

## Supporting Information

### Site-Specific Thrombus Formation: Advancements in Photothrombosis-on-a-Chip Technology

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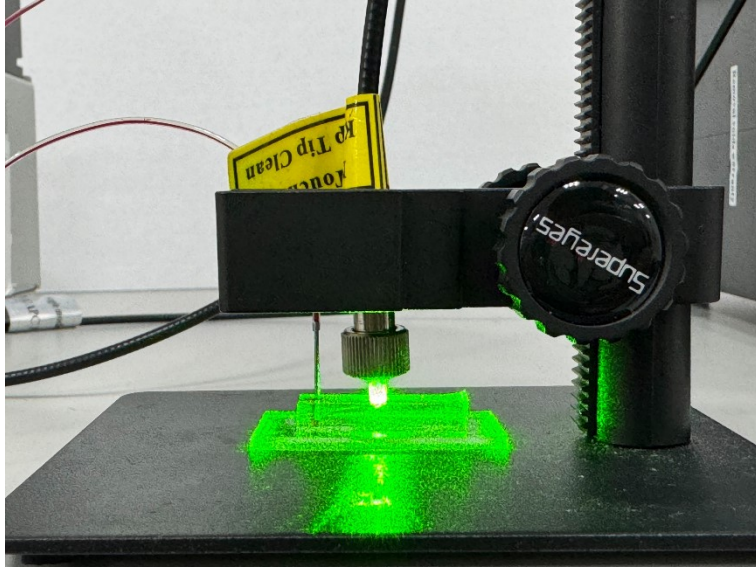
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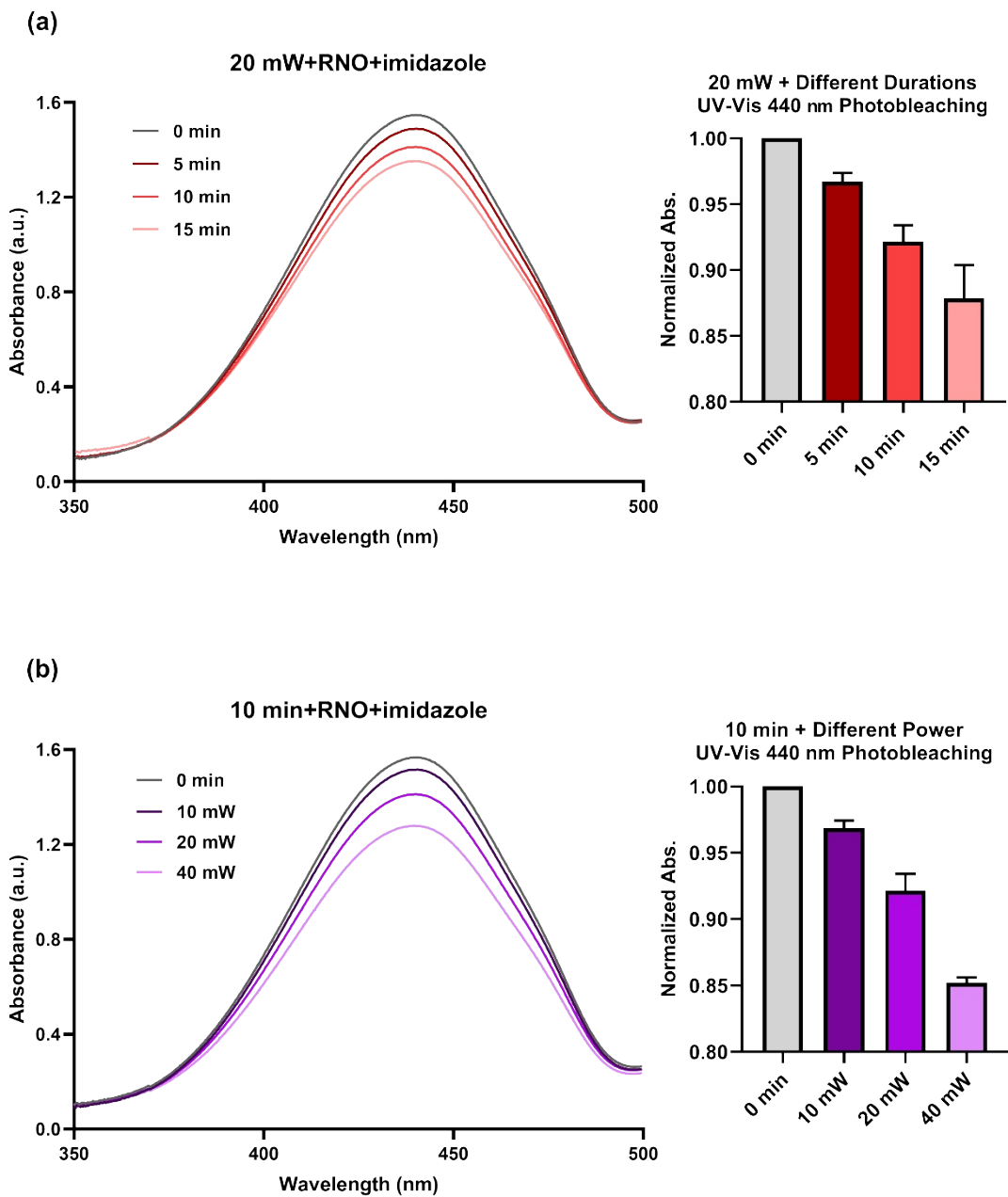
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**Keywords:** photothrombosis, thrombosis vessel-on-a-chip, animal use alternatives (3Rs), thrombosis therapy development



