SUPPLEMENTARY INFORMATION

An Extracorporeal Blood Cleansing Device for Sepsis Therapy

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Supplementary Fig. 1. **a**, Various pathogens, including *C. albicans* and *S. cerevisiae* fungi, gram-negative *E. coli* bacteria and gram-positive *S. aureus* bacteria were efficiently (>90%) captured by FcMBL magnetic opsonin beads when tested in a batch assay using a single biospleen magnetic separator unit (also see **Supplementary Table 1**). **b**, The binding capacity of FcMBL magnetic opsonins for LPS-endotoxin (extracted from *E. coli*) was found to be proportional to the concentration of LPS added when detected by a sandwich immunoassay using FcMBL magnetic beads and HRP-conjugated FcMBL proteins. **c**, FcMBL lacks the
coagulation-promoting, thrombin-like activity of the MASP regions of native rhMBL that were deleted during production of the genetic engineered opsonin. Thrombin-like activity of the MBL and MASP complex was measured by incubating rhMBL or FcMBL on mannan-coated wells in 1% MBL-null serum (that contains MASPs), washing extensively, and adding a rhodamine 110-labeled thrombin substrate (tosyl Gly-Phe-Arg-amide, R22124, Invitrogen); thrombin (King Pharmaceuticals, TN) was used as a control. FcMBL lacks the complement component 4 (C4) cleaving activity of rhMBL. Microtiter wells were coated with mannan and different amounts of FcMBL and rhMBL in 2.5% MBL-null serum were added to the wells. After incubation at 37°C and rinsing, deposited C4 fragments were detected with biotinylated monoclonal anti-human C4 followed by streptavidin-labeled horseradish peroxidase and measurement by ELISA.
Supplementary Fig. 2. a, A schematic drawing of the assembly of the biospleen device. The fluidic unit machined from aluminum, medical grade polysulfone, or PMMA was covered with thin transparent PMMA adhesive film tape (76 μm thickness, McMaster-Carr, IL, USA),
aligned with four connector blocks using their dowel pins, assembled together, and held in place using socket head cap screws (McMaster-Carr, 92290A117, IL, USA). Four o-rings were used to prevent leakage between the connector blocks and the biospleen device, and luer locks were placed at the openings of the channels on the surface of the device to permit fluidic coupling. **b**, Photograph of a polysulfone fluidic unit of the magnetic separator unit containing the micromachined separator channels. **c**, Photograph of an assembled magnetic separator unit composed of PMMA with luer lock connectors to fluidic inlets and outlets. **d**, Circuit diagram of the experimental animal setup used to carry out extracorporeal blood cleansing of septic rats with the biospleen device. Blood is drawn through one jugular catheter of a rat using a peristaltic pump and then mixed with heparin (50 units kg\(^{-1}\) h\(^{-1}\)) and magnetic opsonins (0.5 mg ml\(^{-1}\)) that are continuously injected into the extracorporeal circuit. After passing through the static inline mixer and a helically coiled tubing segment for mixing and incubation, the pathogens and endotoxins bound to the magnetic opsonins pass through the magnetic separator unit where they are pulled out of the blood stream, and the cleansed blood flows back to the rat via the other jugular catheter.
Supplementary Fig. 3. Optimization of biospleen device design and function. a,

Magnetic opsonin capture efficiency was increased by more than 2-fold in banked human
whole blood by integrating the static mixer (inset image) with the biospleen device. \( p < 0.005 \)

