Electronic Supplementary Material (ESI) for Lab on a Chip.

Broad spectrum immunomodulation using biomimetic blood cell margination for sepsis therapy

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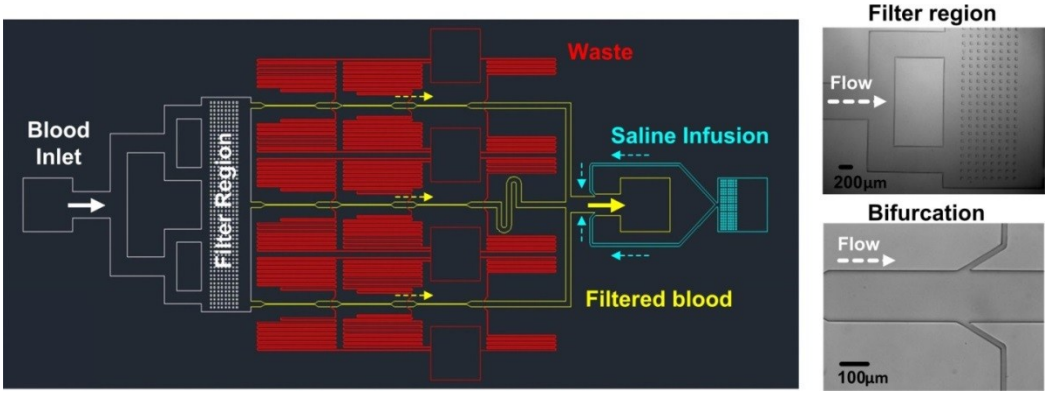
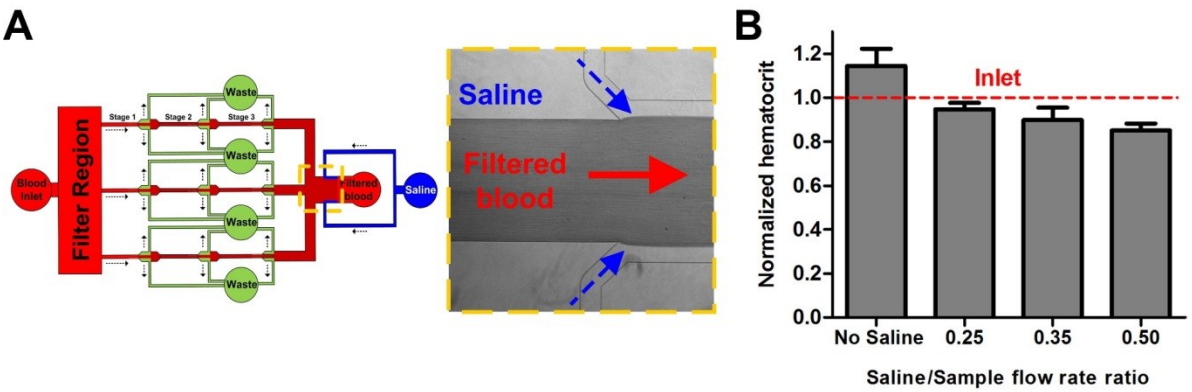
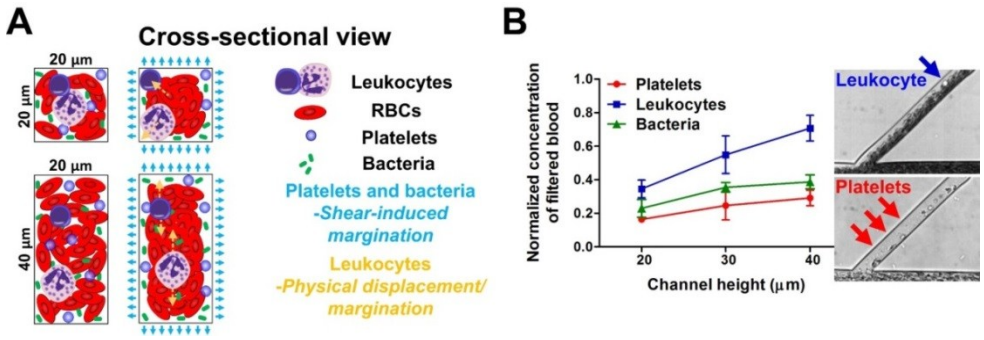
Supplementary information



