Supplementary Information

Binding properties of flowing fibrin-targeted microbubbles evaluated with a thrombus-embedded microchannel

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Experimental protocols for evaluation of the binding and detachment properties of MBs.

Experimental protocols for investigation of the binding and detachment properties of targeted microbubbles (MBs) on thrombus surface are summarized as follows.

Dimensions of the thrombus-embedded microchannel

The design of thrombus-embedded microchannel is shown in Fig. 1. The dimensions of Channel (a) are 400 μm (W) x 100 μm (H) x 20 mm (L); the distance between the two inlets is 1 mm. The dimensions of Channel (b) are 500 μm (W) x 100 μm (H) x 21 mm (L).

Flow conditions for evaluation of the binding and detachment properties of MBs

Inlet_1 is connected to the syringe of the MB suspension, while Inlet_2 is connected to the syringe of the phosphate buffered saline (PBS). The MB suspensions are in the original concentration after preparation without dilution.

In the experiments for evaluation of binding properties, MB suspension is infused into the channel from Inlet_1 while the infusion of PBS from Inlet_2 is stopped. The flow rate is set to 5, 25 or 50 ul/min for different wall shear rates 100, 500 and 1000 s^{-1} at the thrombus surface, respectively. The infusion of MB suspension lasts 10 min, and images of the middle area of the channel are taken with a 40x objective at a 5-s interval in the first 5 min for the characterization of the binding properties. Then stop the infusion of MB suspension, and wait for another 5 min, so that the MBs in the channel have enough time to float to the thrombus surface on top, and the microchannel can be used in the experiment for evaluation of detachment properties of MBs immediately.

In the experiments for evaluation of detachment properties, PBS is infused into the channel from Inlet_2 while the infusion of MB suspension from Inlet_2 is stopped. The flow rates are set to 25, 50 and 100 ul/min to obtain different wall shear rates 500, 1000 and 2000 s^{-1} at the thrombus surface, respectively. The infusion of PBS lasts 5 min, and images are taken at a 5-s interval for the characterization of the detachment properties.

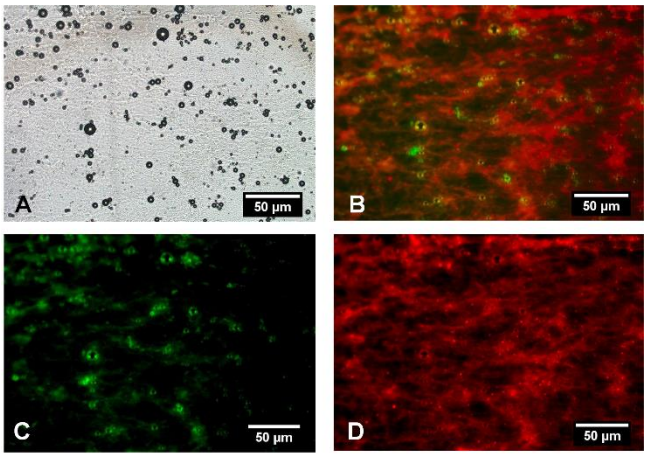

Figure S1 Bright-field and corresponding fluorescent microscopic images of the CREKA-modified MBs adhered on the thrombus surface. (A) Bright field image. (B) Composite fluorescent image of the green and the red channels. (C) Green-channel fluorescent image of the FITC-labelled CREKA. (D) Red-channel fluorescent image of the ALEXA-594-labeled fibrin network of the embedded thrombus.

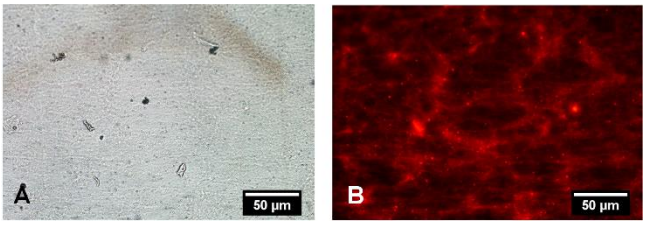

Figure S2 Bright-field and corresponding fluorescent microscopic image of the thrombus surface after the continuous infusion of the plain SonoVue MBs. (A) Bright-field image of the thrombus surface. (B) Fluorescent image of the fibrin network dyed red.

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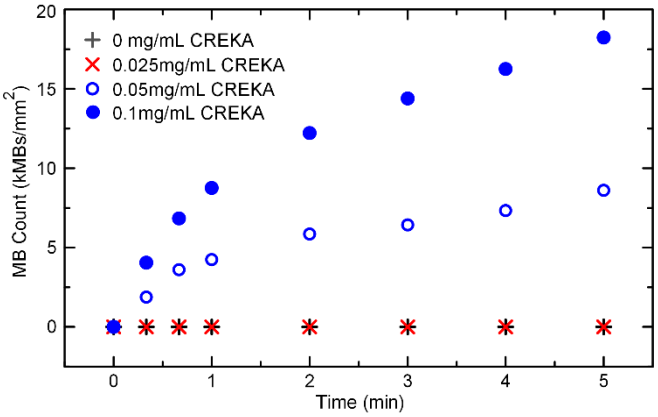

Fig. S3 Effect of the concentration of CREKA on the binding capacity of flowing MBs with wall shear rate of 500 s^{-1}. Variation of the number of adhered MBs with time for the SonoVue MBs treated with 0, 0.025, 0.05 and 0.1 mg/mL CREKA are compared. The number of MBs is characterized with the count of MBs per field in the unit of 1×10^3 MBs/mm^2; while for the concentration of 0.1 mg/mL CREKA, the MB count, n, is estimated with $n = 4k/\pi d^2$, where k is the area fraction (%) of the thrombus surface covered with adhered MBs, d is the average diameter (mm) of a single MB. For the CREKA-modified SonoVue MBs, there is d = 3.34 μm.

Supplementary Movies

Movie S1 The suspension of plain SonoVue MBs flows in the thrombus-embedded microchannel, where the top wall, i.e. the thrombus surface, is observed. The movie corresponds to the plot of 0 mg/mL CREKA in Fig. 3B. The playback speed of the movie is 25x the original speed, while the image size is 220 μm × 150 μm.

Movie S2 Movie of Fig. 3C. The suspension of SonoVue MBs modified with 0.05 mg/mL CREKA flows in the thrombus-embedded microchannel, where the top wall, i.e. the thrombus surface, is observed. The movie corresponds to the plot of 0.05 mg/mL CREKA in Fig. 3B. The playback speed of the movie is 25x the original speed, while the image size is 220 μm × 150 μm.

Movie S3 Movie of Fig. 3E. The suspension of SonoVue MBs modified with 0.1 mg/mL CREKA flows in the thrombus-embedded microchannel, where the top wall, i.e. the thrombus surface, is observed. The movie corresponds to the plot of in Fig. 3D. The playback speed of the movie is 25x the original speed, while the image size is 220 μm × 150 μm.