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Short communication

Separation of model mixtures of epsilon-globin positive fetal nucleated red blood cells and anucleate erythrocytes using a microfluidic device

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ABSTRACT

Microfluidic devices are capable of separating microparticles and cells. We developed and tested the efficiency of silicon cross-flow microfilters for the separation of primitive fetal nucleated red blood cells (FNRBCs) and adult anucleate red blood cell (AARBCs) from model mixtures. Stepwise improvements over three generations of device design resulted in an increasing trend in the recovery of FNRBCs. We obtained a recovery of FNRBCs (74.0 ± 6.3%, $p < 0.05$, $n = 5$) using the third generation device, with a depletion of 46.5 ± 3.2% AARBCs from the cell mixture. The purity of FNRBCs in the enriched fraction was enhanced by a factor of 1.7-fold.

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1. Introduction

Microfluidic devices show considerable promise for the separation of cells, with potential applications in biology and medicine [1,2]. Separation of the blood components have focused primarily on obtaining plasma [3] or white blood cells [4,5]. Cell size, shape and deformability were considered in the design of microfluidic devices for blood cell separation: pillars [4], cross-flow filtration [5], hydrodynamic filtration [6], pinched flow fractionation [7] and lateral displacement [8] have all been examined.

Isolation of rare cells from blood such as circulating tumour cells (CTCs) [9] and fetal cells in maternal blood [10] has potential importance in disease diagnosis and monitoring. To date, these applications have been limited by being time and resource intensive, with limited efficacy. Recently, microfluidic devices have been explored for the isolation of cancer cells from model mixtures and patient blood [11].

Non-invasive prenatal diagnosis using fetal cells enriched from maternal blood would eliminate the risk of miscarriage associated with invasive testing such as amniocentesis and chorion villus sampling [10]. There is some evidence of the value of microdevices in sorting fetal cells. Mohamed et al. [12] sorted cord blood fetal nucleated red blood cells (FNRBCs) from WBCs in a microfluidic device with varying size channels. Huang et al. [13] demonstrated NRBC enrichment from maternal blood using a microfluidic device. Epsilon-globin positive(e+) FNRBCs are the ideal fetal cell target for non-invasive prenatal diagnosis. Separation of e+FNRBCs from abundant adult anucleate red blood cells (AARBCs) in maternal blood would enhance yield, as most e+FNRBCs are lost into the RBC pellet during density gradient centrifugation, the first step in enrichment protocols [10,14,15].

To date, there is no method that can satisfactorily separate e+FNRBCs from AARBCs. We hypothesized that a microfluidic device could be developed that would separate the two target cell types, e+FNRBCs and AARBCs based upon their physical properties such as size and deformability. We have designed, fabricated and tested the efficiency of a silicon-based cross-flow microfilter device for the separation of e+FNRBCs (∼Ø15 μm) from model mixtures containing AARBCs (∼Ø6.5 μm), and assessed recovery and purity of sorted samples. Flow rates were optimized to handle larger sample volumes in a shorter time period, as rare e+FNRBCs in maternal blood may require larger starting volumes for clinical diagnosis. Stepwise improvements over three generations of device design resulted in an increasing e+FNRBCs recovery. The microfilter device presented here recovered 74.0% e+FNRBCs and depleted 46.5% AARBCs from model mixtures.

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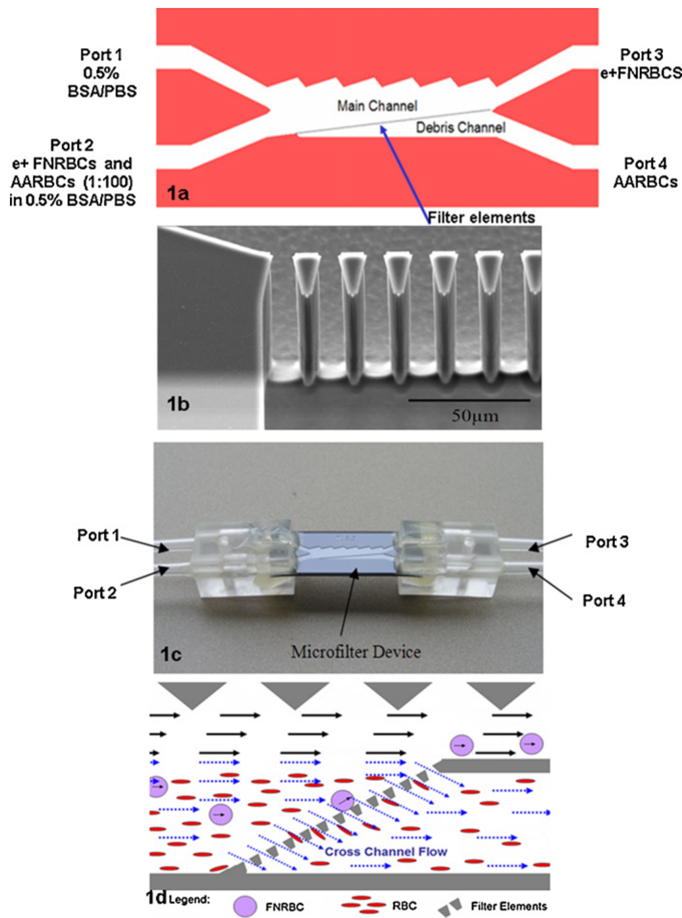

