Supplementary Materials

Microfluidic assessment of red blood cell mediated microvascular occlusion

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SUPPLEMENTARY METHODS

Experimental procedure

The OcclusionChip was placed on an Olympus IX83 inverted motorized microscope stage for high-resolution imaging. An inflation syringe with a cuff pressure indicator was employed to generate a positive pressure of 60 cm2 H2O at the inlet of the microchannel to drive the blood flow (Fig. S2A). OcclusionChip system is shown in Fig. S2B. Valves 1 and 2 are off initially. After the RBC sample was loaded into a disposable syringe (sample reservoir) and tubing was connected, the measurement was conducted with the following five-step protocol: (1) 500 µl of blood sample was loaded and pressure was enabled by opening valve 1. (2) Valve 2 was opened and blood flow was released for 20 min under a constant pressure before valve 2 and valve 1 were sequentially switched off again. It is critical to seal the microchannel during the solution change. (3) The residual of blood sample was discarded and 500 µl washing buffer (PBS) was loaded. (4) Valve 1 and valve 2 were then opened sequentially, and the washing buffer was allowed to perfuse until all non-retained cells were completely cleared out of the channels. (5) Phase contrast, and fluorescent images were recorded at 20X using the Olympus Cell Sense live imaging software (excitation/emission wavelength, 488/505–580 for EGFP). Post processing of recorded images and cell counting were performed using Adobe Photoshop CS5 (San Jose, CA, USA).

Glutaraldehyde exposure of red blood cells (RBCs)

The glutaraldehyde solution was diluted with PBS to obtain a stock solution at a concentration of 0.16% (w/v), which was further diluted with PBS and mixed with the isolated RBCs to adjust the hematocrit to 20% and the glutaraldehyde concentration to 0.00% (as control), 0.02%, 0.04%, or 0.08% (w/v). The RBCs were incubated in glutaraldehyde for 10 min at room temperature. The protein cross-linking reaction was thereafter quenched by GASP buffer (PBS with 1% (w/v) BSA and 5.5mM glucose, pH 7.4), and the fixed RBCs were re-suspended in PBS at a 20% hematocrit. Next, the suspension with glutaraldehyde-fixed RBCs was mixed with a 20% hematocrit suspension of fresh, untreated RBCs in PBS to obtain the RBC suspension containing 1% treated cells, which was then analyzed using the OcclusionChip.

Diamide exposure of RBCs

Diamide (Sigma Aldrich) lyophilized powder was dissolved in PBS to obtain a 0.16% (w/v) stock solution. Similarly, the diamide stock solution was diluted with PBS and mixed with isolated RBCs to adjust the hematocrit to 20% and the diamide concentration to 0.00% (as control), 0.02%, 0.04%, 0.08% (w/v). The samples were incubated for 20 min at room temperature and thereafter quenched by GASP buffer. The fixed RBCs were re-suspended in PBS at a 20% hematocrit and analyzed using the OcclusionChip.

Mercuric ion exposure of RBCs

We used healthy blood samples anticoagulated with sodium citrate instead of EDTA, since mercuric ion binds to EDTA with higher affinity than RBC membrane. 1 mM mercuric ion (Hg^{2+}) stock solution was prepared using mercury chloride ($HgCl_2$, Sigma Aldrich) and PBS. The mercuric ion stock solution was diluted with PBS until 10 times of the final concentration, and then mixed with whole blood at a ratio of 1:9 (v/v). The blood sample was incubated at 37°C for three hours. No hemolysis was observed within the duration of incubation. The mercuric ion-exposed RBCs were then re-suspended (as described above) and analyzed.

Gas exchange setup to assess microvascular occlusion meditated by hypoxia in sickle cell disease

Briefly, the gas exchanger is composed of a gas permeable inner tubing placed in concentric with a gas impermeable outer tubing. A piece of 1.5 mm thick poly(methyl methacrylate) (PMMA) layer with the dimension of 50 mm x 25 mm was designed as the top cover of the gas chamber, while the height of the gas chamber was adjusted with a 3.175 mm thick PMMA layer. The above two layers were bonded to the OcclusionChip through double-sided adhesive (DSA), and the inlet was sealed with epoxy after assembly with the gas exchanger.

Scanning electron microscopy procedures

Fresh human whole blood was collected in Na-citrate tubes for mercuric ion treatment. Control or mercuric-ion exposed blood samples were centrifuged at 500g for 5 minutes followed by removal of plasma and