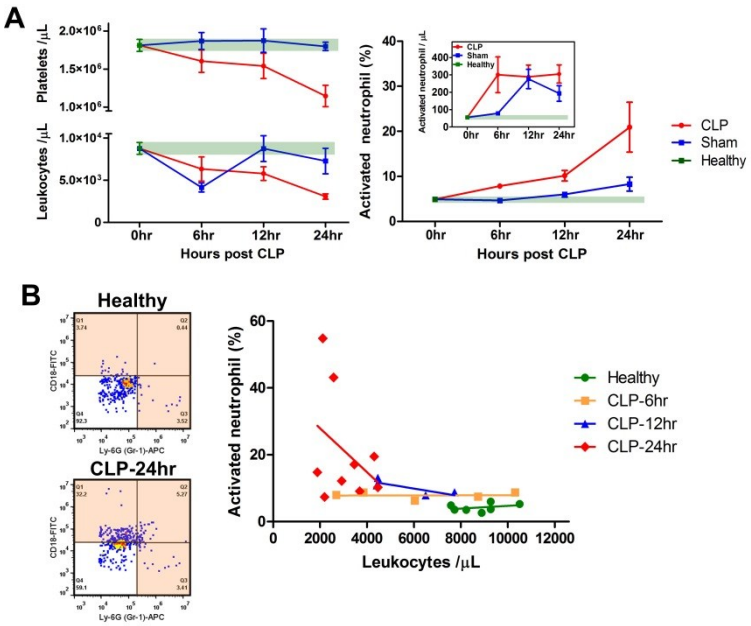
Supplementary Figure S1 (*left*) CAD layout of the developed μBM device. Filtered blood flows along the main channels (yellow) towards a single outlet while marginated cell components are removed via the side channels (red) as waste. A saline infusion port (blue) is added next to the filtered outlet to compensate for volume loss. (*right*) Optical images of the inlet filter region and bifurcation at each stage.



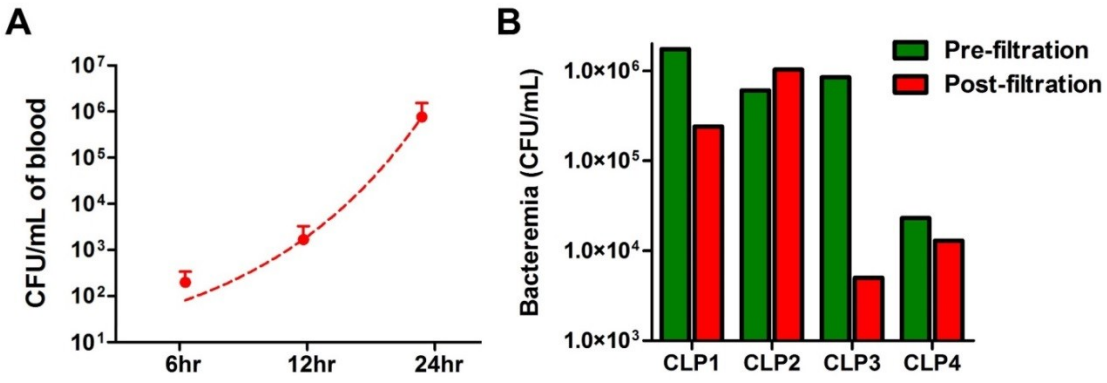
Supplementary Figure S2 Characterization of saline infusion. (**A**) Optical image of the filtered blood merged with saline infusion from both sides. Corresponding region (yellow dotted box) is indicated on the device. (**B**) Normalized change in hematocrit at the filtered outlet at different saline flow rates. A final saline/sample ratio of 0.35 (10 μLmin^{-1} saline infusion and 30 μLmin^{-1} sample flow rate) was chosen to compensate for equal volume loss (~30%) into side outlets. Mean ± s.d. from $n \geq 3$ separate experiments.