**Fig. S1.** Laser exposing apparatus.

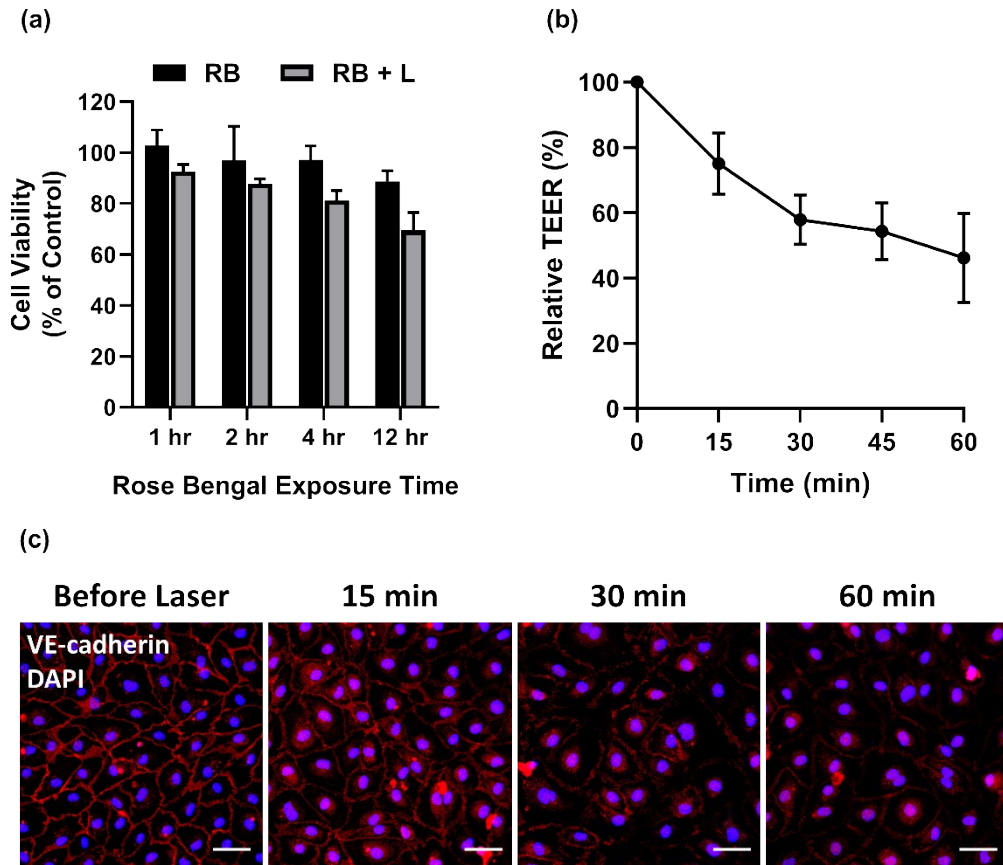


**Fig. S2.** Investigation of ROS generation using the RNO bleaching (RNO-imidazole) method.

Includes UV-vis absorption spectra and normalized absorption at 440 nm.