buffy coat. Pelleted RBCs were washed twice with PBS and re-suspended in PBS at a hematocrit of 20 %. Next, the processed RBCs were fixed with 2.5% glutaraldehyde solution (8% w/v, Sigma Aldrich, diluted in PBS) for 1 hour in room temperature. The suspension was then centrifuged at 500g for 5 minutes to collect RBCs which were re-suspended 1% osmium tetroxide solution (diluted in DI water) for 1 hour in room temperature. Thereafter, the RBCs were rinsed twice with DI water and dehydrated serially in 30%, 50%, 70%, 80%, 90%, 100% and 100% ethanol. Finally, after a 20 min incubation with 100% hexamethyldisilazane (HMDS, Sigma Aldrich), the samples were transferred into a desiccator and kept overnight. The samples were coated with palladium at 55mA for 60s and examined using Helios NanoLab™ 650 (FEI, Field Emission SEM). HbS-carrying RBCs were suspended in PBS at 20% hematocrit and perfused through the microchannel for 5 minutes under either hypoxic or normoxic conditions. Non-retained cells were washed away with PBS. A 2.5% glutaraldehyde solution was then introduced into the channel and the retained RBCs were incubated for 1h. Next, 1% osmium tetroxide solution was injected into the channel and the retained RBCs were incubated for 1h. After the channel washed with DI water, dehydration with gradient series of ethanol was performed within the channel. The PDMS was thereafter peeled-off from the glass slide and further dehydrated with HMDS for 20 min. Samples were kept in a desiccator until surface palladium coating and imaging as described above.

SUPPLEMENTARY FIGURES

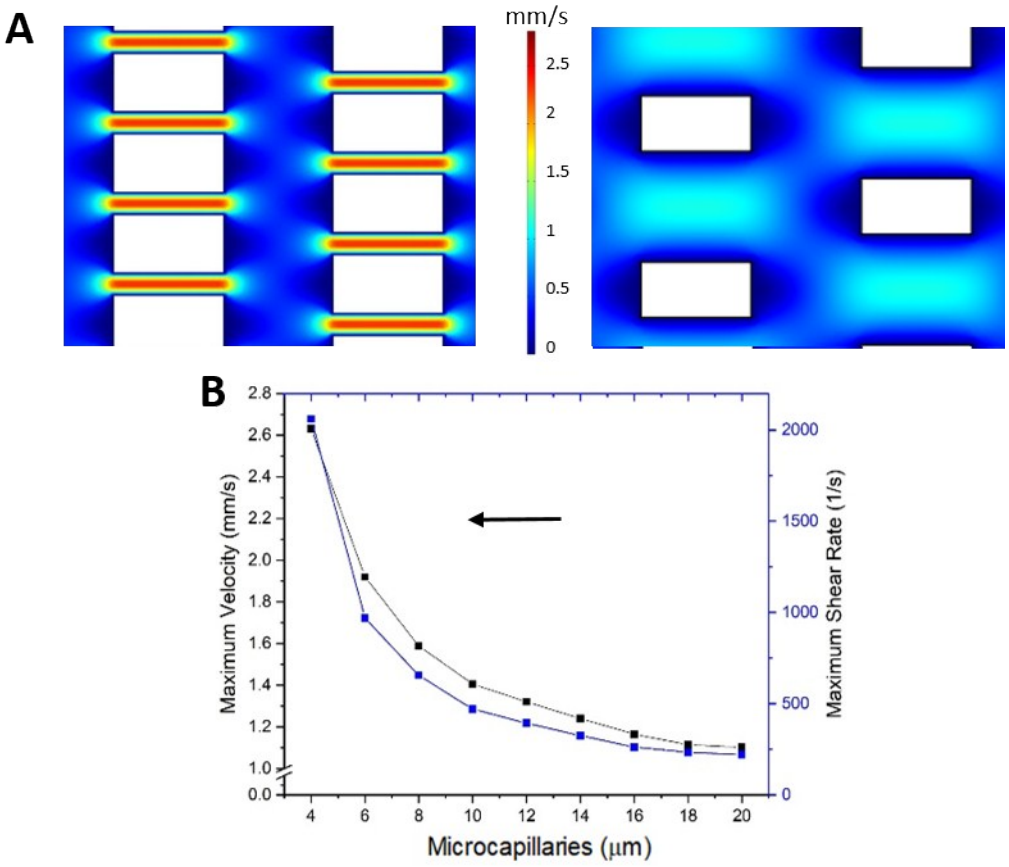


Figure S1. Velocity and shear environment created by the microcapillary features. (A) Velocity profiles of the initial flow condition in micropillar arrays with 20-μm (left) and 4-μm (right) microcapillaries. (B) Maximum velocity and shear rate contours across the microchannel. Arrow indicates flow direction.