b, Magnetic flux density \( (T, \text{Tesla}) \) gradients estimated for the assembled stationary magnets, which guided device design. c, The theoretically estimated traveling distance \( (D_t, \text{black solid line}) \) that pathogens (1 μm in diameter) opsonized by nanomagnetic opsonin beads (128 nm) will travel in blood as a function of flow rate. This analysis predicts that for the 600 μm high blood channel (dashed line) used in the device, > 99% of magnetically opsonized pathogens will be removed from blood flowing at \(~560 \text{ ml h}^{-1}\) assuming 40 opsonin beads bind each pathogen. The experimental data (black circle, 535 ml h\(^{-1}\)) corresponds to the theoretically estimated flow rate. d, The pathogen removal efficiency of the blood cleansing microdevice determined at different flow rates using whole human blood versus saline when spiked with \( S. \text{ aureus} \) and 128 nm FcMBL magnetic opsonins. e, Pressure drop \( (\Delta P) \) measured in the biospleen device alone, the inline mixer alone, and the two integrated together as a function of flow rate using saline. The measured pressure drop of the biospleen device was similar to that exhibited by extracorporeal devices that are currently used clinically. f, Maximum blood loss into the saline channel estimated based on the volume of the saline channel compared to the whole blood volume of a rat passed through the device (black line), and the blood loss experimentally measured in the system (open squares), which is below a blood loss range (10–15%) that a healthy animal or human patient can endure\(^1\).
Supplementary Fig. 4. a, Fluid shear stress (τ) measured in the biospleen blood channel as a function of flow rate, which is below the physiological shear range (< 15 dyne cm⁻²). b, Non-specific interaction of the beads to blood cell components was evaluated by analyzing complete blood cell counts. There was no significant depletion of blood cells by the FcMBL-coated magnetic beads (WBC, white blood cells; RBC, red blood cells; PLT, platelets). c, Increase
in efficiency of capture of 128 nm magnetic opsonin beads (FITC-labeled; 5 mg ml⁻¹) in saline observed when the nanobeads are combined with larger (1 μm; 5 mg ml⁻¹) magnetic beads and flowed through the biospleen device at 10 ml h⁻¹ (p < 0.05). d, Free hemoglobin levels in plasma of rats infected with S. aureus with or without biospleen treatment. Blood cleansing treatment of rats with bacteremia significantly (*p < 0.05) reduced free hemoglobin levels induced by sepsis. e, There was no detectable change in the concentration of thrombin-antithrombin (TAT) complexes measured in blood from healthy rats that were either not treated or treated with the biospleen device for 5 hours.
Supplementary Fig. 5. Rapid clearance of fungal pathogens by the spleen in healthy rabbits. *C. albicans* fungal cells genetically engineered to express yeast-GFP (gift from Dr. Julia R. Koehler, Boston Children’s Hospital, MA, USA) were introduced intravenously through the ear vein into a healthy rabbit using 6 serial injections (every 30 min) of 1 ml of PBS, each containing $10^8$ cells. Samples (1 ml) of blood were collected from the femoral vein in heparinized vacutainers within 5 min of each injection, and analyzed using a FACS Calibur flow cytometer to determine the presence of fluorescent *C. albicans* in the bloodstream. No pathogens were ever detected in the bloodstream after any of the 6 injections; however, the rabbit died soon after the 6th injection due to cardiac arrest. The rabbit spleen was immediately harvested, fixed in formalin, cryosectioned, and stained with DAPI. Analysis of the results shown here indicate that the injected pathogens (green) were captured and cleared by spleen with great efficiency, as the green fungi can be seen filling the sinusoidal spaces of the spleen (nuclei of spleen cells are stained with DAPI) (Scale bar, 150 μm).
Supplementary Table 1: The list of the pathogens that are bound by FcMBL. We tested binding capacity of FcMBL to pathogens by ELISA, and found that FcMBL binds a broad range of pathogens, including gram-positive bacteria, gram-negative bacteria, and multiple fungi. Importantly, these included multiple antibiotic-resistant organisms (indicated in bold)\(^2\text{--}^4\) as well as many organisms that are most frequently found in clinical samples in patients with sepsis (e.g., *E.coli*, *S.aureus*, MRSA, coagulase-negative *Staphylococcus* species).

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<th>Pathogens bound by FcMBL</th>
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<tr>
<td><strong>Gram positive bacteria</strong></td>
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<td><em>C. difficile</em></td>
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<td><em>S. epidermidis</em></td>
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<td><em>S. lugdunensis</em></td>
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<td><em>S. aureus</em></td>
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<td>- Methicillin-Resistant (MRSA)</td>
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<td><em>M. leprae</em></td>
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<td><em>M. tuberculosis</em></td>
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<td><em>S. pyogenes</em></td>
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Supplementary Video 1: The fabrication and the magnetic separation principle of the biospleen. Schematic drawing and microscopic video showing how the biospleen device is fabricated and how the magnetically opsonized pathogens are separated from the blood channel under flow. Because it is difficult to observe the cell movement across the blood channel in the biospleen device, we demonstrated this in a microfluidic device fabricated from optically clear poly(dimethylsiloxane) (PDMS). To mimic pathogens captured by the magnetic opsonins, fluorescent magnetic particles (8 μm, 1.1 g ml\(^{-1}\), UMC4F, Bang Laboratories, Inc., IN, USA) were spiked into human banked blood (1 ml) and flowed at 10 μl min\(^{-1}\).
Supplementary References


