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Portable vibration-assisted filtration device for on-site isolation of blood cells or pathogenic bacteria from whole human blood

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ABSTRACT

Isolation of specific cells from whole blood is important to monitor disease prognosis and diagnosis. In this study, a vibration-assisted filtration (VF) device has been developed for isolation and recovery of specific cells such as leukocytes and pathogenic bacteria from human whole blood. The VF device is composed of three layers which was fabricated using injection molding with cyclic olefin copolymer (COC) pellets consisting of: a top layer with coin-type vibration motor (Φ = 10 mm), a middle plate with a 1 μm or 3 μm-pore filter membrane to separate of *Staphylococcus aureus* (*S. aureus*) cells or leukocytes (i.e. white blood cells) respectively, and a bottom chamber with conical-shaped microstructure. One milliliter of human whole blood was injected into a sample loading chamber using a 3 μm-pore filter equipped in the VF device and the coin-type vibration motor applied external vibration force by generating a rotational fluid which enhances the filtration velocity due to the prevention of the cell clogging on the filter membrane. The effluent blood such as erythrocytes, platelet, and plasma was collected at the bottom chamber while the leukocytes were sieved by the filter membrane. The vibration-assisted leukocyte separation was able to finish within 200 s while leukocyte separation took 1200 s without vibration. Moreover, we successfully separated *S. aureus* from human whole blood using a 1 μm-pore filter equipped VF device and it was further confirmed by genetic analysis. The proposed VF device provides an advanced cell separation platform in terms of simplicity, fast separation, and portability in the fields of point-of-care diagnostics.

1. Introduction

Separation, sorting, and manipulation of target biomarkers, especially blood cells and pathogenic bacteria, have brought great interests to scientists and engineers due to their valuable clinical information in blood-based disease diagnosis [1–3]. Recovering specific cells from human whole blood including leukocytes (i.e. white blood cells), erythrocytes (i.e. red blood cells), circulating tumor cells, and pathogenic bacteria become important to provide valuable information on diagnosis of viral and bacterial infection such as Chikungunya fever, HIV, and febrile disease in young children living in developing countries [4–6]. Therefore, because of the limited access to expensive equipment and experts in those countries, simple, portable, and efficient isolation of specific cells from unprocessed whole blood is urgent and important not only in the field of biological research but also the clinical diagnosis, which is especially important to realize the point-of-care analysis.

Up-to-date, considerable effort has been reported regarding cell isolation from whole blood to increase the isolation efficiency and specificity using various methods including hydrodynamics, microfiltration, micro/nano-topology, and electrophoresis [6–10]. Among them, size-based mechanical filtration is a well-known method in industries with numerous advantages of simple operation, easy integration, and high-throughput capability compared to other separation methods which isolate cells using its physical properties such as density and deformability [11–16]. Although the process of conventional filtration is considered as a gold standard method, it is still a tedious, time-consuming process and can suffer from membrane fouling because of rapid stacking of biomaterials [17–19]. In a similar manner, common

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clogging of blood cells inside of filter pores may decrease filtering efficiency and increase significant cellular damages due to the external pressure. Moreover, the inevitable solid fouling often ruptures blood cells leading to build up pressure inside of the device.

To overcome such challenges, a combination of vibrational motion and a mechanical filter could be a solution to enhance filtering efficiency without severe cellular damages. Previously, the continuous vibrational motion has successfully demonstrated separation of fluid from a solid-liquid mixture by prevention of pore clogging via continuous particle floating and fluidizing [20–25]. In vibrational filtration, the effluent flux is assisted by induced flow field through the filter membrane. The shear stress improves the permeability via prevention of the filter fouling which resulted from concentration polarization, pore blocking, and solid cake formation. Therefore, a vibration-assisted filtering device (VF device) with its anti-fouling and concentration phenomena could be effective in selective isolation of cells within cell manipulation technology.

Herein, inspired from such findings, we proposed a novel method for efficient isolation and recovering of leukocytes or pathogenic bacteria cells that cause serious infection associated with high morbidity and mortality from unprocessed whole blood by using a coin-type vibration motor which is currently used in mobile phones. In particular, the vibration module allows us to facilitate the enhancement of shear stress limitations on the membrane and conserve effective filtration area. The combination of vibration and induced fluid rotation improves the cell recovery and filtration efficiency. To identify the recovered leukocyte, and pathogenic bacteria cells from whole blood, genetic analysis was carried out via polymerase chain reaction (PCR).