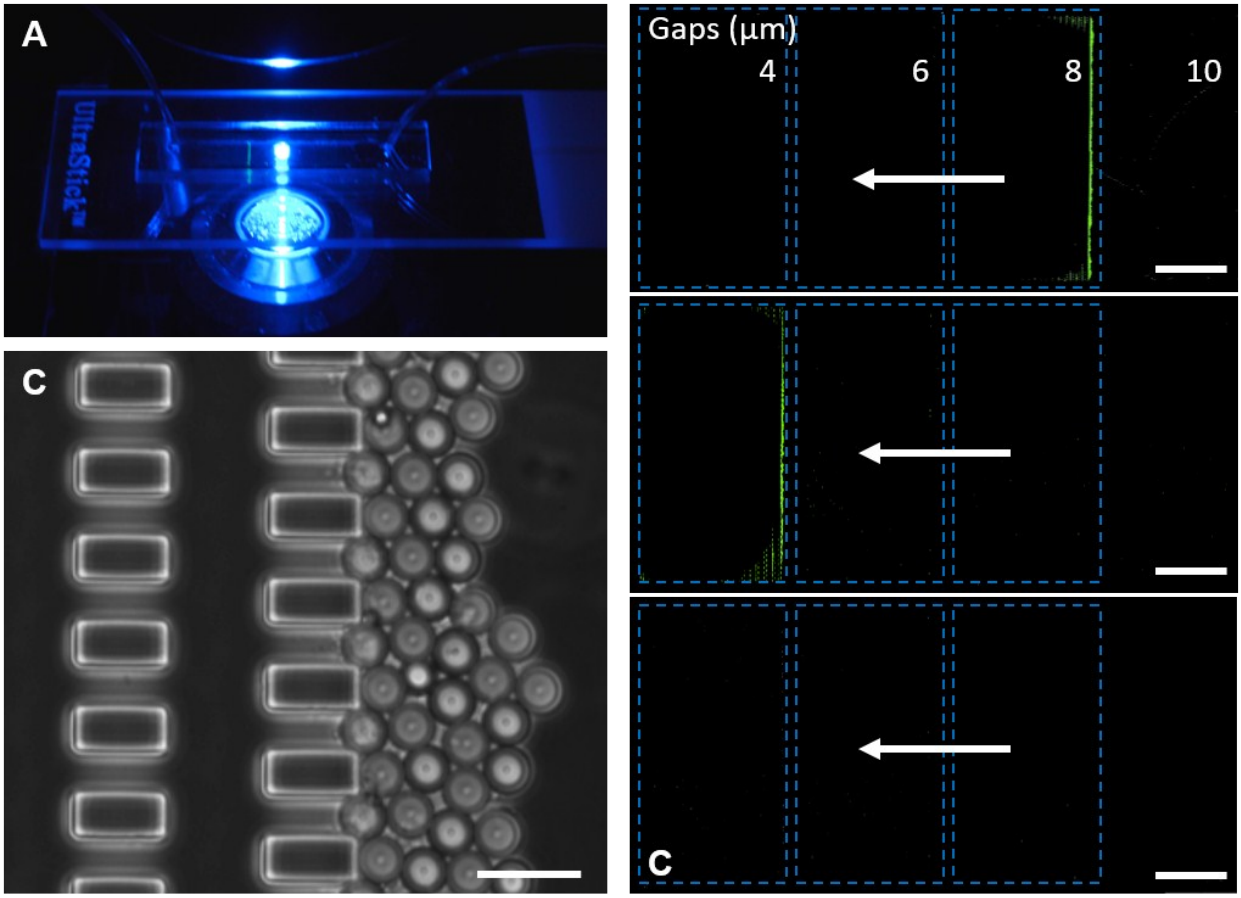


Figure S2. Validation of OcclusionChip micropillar array functionality using rigid fluorescent beads. This initial assay primarily focused on the verification of the retention mechanism of the OcclusionChip. (A) The OcclusionChip was placed on the automated microscope stage for high resolution image recording. A visually clear line formed by 10-µm microbeads. (B) Close-up view of microbeads captured in the micropillar array with 8-µm microcapillaries. Scale bar represents a length of 20µm. (C) Fluorescent images show that microbeads are able to clear the microcapillaries larger than or equal to their diameters but can block those smaller than their outer diameters. (From top to bottom) Tests were performed with 10-µm, 6-µm, and 4-µm microbeads. Arrows indicate flow direction. Scale bars represent the length of 1mm.

