A NOVEL MICROFLUIDIC "CELL-BASED" BLOOD DIALYSIS PLATFORM FOR MURINE MODEL OF SEPSIS

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ABSTRACT

Sepsis represents an adverse systemic inflammatory response caused by microbial infection in blood. Current extracorporeal blood purifications based on removal of cytokines and endotoxin have had limited success in clinical trials which calls for alternative target removal. In this work, we demonstrate non-specific removal of bacteria , inflammatory, and other cellular components (platelets and leukocytes) from whole blood using cell margination and applied *in vivo* in a mouse model of sepsis. We show, for the first time, the feasibility of interfacing microfluidic device with mouse in a closed extracorporeal circuit for continuous blood filtration.

KEYWORDS: Sepsis, Blood separation, Inflammation, Margination

INTRODUCTION

Sepsis represents an adverse systemic inflammatory response caused by microbial infection in blood [1]. Current extracorporeal blood purifications based on removal of cytokines and endotoxin have had limited success in clinical trials which calls for alternative target removal [2]. Microfluidic approaches for microorganism separation from blood have been reported but laborious sample preparations (blood dilution and magnetic labeling of microorganisms) limit their applications for continuous blood dialysis and therapeutic validation in sepsis animal model [3-7]. We have previously developed a label-free separation approach for removal of malaria-infected red blood cells (RBCs) [8] and pathogens [9] from human whole blood, inspired by the *in vivo* phenomenon of leukocyte margination. In this work, we demonstrate non-specific removal of bacteria, inflammatory and other cellular components (platelets and leukocytes) *in vivo* using a mouse model of sepsis. We show, for the first time, the feasibility of interfacing microfluidic device with mouse in a closed extracorporeal circuit for continuous blood filtration. Our hypothesis is this "cell-based" dialysis approach can modulate the host inflammatory responses ('cytokine storm') more effectively than conventional cytokine removal therapy by removing activated immune cells directly. If validated, this technique can be used as a blood cleansing method for the treatment of sepsis and other inflammatory diseases (e.g., autoimmune disease, acute respiratory distress syndrome (ARDS)).

DESIGN PRINCIPLE

In blood vessels with luminal diameter less than 300 μm, deformable RBCs migrate to the axial centre of the vessel due to Poiseuille flow profile (Fahreaus effect), resulting in displacement of larger and stiffer leukocytes towards the vessel wall; a phenomenon aptly termed as margination [10]. Here, we develop a multiplexed margination device which consists of 3 channels 2 mm long, 20×40 μm (W×H) with 3 stages of bifurcation (Fig. 1A). A filter region with an array of square pillars (100 μm gap) is used to trap large thrombi or cell clusters to ensure smooth flow within the device (Fig. 1B). As blood flows through the margination channel, deformable RBCs migrate to the axial centre of the channel and mechanical collisions between the migrating RBCs and other cell types (bacteria, platelets and leukocytes) result in their margination towards the channel walls which are removed via the smaller side channels as waste (Fig. 1C). The relative skimming volume is progressively lower at each stage (15%, 10%, 5%) by varying the resistance/length of the side channels for each bifurcation. By cascading three margination channels in series, blood sample undergoes a 3-stage separation in a single pass through the device with a total skimming volume of ~30%. To account for the volume loss into the side channels, an infusion port is added to the filtered centre outlet which is controlled by another syringe pump (Fig. 1D). Therefore, the system is capable of exchanging up to ~40% of plasma (reducing plasma borne factors such as cytokines) from the incoming blood, in addition to cell separation by margination.

EXPERIMENTAL

The microfluidic device was fabricated in poly-dimethylsiloxane (PDMS) using standard soft-lithographic techniques. *In vitro* device characterization was performed using fluorescein isothiocyanate (FITC)-conjugated *E. coli* BioParticles® (~10^7/mL) spiked in whole blood and pumped through the microfluidic device using a syringe pump at 30 μL/min. To determine bacteria separation efficiency, the filtered centre outlet was collected for FACS analysis using Accuri™ C6 flow cytometer (BD Biosciences, USA) and normalized with the inlet sample. For whole blood analysis, blood was incubated at 4 °C for 30 minutes with FITC conjugated CD41a antibodies (1:50, BD Biosciences, USA) and allophycocyanin (APC) conjugated CD45 marker (1:100, BD Biosciences, USA) to identify platelets and leukocytes respectively using flow cytometry. For *ex vivo* testing, we used male C57BL/6 mice (Charles River Laboratories, Wilmington, MA) and per-

formed cecal ligation and puncture (CLP) surgery, a polymicrobial sepsis model. Experiments were done 24 hours after CLP as it resembles most closely with when a patient clinical sepsis might present for care. Bacteria clearance was determined by plating diluted blood at the inlet (baseline), filtered centre outlet and waste side outlet on LB agar, and colony-forming units (CFUs) were counted after 24 hour incubation at 37 °C and normalized to baseline. Mouse whole blood analysis was performed using flow cytometry similar to the *in vitro* experiment described above. For continuous filtration, the microfluidic device was coupled to a catheterized mouse (carotid artery and jugular vein catheters) under isoflurane anesthesia using a peristaltic pump (P720, Instech Laboratories, Inc., Plymouth Meeting, PA).

