Supplemental figures

Microthrombi Preparation

Fresh porcine blood in citrated buffer was obtained (Lampire Biological Laboratories, Pipersville, PA) and used within 14 days. On the day of the experiment, blood was reconstituted with 25 mM $CaCl_2$ in type 1 borosilicate glass vials (Supelco Analytical, Bellefonte PA) and stored at room temperature for 2 h. The clotted blood was then shaken using a vial mixer (Vialmix, Lantheus Medical Imaging) for 10 sec, centrifuged at 1000 g for 5 min. The buffy coat and pellet were collected and successively passed through needles with progressively decreasing diameters (20G, 25G, 27G and 30G). The suspension containing the microthrombi was then filtered though a 200 µm pore mesh and counted (Beckman Coulter Multisizer 3, 400 µm aperture), yielding a large fraction of microthrombi in the 10-30 µm range [$(6.3 \pm 1.6)\times10^5$ clots/mL] and a tail in the 30-200 µm range [$(6.5 \pm 3.4)\times10^4$ clots/mL] (Figure S1).

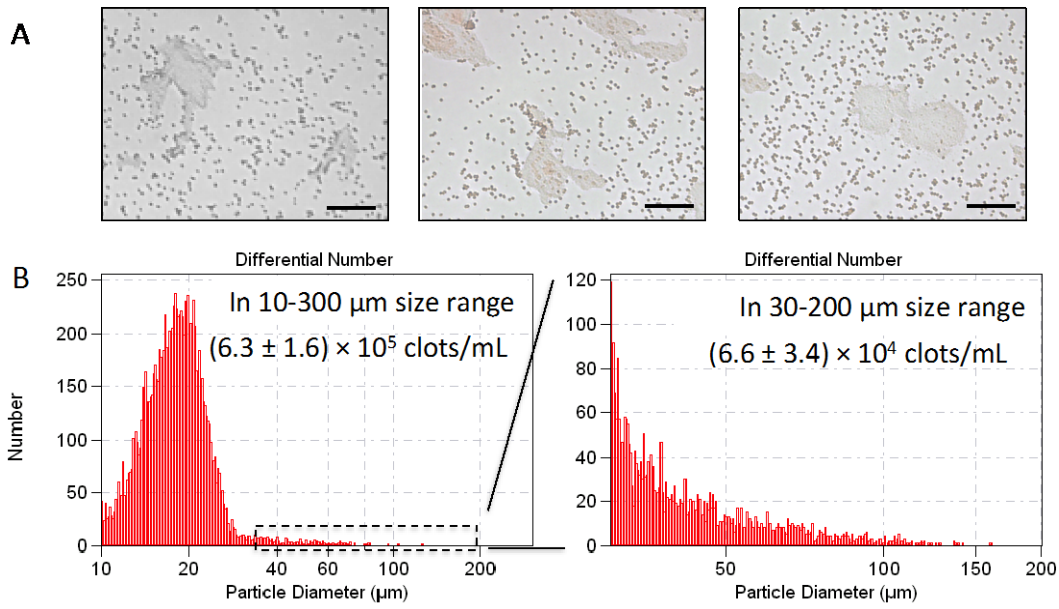

Figure S1: Characterization of microclots used to create MVO. (A) Images of clots using microscopy (bar is 100 µm). Size distribution (Coulter counter) of the clots showing (B) a peak at 20 µm in the 10-300 size range and (C) a tail containing 10% of the clot count in the 30-200 size range.

Image Analysis

A large area excluding the feeding vessels (microcirculation only) was selected and the average video intensity in the microcirculation was quantified following the burst, up until the video intensity plateaued (typically < 30 sec). It has been shown that the blood volume (A) and perfusion rate ($A \times B$) could be estimated by fitting a mono-exponential function to the kinetics of video intensity (VI) using:

$$VI(t) = A(1 - e^{-Bt}) \qquad \text{(Eq. 1)}$$

In this expression A is the maximal peak plateau video intensity and $A \times B$ is the slope of the video intensity at t=0 and is consistent with perfusion rate immediately following the burst. Typical perfusion data and a fitted model are shown Figure S2 D and E.