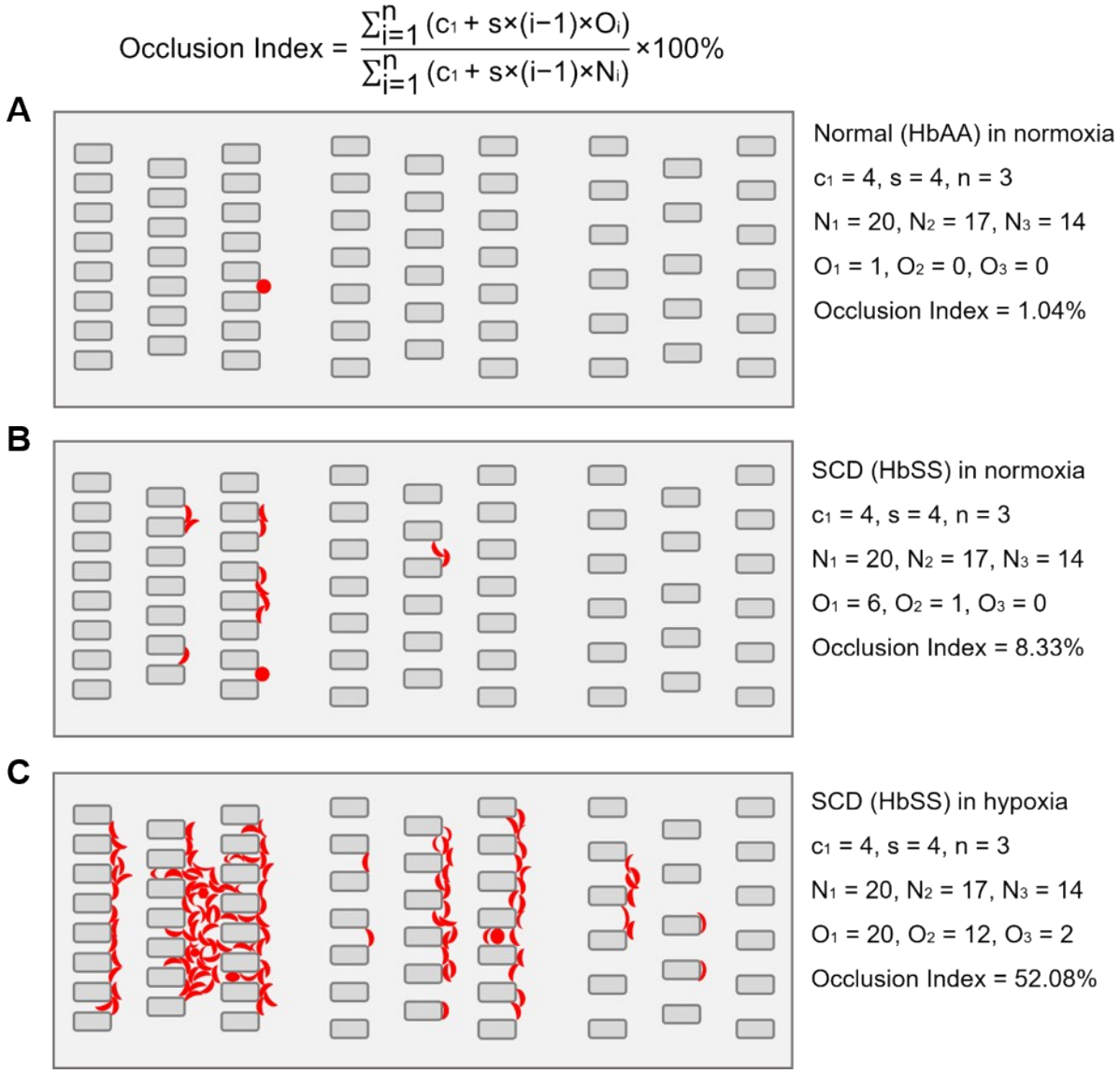


Figure S3. Generalizable Occlusion Index for assessing microcapillary network occlusion. Three identical microfluidic models employing micropillar arrays assessing RBC deformability in three different scenarios, normal (HbAA) in normoxia, SCD (HbSS) in normoxia, and SCD (HbSS) in hypoxia, are shown. Occlusion Indices for these three examples were calculated as 1.04% (A), 8.33% (B), and 52.08% (C).

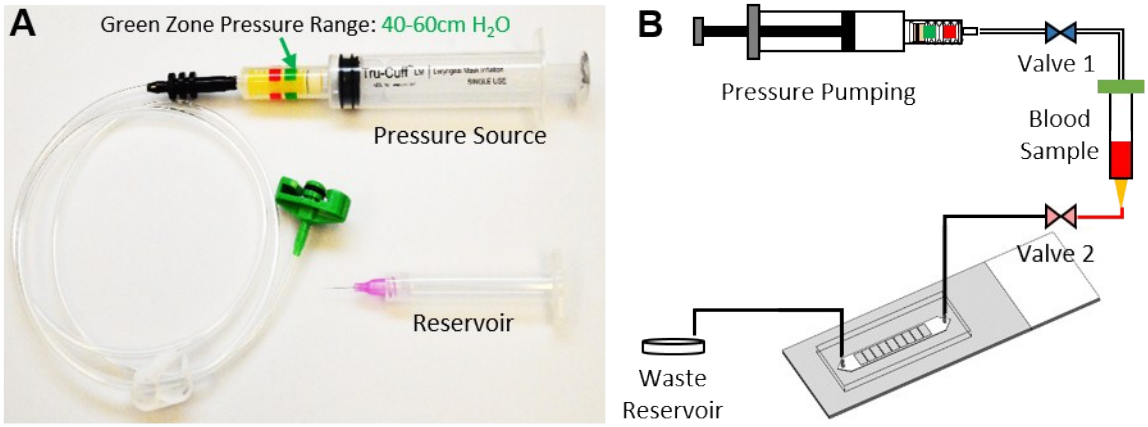


Figure S4. OcclusionChip experimental setup. (A) A cost-effective inflation syringe was employed as the pressure source. The pressure was adjusted by applying the indicator to the end of the green zone, which maintained a constant pressure of 60cm H2O at the inlet of the microchannel. (B) Pre-prepared RBC suspension was input into the sample reservoir and connected to the inlet of the OcclusionChip. The pressure was controlled by valve 1. Valve 2 was used for back flow prevention. Switching off the two valves can realize the solution change. A waste reservoir was connected to the outlet of the OcclusionChip.