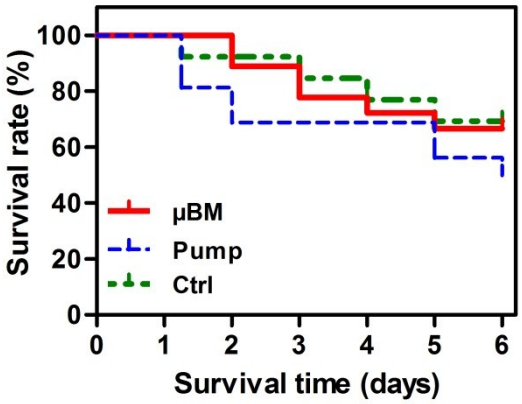
Supplementary Figure S3 *In vitro* characterization of blood margination in channel with different heights using human whole blood. (**A**) Schematic representation of different margination mechanisms in microchannel of different heights. (**B**) Normalized target cell concentrations at the filtered centre outlet at 30 µLmin^{-1}. Mean ± s.d. from $n \geq 3$ separate experiments. Leukocyte removal efficiency decreased with increasing channel height due to enhanced margination/physical displacement along the height (yellow arrows in **A**). Representative high speed images illustrate removal of marginated leukocyte (blue arrow) and platelets (red arrow) into side channel.



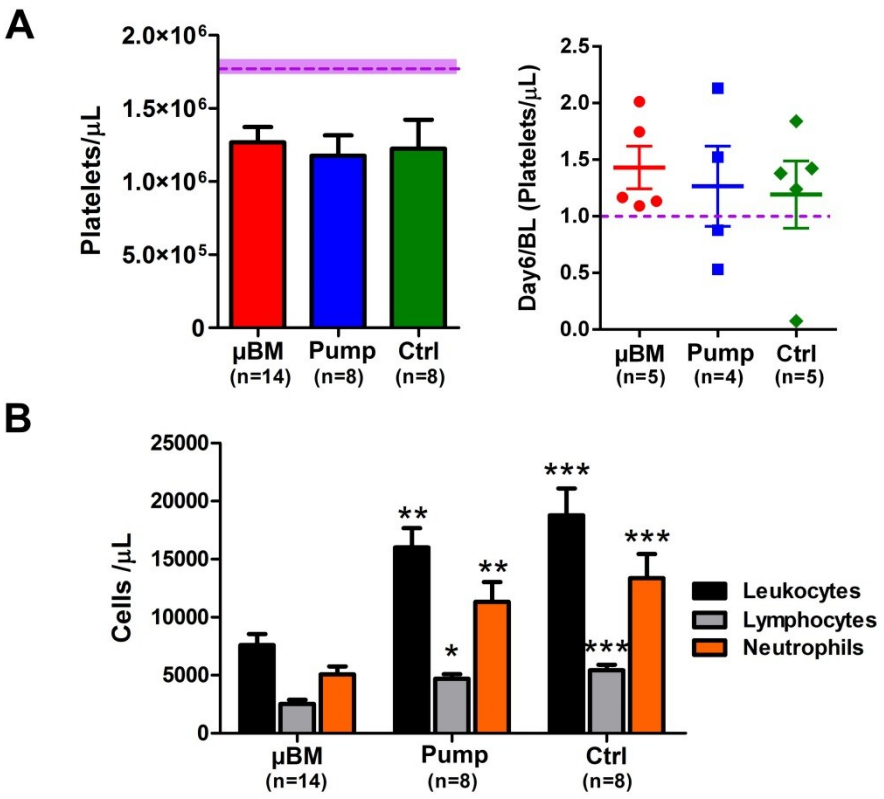
Supplementary Figure S4 Profiling of immune cell concentration in mice following cecal ligation and puncture (CLP) surgery. (**A**) Platelet count, leukocyte count and neutrophil activation (inset shows activated neutrophil concentration) for CLP-operated mice over 24 hours (n = 4 for 6hr, 12hr and n = 9 for 24hr). Sham-operated mice (laparotomy without cecum perforation, n = 4 for all timepoints) recovered and had similar cell concentrations as healthy mice after 12 hours. 0 hr timepoint corresponds to healthy mice (n = 7) and approximate healthy ranges are highlighted (green region). All data represent mean ± s.e.m. (**B**) Representative gated flow cytometry plots indicating quantification of neutrophil activation (upregulation of CD18 and Ly-6G (Gr-1), coloured region) for healthy and CLP24hr mice. Activation was gated using PMA-activated neutrophils (data now shown). Neutrophil activation (%) and leukocyte count in healthy mice and CLP mice over time. Each dot corresponds to 1 mouse and data were linear fitted for each timepoint.