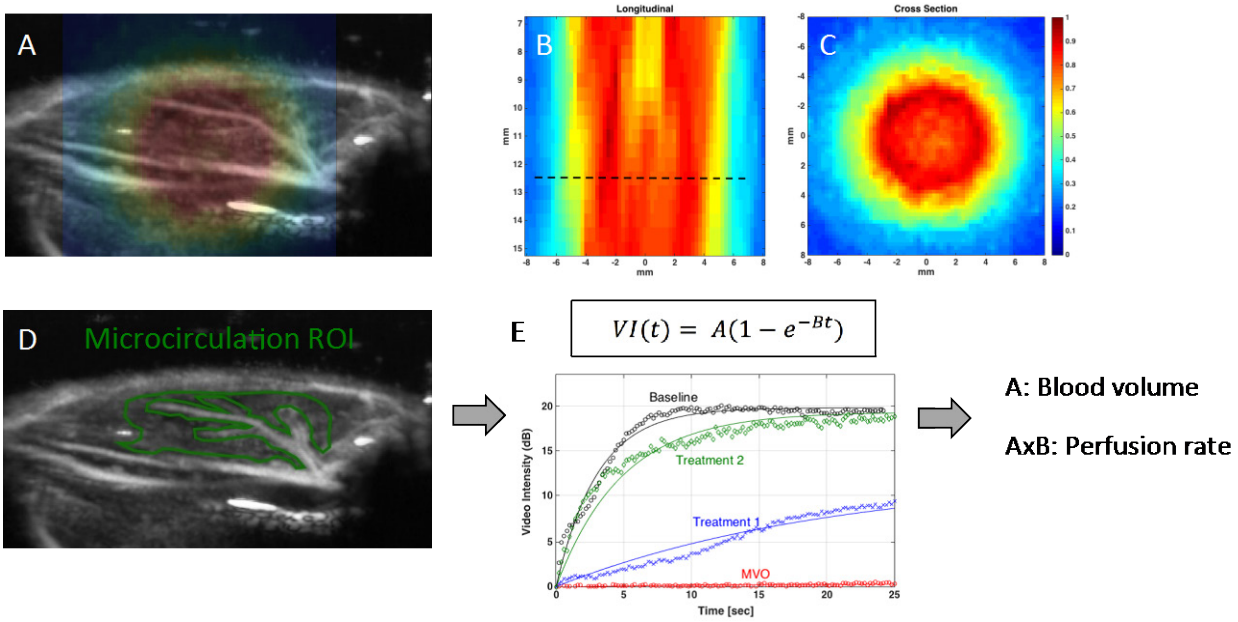

Figure S2: (A) Co-localization of CEUS imaging (grayscale) and therapeutic (color scale) ultrasound beams used for SRP theray; (B) Longitudinal and (C) cross section of the therapeutic ultrasound beams using a hydrophone; (D) Videointensity in the microcirculation was analyzed in the burst replenishment video sequences at baseline, after MVO and after 2 rounds of SRP therapy. Video intensity kinetics were curve-fitted to a mono exponential equation were A reflects the microvascular volume and $A \times B$ estimates the microvascular perfusion flow rate.

Duration of enhanced flow rate after SRP

Three rats received 2 min of therapeutic US (5,000 cycles every 3 s at 1 MHz, 1.5 MPa) during simultaneous infusion of our custom lipid MB into the femoral artery catheter via syringe pump. Contrast ultrasound burst/replenishment was performed at baseline (BL), post US (Post) and at 3 min, 15 min, 30 min, 1 h, 2 h, 3 h and 4 h post US. The flow rate ($A \times B$) is reported in Figure S3 and was still increased 4 h after treatment.