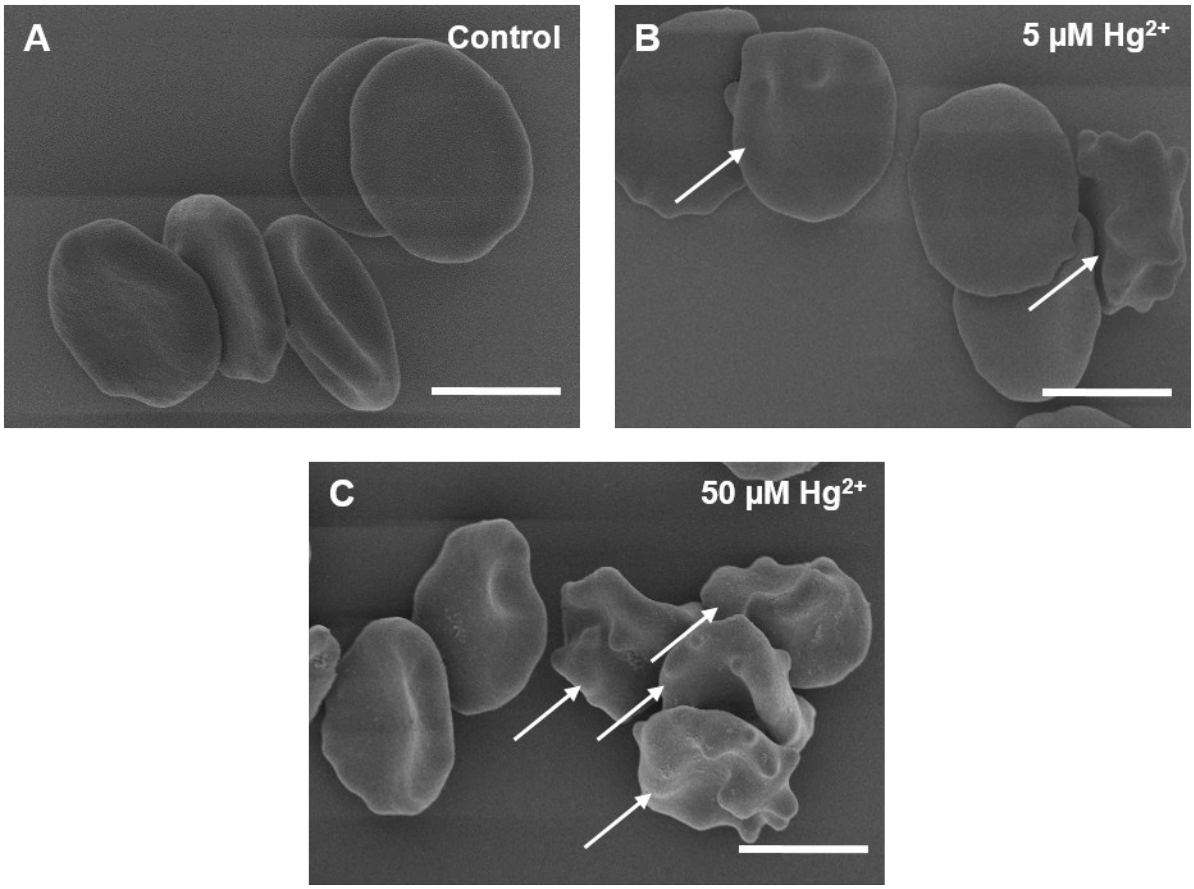


Figure S5. Scanning Electron Microscopy images of normal and $HgCl_2$-treated RBCs at 6500X magnification. (A) Non-treated RBCs (control) maintained their intact biconcave morphology. Hg^{2+}-exposed RBCs underwent morphology changes and turned into echinocytes (indicated by arrows) after 3h at 37 °C incubation with 5 µM (B) and 50 µM (C) mercuric ion. Scale bars represent a length of 5 µm.

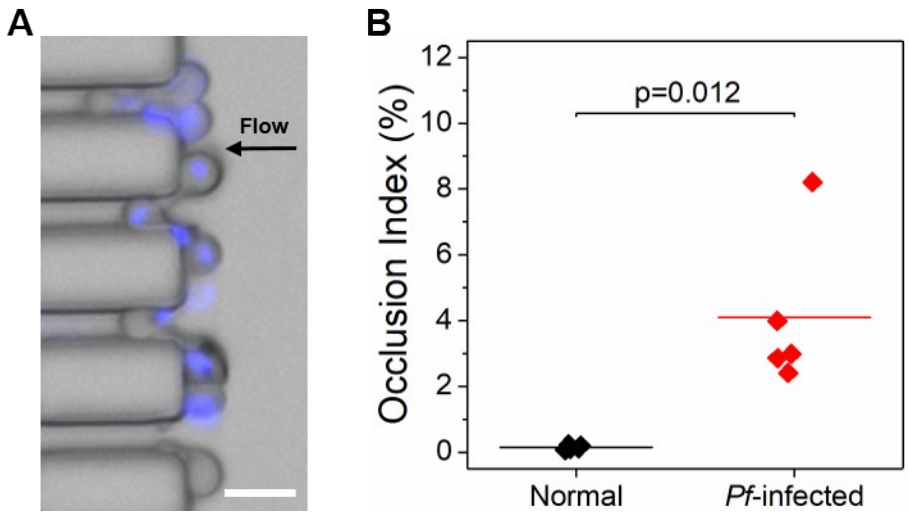


Figure S6. Microvascular occlusion mediated by *Plasmodium falciparum*-infected RBCs (*pf*-iRBCs). (A) Close-up view of parasitized RBCs retained by the 4-μm microcapillaries. Parasites were labeled with Hoechst stain. Scale bar represents a length of 10μm. (B) The Occlusion Index (OI) of *pf*-infected RBCs is significantly higher compared to that of normal RBCs (p=0.012, Mann-Whitney). Data point cross bars represent the mean.