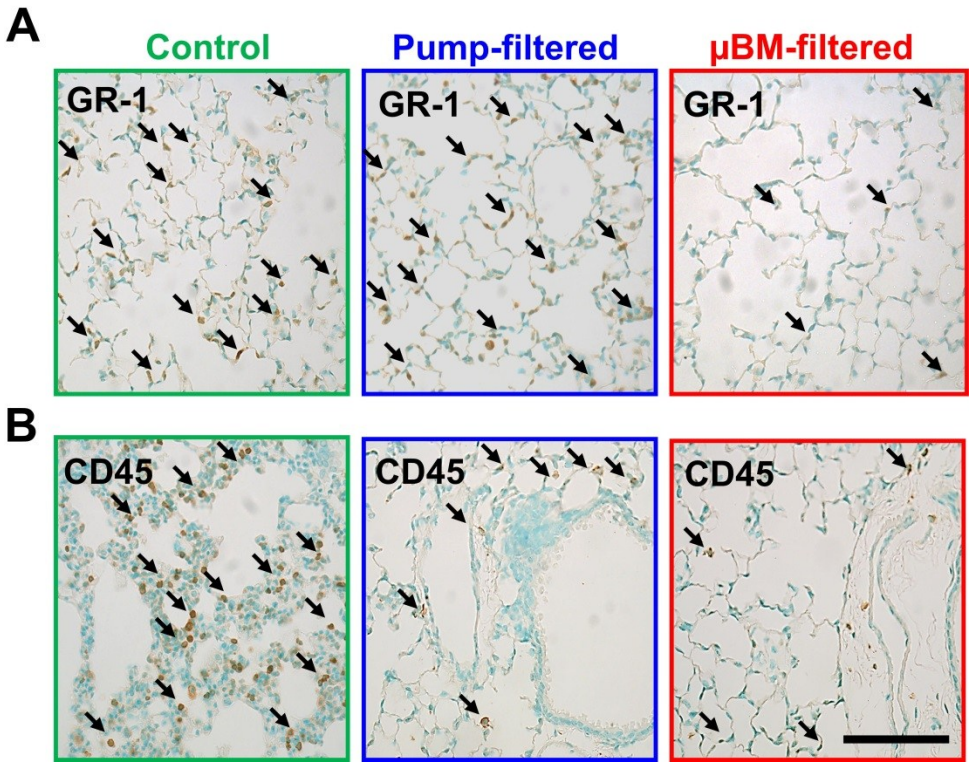
Supplementary Figure S5 Profiling of blood bacteremia in CLP mice (6-24 hours). (**A**) Exponential increase (log scale) in bacteremia in CLP-operated mice over time. Mean ± s.e.m from n = 2 for 6, 12hr and n = 3 for 24hr. (**B**) Change in bacteremia for 4 mice (CLP1-4) before and after μBM intervention in an extracorporeal circuit.



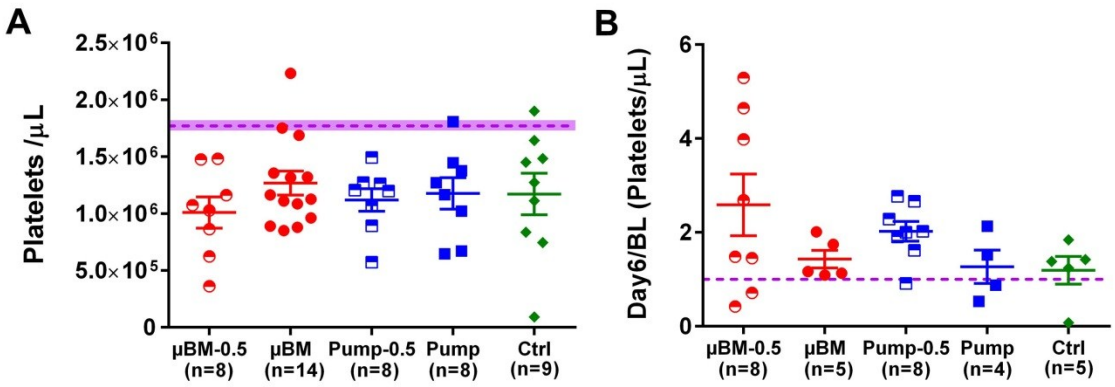
Supplementary Figure S6 Kaplan-Meier plot showing no significant difference in long-term survival in CLP mice 6 days post CLP (5 days after µBM intervention). µBM-filtered mice (red solid line), n = 18; pump-filtered mice (blue dashed line), n = 16; ctrl mice (green dashed line), n = 13).