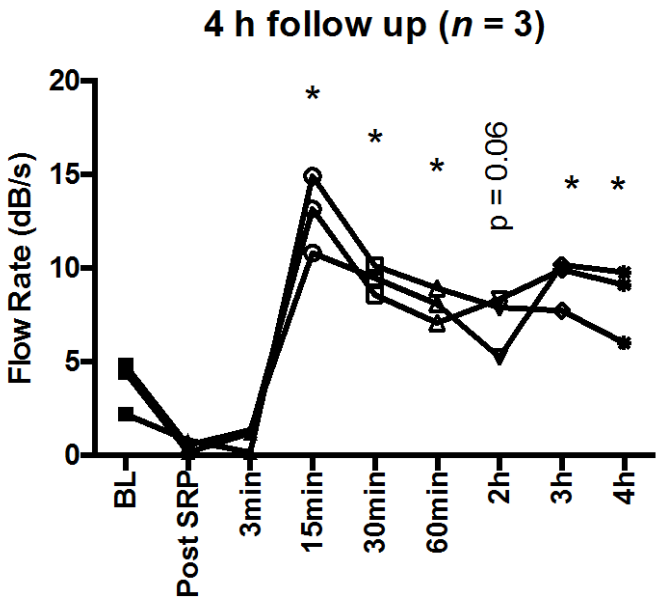

Figure S3: Hindlimb flow rate measured by burst/replenishment contrast ultrasound imaging at different time points after 2 min of SRP. Data was analyzed using a one-way analysis of variance (* $p<0.05$).

Effect of repeated burst replenishment imaging on flow rate compared to 2 min of therapeutic SRP ultrasound.

In three rats, burst/replenishment was serially applied 35 times [4 times more than the total used in our MVO model experiment (2 burst/replenishment videos per time point)]. The flow rate did not change between the average of the first 5 bursts and the average of the last 5 bursts. Additionally, in the same rats, burst/replenishment imaging was performed at 30 min, 1 h, 2 h, 3 h, and 4 h post SRP. The flow rate increased following the therapeutic pulse (1 MHz, 5000 cycles, 1.5 MPa, pulse interval of 3 s, 2 min duration) compared to before SRP. As specified in the methods, each burst consisted of 5 frames at 1.9 MI in CPS mode to burst the MB during burst/reperfusion, which was different from other publications using a diagnostic scanner for sonoreperfusion therapy such as in [2, 8, 10].

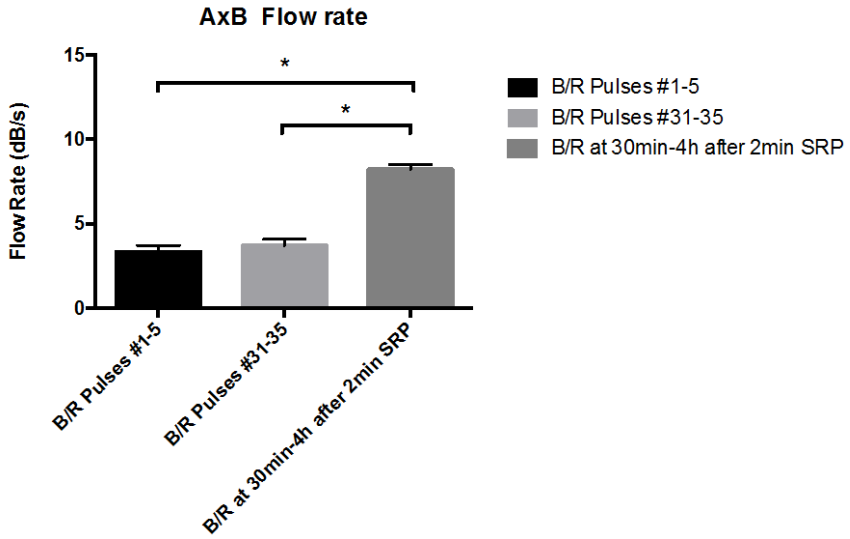

Figure S4: Flow rate measured using contrast ultrasound burst/replenishment imaging after repeated burst/replenishment (B/R) #1-5, #31-35, and in plateau between 30 min and 4 h following SRP.