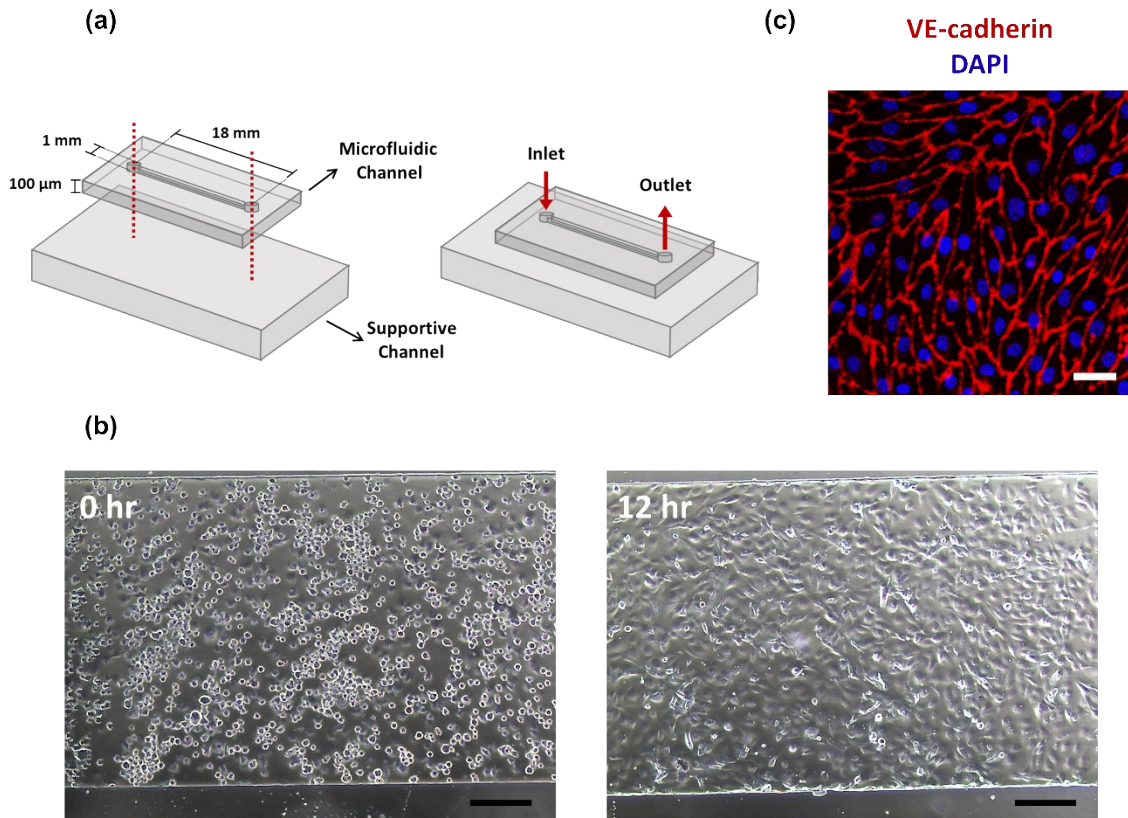
(a) Laser intensity kept at 20 mW with varying laser durations.

(b) Laser duration kept at 10 mins with varying laser intensities.



**Fig. S3.** The effect of photothrombosis on seeded HUVECs.

- (a) Evaluation of cytotoxicity of Rose Bengal and laser exposure using the CCK-8 assay. (n = 4)
- (b) Measurement of relative TEER value in HUVECs in a transwell culture insert model after photothrombosis.
- (c) Immunofluorescence staining of HUVECs with VE-cadherin (red) and DAPI (blue) post-photothrombosis. (Scale bars: 50  $\mu$ m)



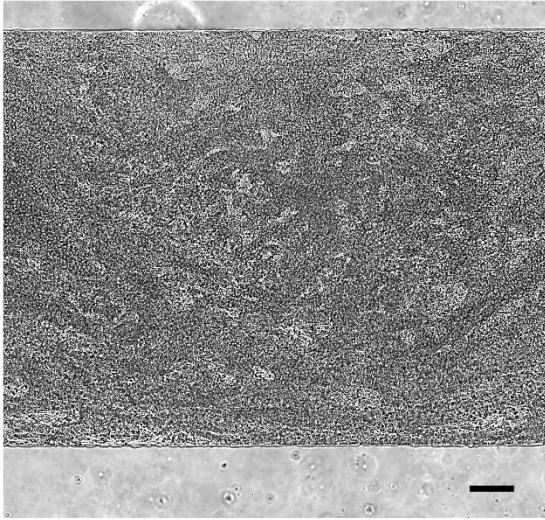
**Fig. S4.** Endothelialization of vessel-on-a-chip.

(a) Construction of the microfluidic device.

