare cell trapping. This device consists of a wide microfluidic channel, separated into three parallel parts along the channel direction by two linear arrays of narrowly spaced micro-columns. In the central part of the channel, patterned microstructures were added on the upside and downside walls of the channel for flow-derivation so that the cells injected into the central part could be dispersed with a great probability to encounter the integrated micro-columns. Consequently, cells might be captured on the surface of the columns by immunological interaction. Numerical simulation has been done to prove this concept. By applying a two-level photolithography and soft lithography techniques, the device could be fabricated. The experimental proof-of-concept has been done using umbilical cord blood-derived EPCs spiked into a buffer solution or a blood sample. The results of both trials showed a EPCs capture rate of more than 50% with a micro-channel of only 1 cm traveling length. Comparing to the previously reported data [4], our device is clearly advantageous in cell trapping efficiency and image processing speed, thereby making a further step toward real applications.

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2. Experimental method

2.1. Materials and chemicals

3-Aminopropyltriethoxysilane (APTES), fibronectin, ethanol, bovine serum albumin (BSA), and phosphate buffered saline (PBS) tablets, trimethylchlorosilane (TMCS) purchased from Sigma (France) were used as received. Antibody against human CD34 was obtained from Santa Cruz Biotechnology (USA). The solution kit of RTV615 purchased from GE (France) was used for polydimethylsiloxane (PDMS) device preparation. The photoresist SU8-2015 and SU8-3035 were obtained from MicroChem (USA). All the solutions were prepared with 18.2 MOhm cm water (DI water) from a Simplicity 185 pure water system (Millipore, USA).

2.2. Devices design and fabrication

Fig. 1 shows a schematic diagram of our device configuration: two arrays of micro-columns are used to divide the main channel into three parallel parts with the same width (a). A blood sample is injected into the center whereas a buffer solution is added in the left and right parts of the main channel through the same inlet (b). On the surfaces of both upside and downside walls of the center part, left- and right-hand diagonal surface structures are patterned to alter the trajectory of the sample flow.

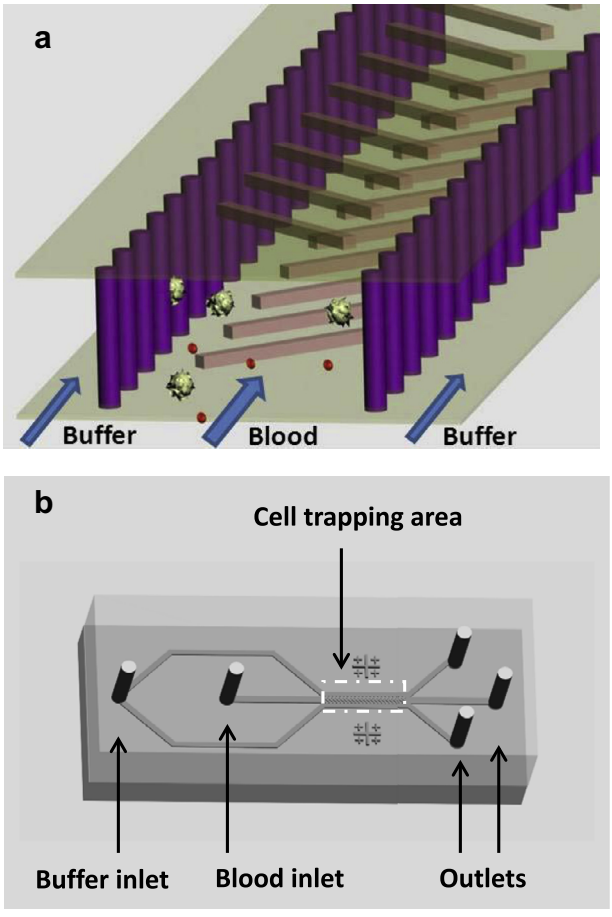

Fig. 1. Schematic diagram of a microfluidic channel for high efficient rare cell trapping. (a) Two micro-column arrays are integrated to divide the main channel into three parallel parts. The blood sample to be analyzed is injected into the center part whereas a buffer solution is added in the adjacent parts. In the center part, diagonal surface structures are patterned on both up- and down-side walls of the channel to derive the flow so that cells will have a large probability to be trapped by the antibody coated micro-columns. (b) Schematic of the device.

Our device was fabricated using standard lithography and thermal bonding processes. The microchannel and the upside-wall flow derivation structures (*cover*) were obtained by soft lithography. The micro-columns and the downside-wall flow derivation structures were fabricated by photolithography on a glass slide. To evaluate the efficiency of the device, the size of the cell trapping area was limited to 1 cm (length) × 1 mm (width) × 100 μm (height). The diameter of the micro-columns is 40 μm, equally spaced by 15 μm which is comparable to the size of EPCs. Both up- and down-side flow derivation structures are periodic stripes of 35 μm width and 110 μm spacing, aligned in diagonal in the center part of the main channel.

The mold for soft lithography was firstly fabricated on a silicon wafer by a two-step photo-lithography. After spin-coating a 70 μm thick photoresist layer (SU8 3050) on a silicon wafer, UV exposure was performed using an optical aligner (MJB4, Suss MicroTec, Swissland) with a Cr photomask which defines the pattern of the first layer of the cover structure (the whole microchannel structure). After development and post-exposure bake, a 27 μm thick photo resist (SU8 2015) was spin-coated on and patterned with another Cr mask to define the same exposed area, except the upside-wall flow derivation structures in the central part of the channel. After development and hard bake, TMCS was evaporated on the mold as release agent. The pre-polymer solution of PDMS was prepared at a base-polymer to cross-linker ratio of 10:1 with a mixer (UM-113, UNIX, Japan). Then, it was poured on the two level SU8 mold. After curing in an incubator of 80 °C for 30 min, the PDMS cover layer was peeled off and inlet and outlet holes were punched, resulting in designed microchannel with the upside-wall flow derivation structures.

Similarly, the micro-columns for trapping and the downside-wall flow derivation structures could be obtained using the two-level photolithography technique on a glass slide. A 27 μm thick photoresist layer (SU8 2015) was spin coated on a clean glass slide and exposed with a photomask which defines the downside-wall flow derivation structures. Afterward, a 70 μm thick photoresist (SU8 3035) was spin coated on and exposed with another photomask which defines the micro-columns. After development, the whole substrate was baked on a 150 °C hot plate for 5 min to improve the mechanical stability of the fabricated patterns. Finally, both PDMS cover layer and the patterned glass slide were exposed to oxygen plasma and then irreversibly bonded in an oven of 80 °C for 1 h.

2.3. Surface functionalization

The microfluidic devices were surface modified with an amino group by injecting 2% APTES in 98% ethanol into the microchannel for 1 h at room temperature [8]. The microchannel was then rinsed with DI water to remove unreacted silane. The amino group (NH_2-) on the microchannel surface could then be used for further bio-functionalization with 100 μg/mL antibody CD34 for 30 min. Afterward, the microchannel was washed again with DI water.

2.4. EPC sample preparation

Human umbilical cord blood samples (30–50 ml each) were collected in a sterile tube containing heparin sodium solution as anticoagulant EDTA-coated Vacuette® tubes (Greiner Bio-One, France) from donors in compliance with French legislation. Mononuclear cells were isolated from cord blood by density gradient centrifugation with Pancoll (1.077 g/mL) (Dominique Dutscher, Brumath, France). The mononuclear cells were depleted of adherent cells by culturing on plastic dishes at 37 °C for 24 h and were directly plated into wells of six-well plates coated with type I collagen (Sigma–Aldrich, Saint Quentin Fallavier, France). Mononuclear cells

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