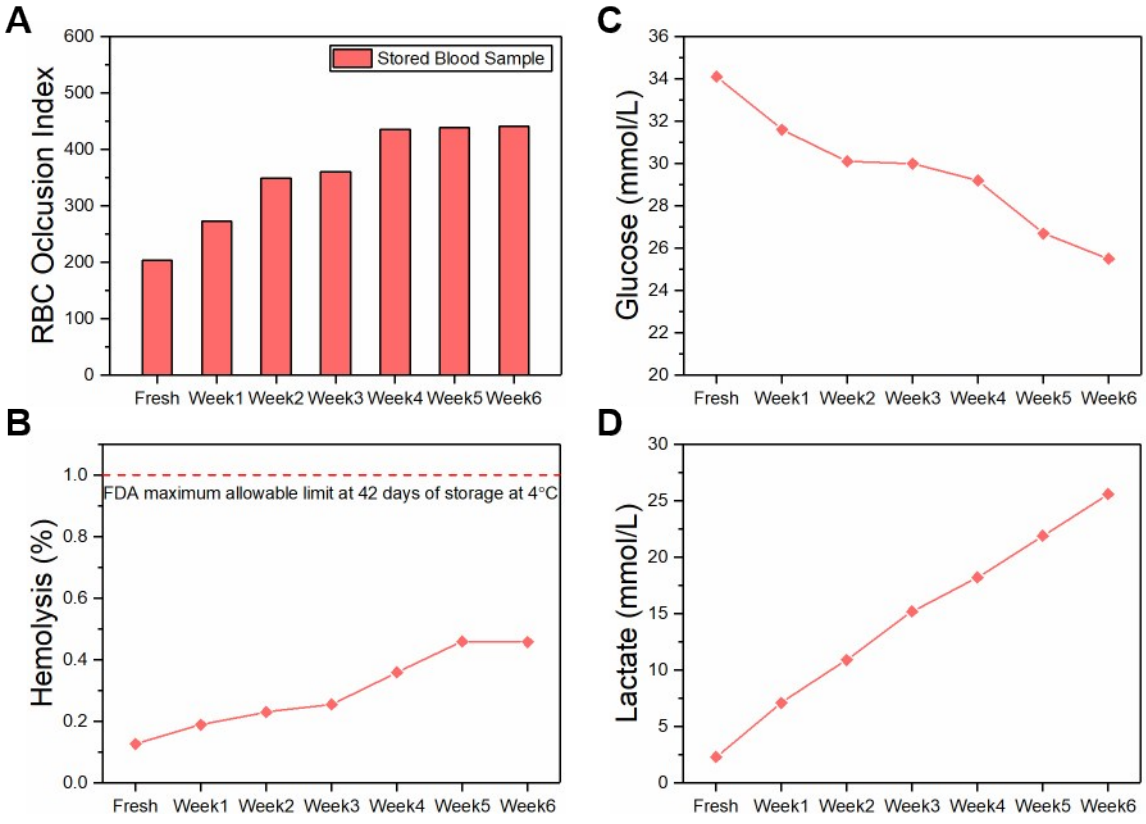


Figure S7. Analysis of RBC Occlusion Index change of stored RBCs as the blood storage progressed. Data of RBC occlusion index (A), hemolysis level (B), glucose consumption (C) and lactate production (D) plotted against storage course.