2. Experimental

2.1. Design of the VF device

The VF device is composed of three plastic layers with a coin-type vibrator and a filter membrane as shown in Fig. 1a: From the top to bottom: a top layer with a 3 mL-volume of cylindrical-shaped sample loading chamber, a 25 mm-diameter membrane holder contained middle plate with 1 μm or 3 μm-pore filter membrane in between the top layer and middle plate to separate *Staphylococcus aureus* (*S. aureus*) cells or leukocytes respectively, from whole blood, and a 4.68 mL-volume bottom chamber with a conical-shaped microstructure in the center. Schematic image of the assembled VF device (70 mm × 30 mm × 12 mm) is illustrated in Fig. 1b.

2.2. Fabrication of the VF device

Fig. 1c shows the digital image of the injection molded plastic components. Three plastic layers of the VF device were fabricated from cyclic olefin copolymer (COC) pellets using injection molding. First, master molds for the each plastic layer were manufactured by CNC milling. The prepared master molds were loaded in an injection molding machine for the production of each plastic layer. The melted COC pallets were injected into the mold cavity with a pressure ranging from 80 to 150 MPa at 260 °C and then the precast plastic layers were cooled down for solidification.

Once the layers of the device were prepared, an ultrasonic welding method was employed to bond each plastic part. For this purpose, we designed welding lines at the edge of the plastic components and around the filter chamber to enhance the interlayer bonding. In order to prevent potential leakage, top, middle, and bottom plastic parts were carefully aligned while the filter membrane was placed between the top layer and middle plates. 20 kHz of ultrasonic force was applied for 0.18 s to welding lines of the injection molded plastic component and the lines were immediately melted down and tightly bonded the plastic layers. After the ultrasonic energy discharged, the product was pressed with 100 MPa for 10 s to enhance the bonding property. The real photograph of as-prepared VF device is displayed in Fig. 1d.

2.3. Cell separation using VF device from whole blood

For isolating the leukocytes on the VF device, 1 mL of blood samples were injected into the loading chamber of the VF device (3 μm-pore filter membrane-inserted). The coin-type vibration motor (NT316040001) was connected with a DC supplier (EDP-1501, PNCYS)

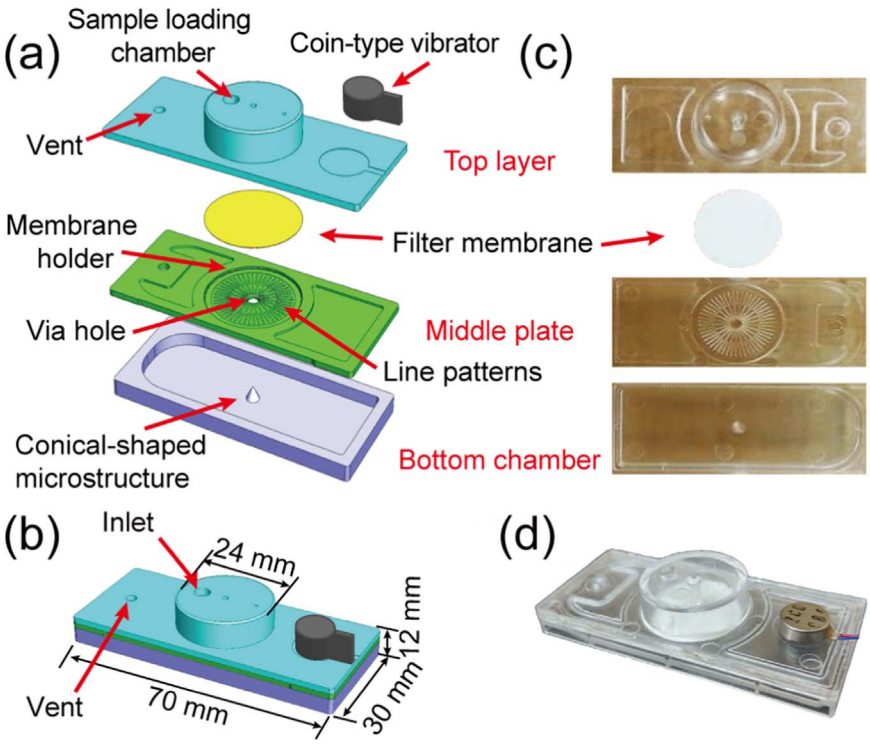

Fig. 1. (a) Exploded view of the VF device with top layer, middle plate and bottom chamber. (b) Schematic image of the assembled VF chip (c) Digital images of the injection molded top layer, middle plate, and bottom chamber with filter membrane. (d) Digital image of the VF device.