MICROFILTRATION DEVICE FOR CONTINUOUS, LABEL-FREE BACTERIA SEPARATION FROM WHOLE BLOOD FOR SEPSIS TREATMENT

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ABSTRACT
This work presents the design, fabrication, and testing of a cross-flow filtration microdevice, for the continuous extraction of bacteria from a whole blood sample for the treatment of sepsis and bacteremia. The system consists of a two-compartment microdevice with two aligned sets of PDMS microchannels, separated by a porous polycarbonate (PCTE) membrane with 2 µm pores. When a whole blood sample flows through the channels on one side of the membrane (Reservoir channels), the larger blood cells are sterically excluded from passing through the membrane while the smaller bacteria are removed by traversing the membrane into the filtration channels.

KEYWORDS: Cross Flow Filtration, Microfiltration, Extracorporeal life support, Sepsis

INTRODUCTION
Sepsis is caused by a severe infection originating from a variety of sources including: pneumonia, urinary track infection, wound infection, infection following burn, or as a complication of chemotherapy or immune compromised state such as those infected with HIV/AIDS. The infection can localize within the kidneys, the lungs, the gall bladder, or the liver. Once pathogenic organisms enter the bloodstream a patient may suffer from septicemia or bacteremia; the presence of bacteria in the blood. A patient with severe sepsis presents with symptoms of systemic inflammatory response syndrome (SIRS) characterized by acute inflammation throughout the body, fever and elevated white blood cell (WBC) count (leukocytosis). In patients with sepsis, regardless of the initial infecting pathogen, a cascade of inflammation with activation of the coagulation system leads to alterations in microvascular circulation, multiorgan dysfunction and death [1]. Sepsis is one of the primary causes of morbidity and mortality in hospitals, with ~750,000 cases per year in the United States [2]. Despite therapeutic advances, powerful antibiotics, blood transfusion and improved intensive care support, the mortality rate still ranges from 30 to 50% and up to 90% for severe cases with multiple organ dysfunction [3]. A key difficulty for antibiotic administration for rapid intervention in septic patients is identifying the infectious pathogen which delays the treatment. Rapid elimination of microbial pathogens, even before they are identified, can be critical to patient survival. Therefore, there is a need for a high throughput blood cleansing device to remove pathogens from whole blood.

Recently, microfluidic and miniaturized lab-on-a-chip separation devices have been introduced to separate pathogenic bacteria from blood. Active actuation has been used to remove bacteria from contaminated blood by generating physical fields based on magnetic activated cell sorting, where specific bacterial pathogens were labeled with magnetic bead coated with pathogen specific antibodies and 80% of pathogens were removed from a contaminated blood sample at a rate of 20 ml/h [4]. This device requires identification of the pathogenic invader and a bead/sample incubation time between 30-60 min to obtain binding of 85 to 90% of the bacteria. Label-free hydrodynamic separations are another attractive separation method because they are cost effective and do not require external fields and labels. The ability to achieve high-throughput separation at high flowrates is important in some clinical applications where large volume of blood needs to be processed in a reasonable amount of time. In one method, a soft inertial force on varying sized particles was used to separate 99.87% bacteria from diluted blood (20x dilutions of RBCs and bacterial cell sample) at a maximum flowrate of 18 µl/min [5]. In another report E-coli bacteria was separated from diluted blood (~0.5% (v/v)) at a high flowrate of 200 µl/min within a single channel [6]. The goal of this study is to design a microfluidic device for cell separation based on size differences between bacteria and other blood components for the continuous separation of bacteria and microorganism from undiluted whole blood for effective intervention in clinical settings for sepsis treatment.