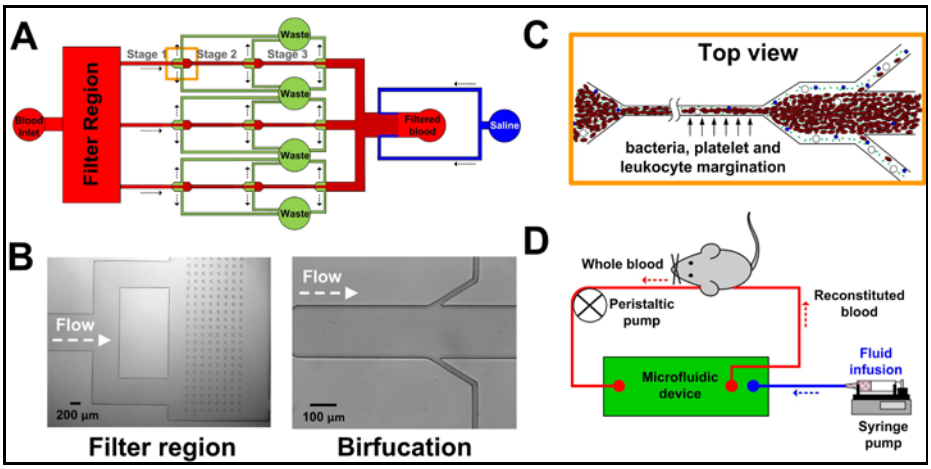

*Figure 1: (**A**) Schematic illustration of 3-channel margination microdevice. (**B**) Images of filter region and bifurcation. (**C**) Separation principle at each bifurcation stage (yellow box in **A**). (**D**) Schematic representation of the closed circuit used for coupling the microfluidic device with a catheterized mouse using a peristaltic pump. A saline fluid infusion is added at the filtered outlet to account for the volume loss (~30%) into waste side outlets during filtration.*

RESULTS AND DISCUSSION

In vitro experiments using human blood spiked with FITC-conjugated *Escherichia coli* (*E. coli*) were carried out to characterize device performance (Fig. 2A). As expected, shear-induced margination of bacteria and platelets had little effect with channel height, achieving ~60-70% removal efficiency while leukocyte removal decreased with channel height due to enhanced margination along the height (Fig. 2B).

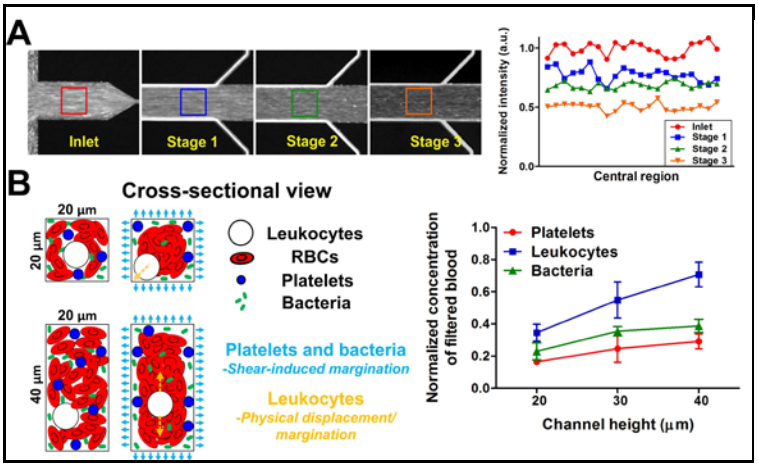

*Figure 2: (**A**) Average composite images and fluorescence intensity plot illustrating margination and successive removal of FITC-labelled E. coli at each bifurcation stage. (**B**) (left) Cross-sectional schematic illustration of cell margination for various cellular components in different channel heights. (right) Plot shows the normalized concentration of different cellular components at the filtered centre outlet at 30 µLmin^{-1}. Leukocyte removal efficiency decreased with increasing channel height due to enhanced margination/ physical displacement along the height.*

For *ex vivo* testing using CLP mice, similar separation efficiencies (~60-70%) were achieved for different targets as compared to *in vitro* experiments using human blood (Fig. 3A). Lastly, CLP-mice were subjected to continuous filtration, and bacteremia was successfully reduced for all filtered mice after a 25 minute intervention without administration of antibiotics (Fig. 3B). This was confirmed by measuring waste outlet bacterial count which was approximately twice of baseline. Our *in vivo* results were also consistent with *in vitro* results (continuous filtration of 1 mL of blood) and standard Monod kinetics, achieving ~40% decrease in bacteremia after 25-30 minutes (Fig. 3C).