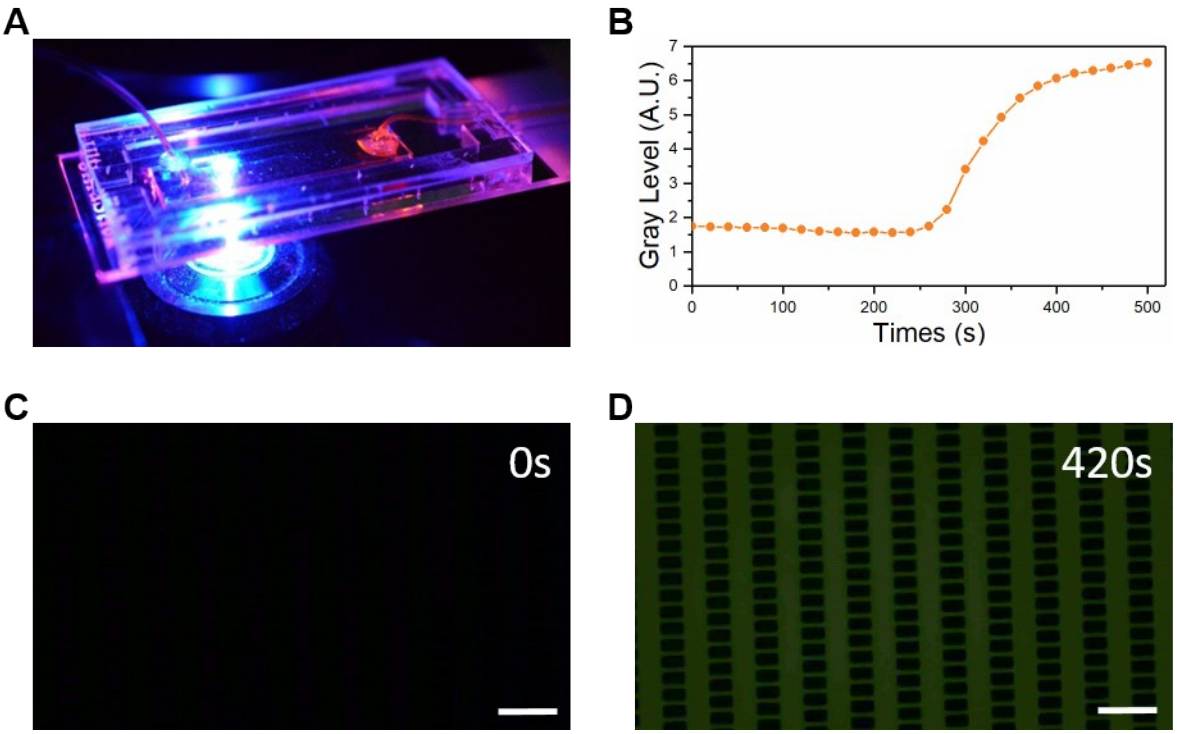


Figure S8. Validation of oxygen diffusion within the microchannel. (A) Tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride complex dissolved in ethanol was injected into the deoxygenation microsystem. Luminescent intensity was measured under illumination at 488 nm. (B) Gray-level intensity near the outlet was analyzed via image processing. Solution was perfused before 0s while the controlled gas flow (95% N_2 and 5% CO_2) was allowed at 0s. (C) Fluorescent image obtained at 0s. (D) Fluorescent image obtained at 420s. Saturation of luminescent intensity indicated completion of gas exchange.

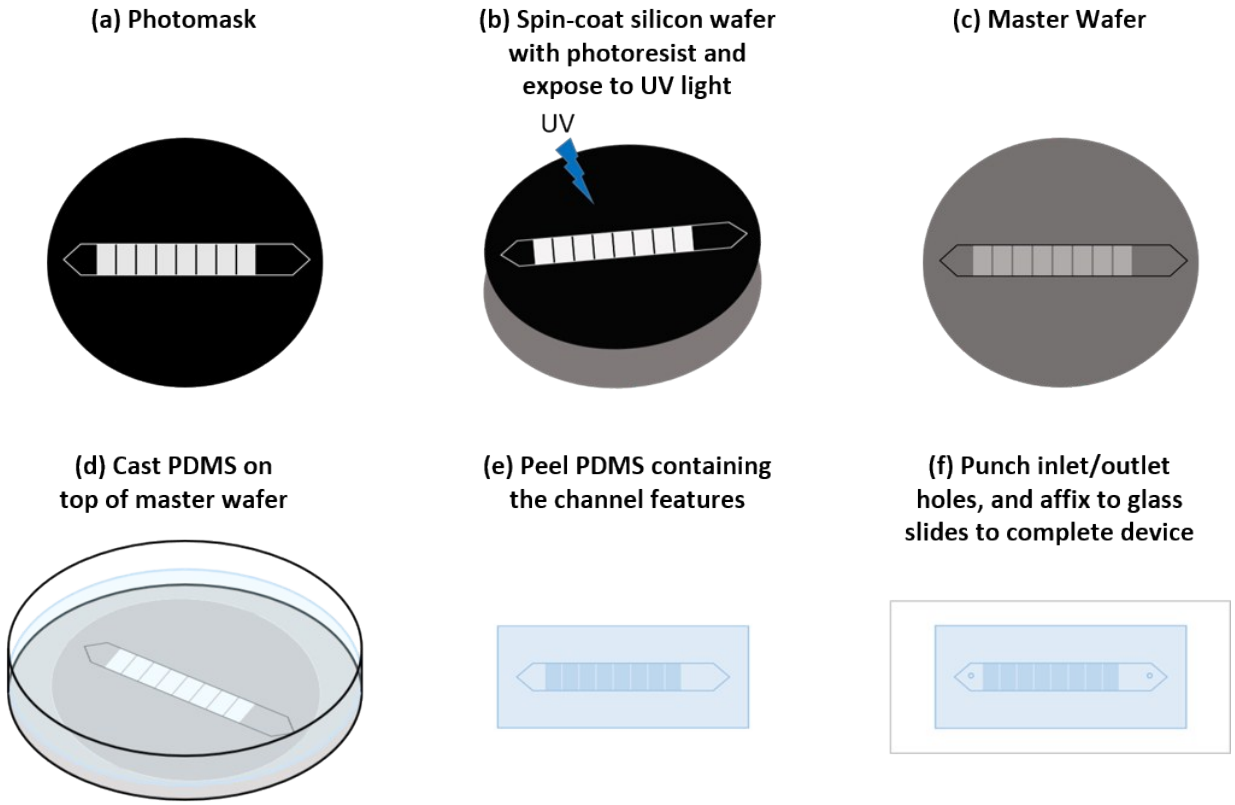
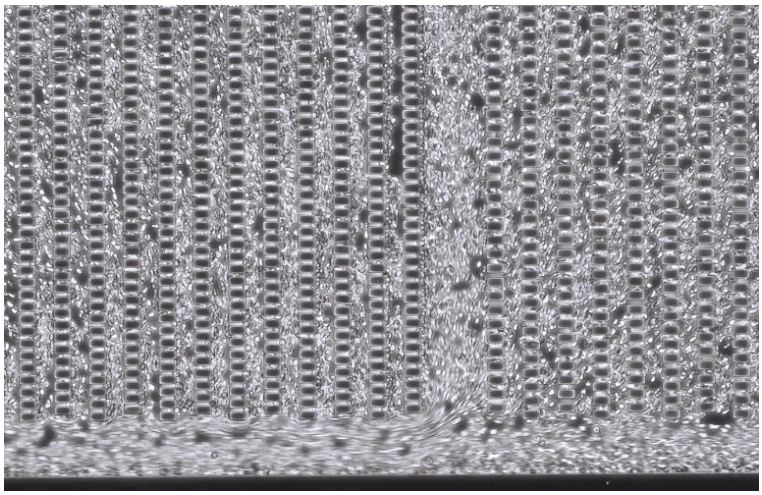
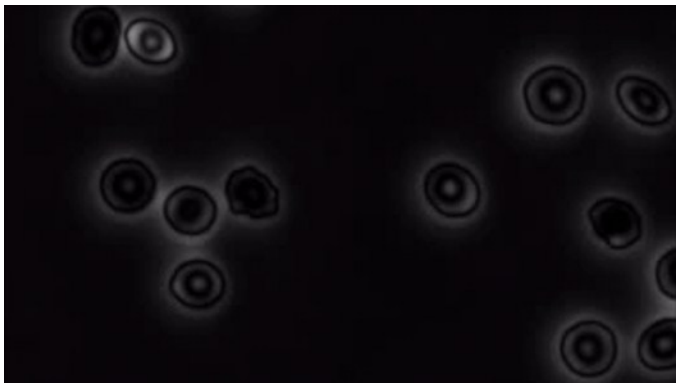