Fig. 1. Microdevice working principle: (a) schematic of microdevice: Ports 1 and 2 are inlets for PBS and model mixture sample(s), respectively. Ports 3 and 4 are collection ports for e+FNRBCs and AARBCs, respectively; a microfilter array in the main channel creates a debris channel as shown; (b) ESEM image of microfilter showing the filter elements; (c) photomicrograph of the microfilter device; (d) dashed arrows indicate flow of 1× PBS containing cells mixture from Port 2. Solid arrows indicate flow of 1× PBS/0.5% BSA buffer from Port 1, flowing alongside the cell mixture from Port 2. The flow from Port 1 pushes the cell mixture to encounter the filter array, whereby the smaller AARBCs can pass through the filter gap and get collected at Port 4; whereas larger FNRBCs cannot pass through the filter gaps and are diverted to Port 3.

While the current enrichment is encouraging, further improvement will be required for the device to be useful for fetal non-invasive prenatal diagnosis.

2. Experimental

2.1. Device structure and principle

The microfluidic filter device used to separate e+FNRBCs from model mixtures containing AARBCs was based on size and known deformability of AARBCs. A main conduit channel with an inclined silicon filter array in its path creates a second debris channel. The device has two inlets (Ports 1, 2; P1, P2) and two outlets (Ports 3, 4; P3, P4). P1 and P2 are for flow of buffer and sample mixture, respectively. P3 and P4 are for e+FNRBC collection and AARBC collection, respectively (Fig. 1a and c).

1× Phosphate-buffered saline (PBS) buffer/0.5% BSA was injected through P1 and flowed alongside the sample mixture injected through P2. The flow from P1 pushed the flow of sample mixture to encounter the filter array. Filter gaps (Fig. 1b) were designed to allow deformable AARBCs to pass through under a hydrodynamic pressure between the main channel and debris channel across the filter and collect at P4.

Larger, less deformable e+FNRBCs get diverted to the cell collection P3 (Fig. 1d). Device characteristics of the three devices designed are described in Table 1. Buffer and sample flow rates were manipulated to obtain maximum e+FNRBC recovery.

2.2. Device fabrication

Silicon microfilter devices were fabricated at the Institute of Bioengineering and Nanotechnology, Singapore, using standard micromachining techniques. Device layout was prepared and a photo-mask created. Silicon dioxide (SiO_2) was deposited on deionised water (DI) cleansed silicon wafers using plasma enhanced chemical vapor deposition (PECVD, 5 min) and coated with hexa-methyl-di-silizane (HMDS) before photo-resist (PR) was spun-coated (2000 rpm, 30 s) and soft-baked (100 °C, 3 min). Filter design was realized on PR-coated silicon wafers by UV exposure (EVG 620). Wafers were soft-baked (110 °C, 3 min) developed in AZ300MIF-developer solution, DI-water rinsed, spun-dried and hard-baked.

Reactive ion etching (RIE) enabled removal of SiO_2 from regions on silicon wafers not protected by PR. Residual PR on silicon wafers were stripped off by rinsing with acetone and N-methyl-pyrrolidone (NMP)-soak/sonication (70 °C, 2 h). Deep reactive ion etching (DRIE, Alcatel AMS100 I-Speeder) on silicon wafer created microchannels and silicon pillars of microfilter devices.

SiO_2 mask on silicon wafers were stripped off using buffered oxide etchant (27 °C, 20 min), DI-water rinsed and spun-dried. Following this a 500 ± 25 μm thick Pyrex 7740 glass wafer (4″) was anodically bonded onto the silicon wafer (305 °C, 1000 V, 40 min, EVG 520 Anodic Bonder).

Silicon microfilter devices were singled-out from glass-bonded silicon wafer by dicing process (DISCO-DAD3350). Tubing adaptors were fabricated using a three-dimensional fast prototyping machine (Objet Eden-2600) and mounted onto both ends of the microfilter device using epoxy resin.

2.3. Preparing e+FNRBCs and AARBCs

Placental tissues were obtained from women undergoing elective first trimester surgical termination of pregnancy (fetal gestational age: 7–10 weeks) at the Department of Obstetrics and Gynecology, National University Hospital, Singapore. Institutional Review Board approved written informed consent was obtained in each case. e+FNRBCs were isolated from trophoblast tissue using our own protocol [16]; AARBCs were prepared from blood obtained from healthy volunteers.

2.4. Sorting e+FNRBCs and AARBCs from model mixtures

Model mixtures comprised 1:100 ratio of e+FNRBCs:AARBCs (Device 1: 10^5:10^7; Devices 2, 3: 10^4:10^6) suspended in 3.0 mL 0.5% BSA/PBS. At start, devices were primed with 0.5% BSA/PBS through both inlets using syringe pumps until the entire volume within the device was filled; care was taken to ensure that no air bubble remained within the device. 3.0 mL sample mixtures

Table 1
Separation element features for devices.

Parameters	Device number		
	Device 1	Device 2	Device 3
Microfilter elements gap size (μm)	7	5	4
Angle of microfilter elements array (°)	30	5	5
Outlet Port 3 channel width (μm)	200	1200	1200

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