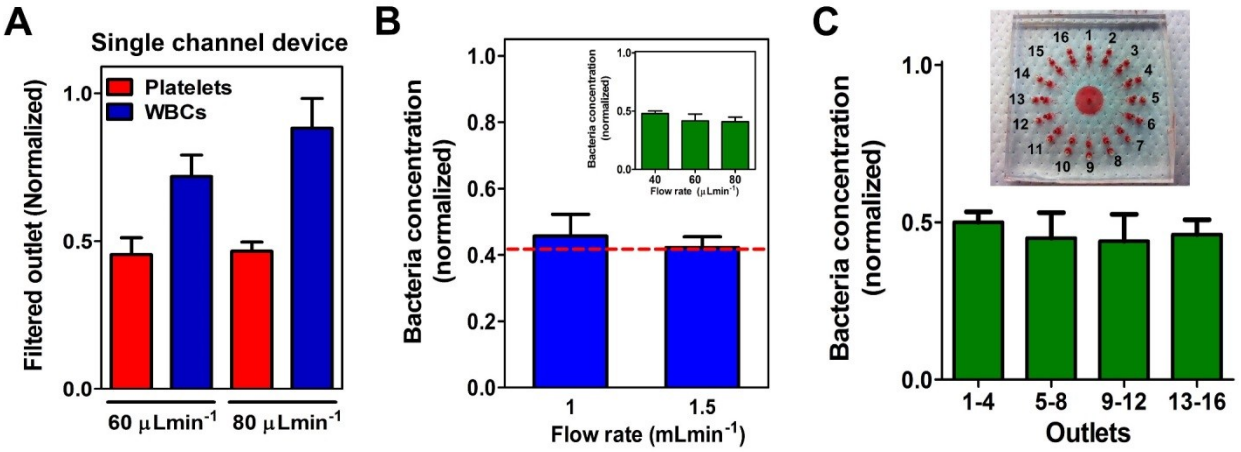
Supplementary Figure S7 Effect of µBM intervention on long term immune cell response. (**A**) Platelet count (*left*) and fold change (*right*) at day 6 when normalized to day 1 (before intervention). Purple dotted line and shaded region indicate mean ± s.e.m for healthy mice. Normalized value of 1 (purple dotted line) indicates no change compared to day 1. (**B**) Similar attenuation of lymphocytes and neutrophils in pump-filtered and control mice at day 5. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005 versus µBM-filtered mice. All data represent mean ± s.e.m.



Supplementary Figure S8 Leukocyte sequestration in mouse lungs at day 6 post CLP. Representative histological sections of surviving mice showing (**A**) GR-1 and (**B**) CD45 staining (black arrows) of lung tissue in control, pump-filtered and µBM-filtered mice. Scale bar represents 100 µm. Representative sections of n=5 mice.



Supplementary Figure S9 Dose dependent long term effect of µBM intervention on platelets at day 6. (**A**) Platelet count and (**B**) fold change when normalized to baseline (day 1 before intervention). Purple dotted line and shaded region indicate mean ± s.e.m for healthy mice. µBM-0.5 and Pump-0.5 groups correspond to mice on shorter intervention time (~13 mins). Mean ± s.e.m from *n* = 8-14 mice in each group. Normalized value of 1 (purple dotted line) indicates no change.



Supplementary Figure S10 Multiplexed μBM system for high throughput blood filtration. (**A**) Platelet and leukocyte removal in a 2-stage, single-channel μBM device. Channel is 2mm long, 20 μm wide and 40 μm height. (**B**) Bacteria removal efficiency in a 2-stage, 16-channel μBM device. Red dotted line indicates filtration performance for a single channel. Inset figure represents the single channel characterization at different flow rates. (**C**) Similar bacteria removal for different channels in the same device. Corresponding channels are numbered on the device (filled with red dye). All data represent mean ± s.d. from $n \geq 3$ separate experiments.

SI Movie Legends

Supplementary Movie 1 Fluorescent video illustrating successive removal of marginated *E. Coli* (fluorescent) into the side channels of μBM device at each bifurcation stage.