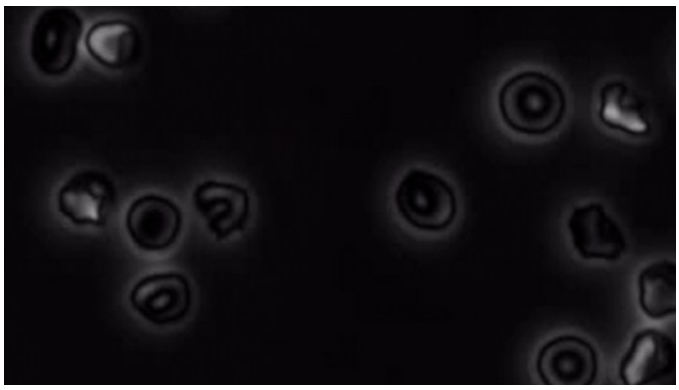
Figure S9. Wafer scale schematics showing the fabrication process of OcclusionChip. (a) A photomask with designed microchannel features was used to pattern onto a silicon wafer. (b) The wafer was spin-coated with a negative photoresist layer (SU8-2010), soft-baked, and exposed to UV light under the photomask. (c) After post-exposure baking, developing and hard baking, the master wafer was completed. (d) The wafer was covered with PDMS and then cured. (e-f) The cured PDMS block was peeled-off from the master wafer, and two holes were punched as the inlet and the outlet. After cleaned, the PDMS block was bond to a glass slide to complete the assembly of OcclusionChip.



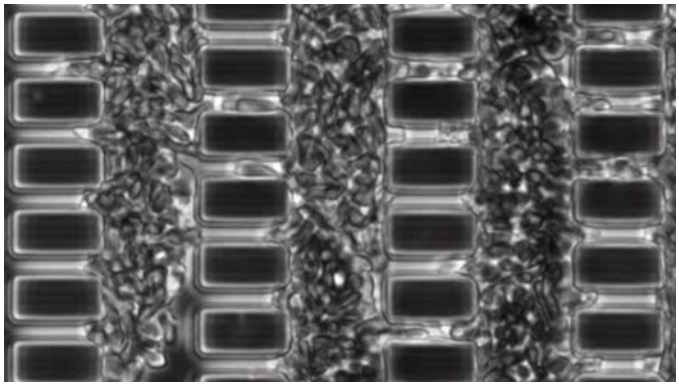
Video S1. This video demonstrates the typical flow of RBCs around occluded microcapillaries through the side microfluidic anastomosis. RBCs uniformly flow through the micropillar arrays when no occlusion is present (as shown in the 6 μm array on the right). If the microcapillary array is occluded (as shown in the near-inlet portion of the 4 μm array on the left), RBC flow diverts to the nearest anastomosis side passage preventing the full blockade of the microchannel.



Video S2. Heterogeneous morphology sickling of HbS-carrying RBCs in response to elevated oxygen tension.



Video S3. Morphology recuperation of sickled HbS-carrying RBCs under recovered oxygen condition.



Video S4. Clearance of retained HbS-carrying RBCs within the microfluidic channel in response to oxygen level recovery.