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Short Communication

Sequestration of bacteria from whole blood by optimized microfluidic cross-flow filtration for Rapid Antimicrobial Susceptibility Testing

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ABSTRACT

A microfluidic device to separate bacteria from blood cells based on size-exclusion through cross-flow channels was designed and performance tested using fluorescently-labeled, heat-killed *Escherichia coli* spiked into whole blood. The device is easy and cheap to fabricate, and simply and robustly purifies bacteria from large blood cells. Thus the device would be an effective sample-preparation stage within a point-of-care system for rapid testing for antibacterial resistance.

1. Introduction

Sepsis causes 751,000 hospitalizations and 215,000 deaths in the United States annually, costing $17 billion [1]. Incidence is increasing 13.7% per year, with the number of deaths tripling between 1979 and 2000 [2]. The high mortality and cost of sepsis are partially caused by blood culture testing taking too long (24–72 h) [3], and potentially producing false negatives [4]. The delay leads to the use of a broad cocktail of antibiotics that encourage drug resistance and may still prove ineffective [3]. Microfluidic approaches to sequester pathogens from whole blood could facilitate Rapid Antimicrobial Susceptibility Testing (RAST) and allow specific therapy sooner, with a higher chance of success [5,6]. RAST in combination with antimicrobial stewardship can reduce hospital costs by $20,000 per patient [7].

Current microfluidic approaches to filter blood components exploit chemical [8–10], physical [11–18] and hydrodynamic [19–22] separation mechanisms. Methods based on molecular binding to bacteria miss untargeted pathogens. The simplest and cheapest physical method is size exclusion; it does not require blood cell lysis, which could interfere with subsequent assays. Hydrodynamic methods, except for margination, require sample dilution. In contrast to other blood separation approaches using cross-flow filtration alone [15,21] or with size-exclusion [18], we aimed to separate bacteria from whole blood without dilution and with minimal clogging, a frequent problem for size-based separation techniques.

In order to sequester bacteria, which cause >95% of sepsis cases [2], from whole blood, microfluidic approaches must be tailored for clinical use. Since the bacteria are in low abundance, the device must process ~1 mL without clogging, and must remove blood cells that could interfere with detection and testing. A portable, point-of-care device should provide a filtered output in minutes without complicated sample preparations or dilutions.

Based on the aforementioned clinical criteria, we rationalized that physical and hydrodynamic separation mechanisms in combination would provide robust sequestration of bacteria from large blood cells. Therefore, the current design is a microfluidic filter ("separator") based on size-exclusion and margination, with continuous, undiluted sample flow. We designed cross-flow filter resistances to minimize clogging by extracting more plasma in the region of the device containing lower hematocrit. Separator performance was evaluated by directly loading with undiluted whole blood spiked with fluorescence-labeled *Escherichia coli*, followed by cell counting. The optimized device removes 97% of RBCs and

Abbreviations: RAST, Rapid Antimicrobial Susceptibility Testing; RBC, Sred blood cells.

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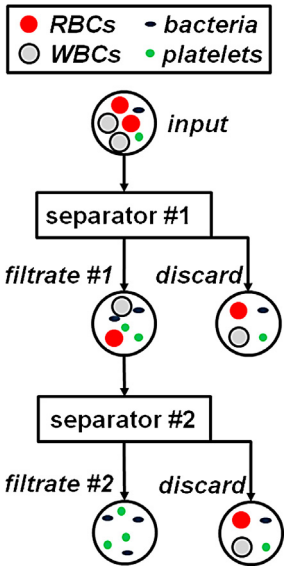

Fig. 1. Block diagram of a microfluidic method to separate bacteria from whole blood. Undiluted, whole blood containing plasma, red blood cells, white blood cells, platelets and bacteria is input to the device. The device separates blood components based upon size. The filtrate receives the smaller fraction, including bacteria. The discard receives most large blood cells. The process can be repeated to further remove blood cells from the filtrate.

retains 30% of bacteria, and so is suitable for sample preparation for point-of-care RAST applications.