THEORY
The microfiltration device was fabricated as a porous membrane sandwiched between and separating two compartments of microchannels. A novel fabrication procedure was developed to irreversibly bond the separation membrane as a laminated structure with aligned microfluidic channels by surface modification of the polymer membrane via 3-aminopropyltriethoxysilane (APTES), followed by plasma activation of the PDMS microchannels (Figure 1-left) [7]. Devices were designed with multiple parallel microchannels to maximize filtration area surface with high levels of cell presentation to the membrane pores. Since the filtration relies on passive fluid and particle permeation across the membrane the device can
operate over a wide range of perfusion flow rates and pressures with no evidence of membrane leakage, cell lysis or clogging over an extended period of operation (over 4 hours). This operational principle overcomes many of the limitations of blood fractionation microdevices which require precise actuating field and/or flow rate control, blood dilution, or have cell throughput limitations. A previously designed microfiltration device using a 200 nm pore size polycarbonate (PCTE) membrane was successfully tested multiple times in clinically relevant settings where it was connected to a simulated cardiopulmonary bypass (CPB) or porcine model of extracorporeal life support (ECLS) circuit to continuously extract pure plasma from whole blood with high sampling frequencies [8].

EXPERIMENTAL
This technology has been extended to continuously extract pathogens from undiluted blood (Hct > 38%) for treating sepsis. A microfiltration device with a 2 µm pore-size PCTE membrane was fabricated and was used to separate E.coli from blood (packed RBCs diluted with lactated Ringer’s solution to 38% Hct). The microfiltration device was first primed with Lactated Ringers solution (Baxter Healthcare Corp, Deerfield, IL) and was then coated with heparin as an anticoagulant for at least 30 min to prevent blood cell adhesion to the membrane surface [8]. Whole blood was doped with 10^8 CFU/ml of E.coli bacteria in 1 ml total volume of blood and was infused into reservoir inlet at a constant flowrate of 50 µl/min via a peristaltic pump and the reservoir outlet was recycled back to an external blood reservoir (Figure 2). The blood inlet and filtrate samples were collected in fractions every 20 minutes and then analyzed via a Coulter cell and particle counter (Beckman Coulter Inc., Hialeah, FL, USA) with counts verified via direct cell counts using a hemocytometer. Bacterial cells in 10 µl of undiluted sample taken from the blood reservoir and filtrate samples were collected in fractions every 20 minutes and then analyzed via a Coulter cell and particle counter (Beckman Coulter Inc., Hialeah, FL, USA) with counts verified via direct cell counts using a hemocytometer. Bacterial cells in 10 µl of undiluted sample taken from the blood reservoir and filtrate samples were incubated in a staining solution containing Hoechst 33342 fluorescent dye and lactated Ringer’s solution for 60 minutes at room temperature. Samples were then placed under the coverslip of the hemocytometer and counted via a fluorescence microscope at 10× magnification. Additionally, to determine the number of RBCs in the filtrate, samples from the blood reservoir and filtrate (at 80 minutes) were diluted (1:500) with lactated Ringer’s solution and were placed under the coverslip of the hemocytometer and counted under bright-field microscopy at 10× magnification.

RESULTS AND DISCUSSION
Figure 3(a) shows the cell sizing and concentration data obtained via Coulter counting of the blood sample doped with E.coli bacteria in the blood reservoir and filtrate samples. These data indicate that after 80 minutes of blood circulation through the microfiltration channels, the reservoir bacteria peak was significantly smaller than at the beginning of the experiment. Additionally, the filtrate bacteria peak after 80 minutes showed the accumulation of bacteria with minimal RBC contamination which indicated that the hematocrit on the reservoir side was maintained. Figure 3(b) shows the cumulative
The amount of bacteria removed in each 20 minute fraction normalized to the blood reservoir. Using this device approximately 5-6% of the *E. coli* was removed from the blood in the reservoir sample in each collected fraction resulting in a cumulative removal of 22% over a period of 80 minutes.

Figure 4 shows representative images taken during hemocytometer counting of cells observed under fluorescence microscopy in undiluted blood and RBCs observed under brightfield microscopy in diluted blood. These images indicate the filtration capability of the microdevice, showing the accumulation of bacteria in the filtrate with minimal RBC contamination in filtrate. A few bacteria cells which can be observed under brightfield illumination are indicated by white arrows.

**CONCLUSION**

These results demonstrate the ability of the microfiltration system described here to continuously remove bacteria from blood. The performance of the microdevice will be improved to increase the separation efficiency of bacteria from larger volume of blood in a reasonable period of time with the ultimate goal of creating a staged microfiltration device with increasing filter stringency which is capable of separating blood components from small pathogens as well as filtering blood plasma.

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