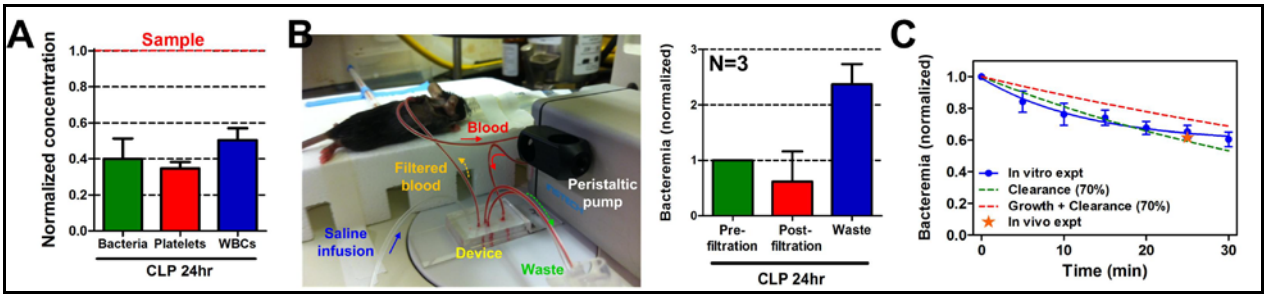

*Figure 3: Filtration performance using septic (CLP-induced) mouse blood in 40 µm height channel system. (**A**) ex vivo test- Normalized plot showing concentration of different cellular components from septic mouse blood at the filtered centre outlet at 30 µLmin^{-1}. (**B**) Photo showing the actual filtration setup for a catheterized mouse with saline infusion under gas anesthesia. In vivo test- Bacteremia quantification of septic mice before and after filtration (~25 mins intervention at 30 µLmin^{-1}) and waste outlets of device. (**C**) Both in vitro (continuous filtration of 1mL of blood at 30 µLmin^{-1}) and in vivo experimental results were similar to standard Monod kinetics model (70% removal efficiency).*

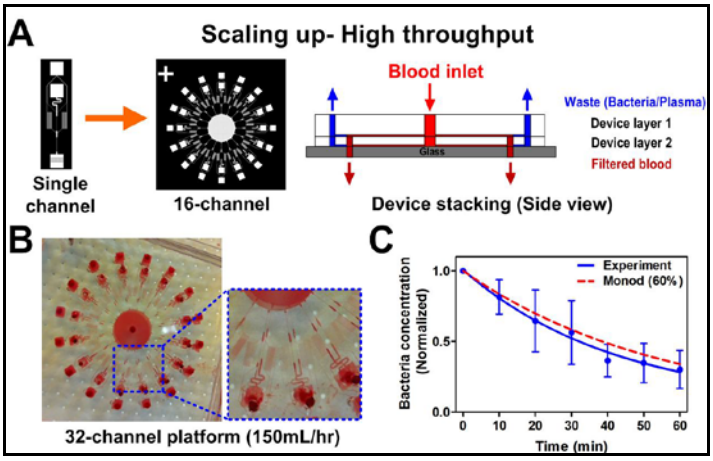

*Figure 4: (**A**) Multiplexed margination platform using channel parallelization and device stacking for "human scale" throughput. (**B**) Optical image of a dual-stacked margination device capable of processing whole blood at 150 mLhr^{-1}. (C) Decrease in bacteria concentration in 50 mL sample at 90 mLhr^{-1} using a 16-channel device.*

CONCLUSION

The developed technique offers significant advantages for non-labeled, non-specific removal of a broad range of blood-borne pathogens. The current platform was developed specifically for a murine experimental model, as the filtration rate (90 mL/kg/hr) is comparable to human hemofiltration (45-60 mL/kg/hr) in clinical settings. We also demonstrated massive channel multiplexing on a single device to process ~100-150 mL/hr of whole blood which is relevant for interventions in human sepsis (Fig. 4). Our hypothesis is that a broad spectrum removal of bacteria and inflammatory cellular components can modulate the host inflammatory response for sepsis treatment. Long-term survival studies using the CLP mouse model are currently on-going, and will be presented at the conference.

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