2. Materials and methods

2.1. Modeling

The separator was designed to remove half of plasma (~30% of blood volume) from the input, minimize clogging, and sequester bacteria from large blood cells. Fluidic flow was modeled with discrete elements like an electronic circuit. Fluidic pressure was analogous to voltage and fluidic resistance to electrical resistance. Flow was determined by applying Kirchoff's laws, assuming rectangular channels throughout the device, constant viscosity and laminar flow. For a rectangular channel with viscosity μ, length L, width w and height h (where $w > h$), the fluidic resistance is estimated:

$$R_h \approx \frac{(12\mu L)}{wh^3(1 - 0.630h/w)} \quad (1)$$

The separator was modeled as a circuit of independent resistors using Matlab (MathWorks, Inc., Natick, MA). Flow through the last in-series cross-flow channel was half that of the first filter. The flow through each cross-flow channel along the length of the device was set to vary linearly and inversely with the hematocrit in the main channel, which increased steadily from input to output. We reasoned that this flow profile would reduce the chance of clogging. The lengths of the filter channels and the width of the intermediate and collection channel were set to match the resistances from the model using (1). Two columns of cross-flow filters were designed, on either side of the main channel. This maximized filtration speed and reduced clogging.

2.2. Device design and fabrication

A flowchart (Fig. 1) shows the filtration steps. The first and second stage separators were identical (Fig. 2). The separator consists of an inlet for whole blood, leading to two or four main channels in parallel. Cross-flow channels lead on either side to

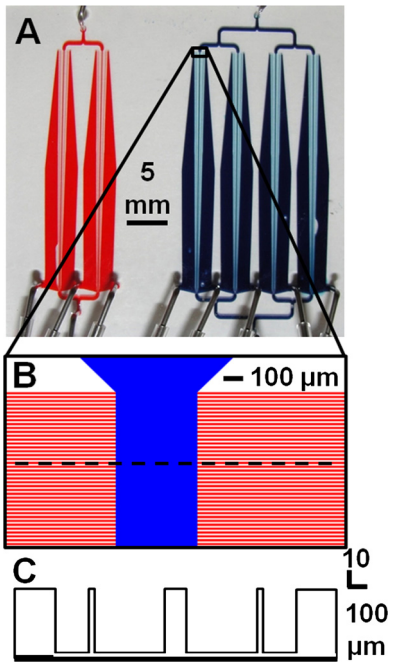

Fig. 2. Separator device design. (A) An *en face* microscope image of two separator devices cast from the same mold, with two (left, red), and four (right, blue) parallel channels to filter blood. (B) An *en face*, and (C) transverse schematic of the filter channel microstructure, depicting main and collection channels (B, blue), and cross-flow filter channel (B, red) dimensions. Scale is indicated. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

two continuous flow collection channels. Filter channel height (1.55 μm) was less than that of RBCs (~2 μm). In the separator, the main channel was 100 μm × 40 μm × 28 mm ($w \times h \times L$); first in-series filter channels were 10 μm × 1.55 μm × 384–45 μm ($w \times h \times L_{FIRST} - L_{LAST}$); first in-series collection channel was 30 μm × 40 μm × 28 mm ($w \times h \times L$); second in-series filter channels were 10 μm × 1.55 μm × 168–179 μm ($w \times h \times L$); and second in-series collection channel was 167–1520 μm × 40 μm × 28 mm ($w_{TOP} - w_{BOTTOM} \times h \times L$). Both feed and collection vials were connected to the device by silastic tubing (0.02 mm ID, VWR International, Radnor, PA) with steel pins.

Negative masks of separator channel microstructures were drawn using AutoCAD (Autodesk, Inc., San Rafael, CA) and printed on transparency masks (CAD ART Inc., Poway, CA). Inverse molds of the required microstructures were fabricated using standard photolithographic steps. Access holes to the inlets and outlets were punched by a 22-gauge punching tool (Technical Innovations Inc., Angleton, TX). The PDMS filter channels were irreversibly bonded to glass slides following 35 s activation with oxygen plasma.

2.3. Sample preparation

Experiments were conducted with human whole blood collected in disodium EDTA-coated vacutainers (Bioreclamation Inc., Charleston, SC, USA). To test the performance of the separator, whole blood was mixed gently with heat-killed, Alexa Fluor 488-conjugated *E. coli* (Life Technologies, Carlsbad, CA) spiked-in at 22 cells/nL. To test the performance of two separators in series, three trials of 1.5, 1.5, and 7.5 mL of whole blood with BioParticles *E. coli* spiked-in was input to the first separators, which had four main channels. The second separators, which had two main channels, received as input the filtrate from the first separators, minus 10 μL, retained for cell counting. The concentrations of BioParticles in stock and RBCs in whole blood were determined by serial dilution and hemacytometer counting.