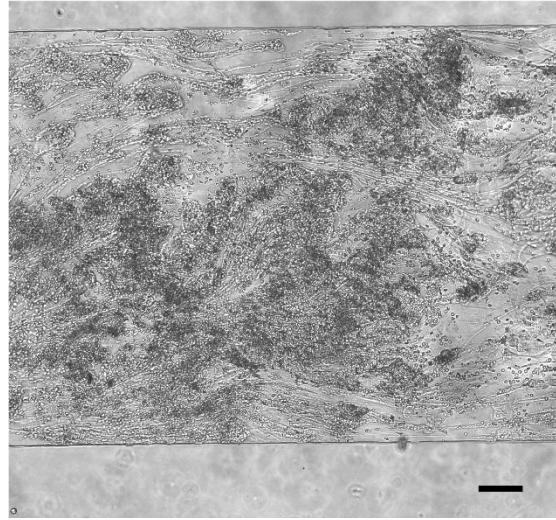
(b) Seeding of HUVECs in the microfluidic channel at 0 hr and 12 hr. (Scale bars: 200 μm)

(c) Immunofluorescence staining of human umbilical vascular endothelial cells (HUVECs) with VE-cadherin (red) and DAPI (blue). (Scale bars: 25 μm)

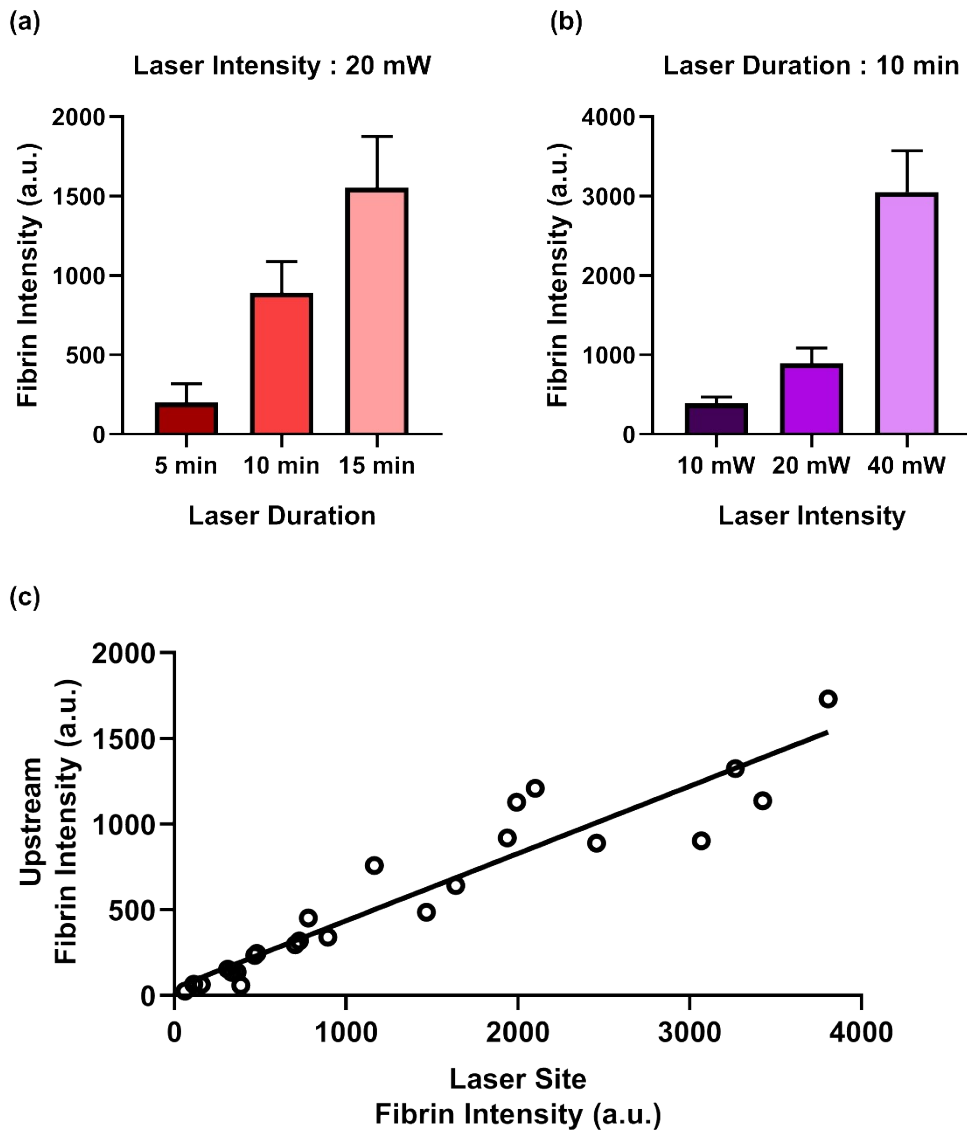
**Before PBS wash**



**After PBS wash**



**Fig. S5.** Photothrombosis-on-a-chip images with and without PBS wash.

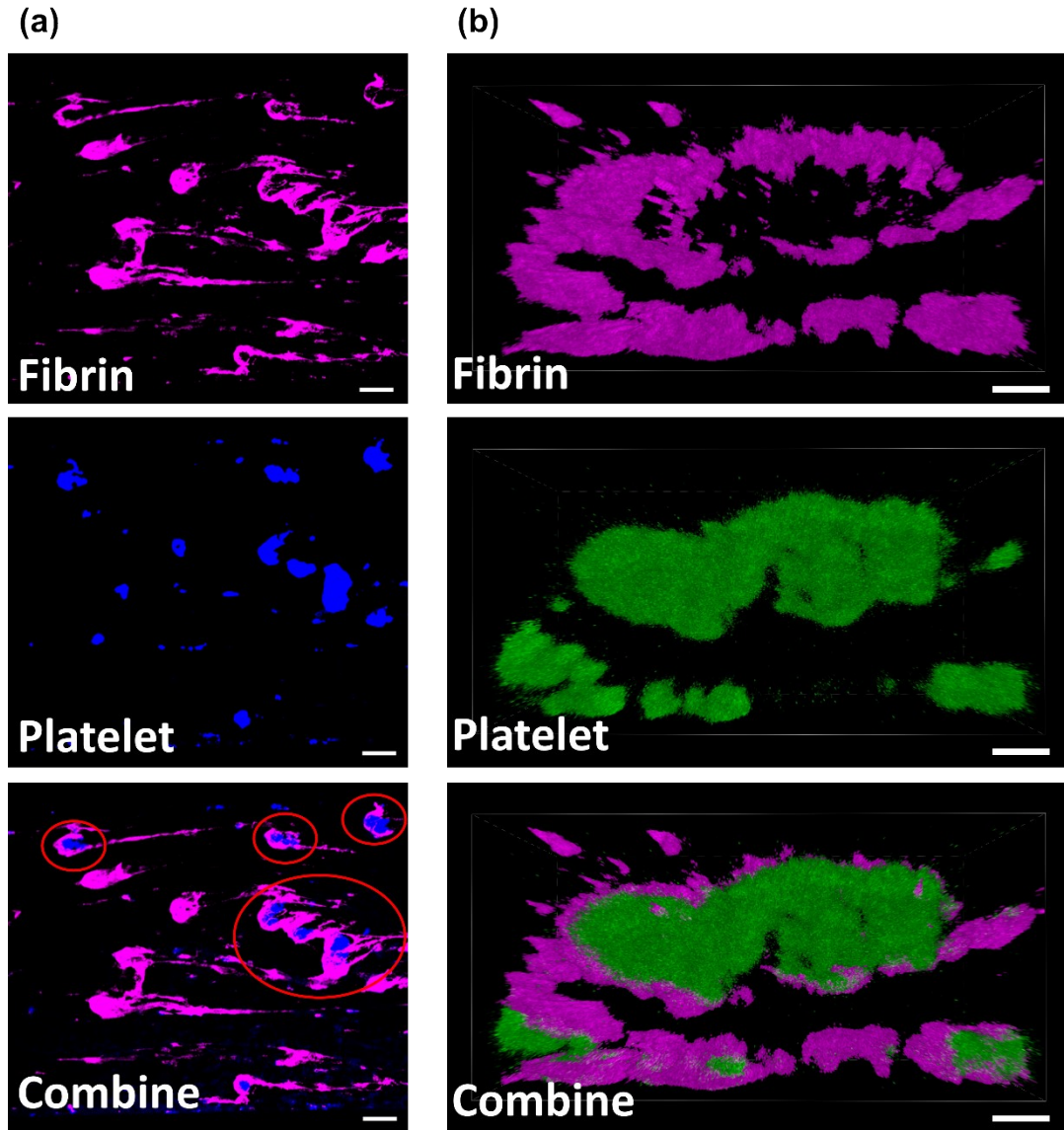


**Fig. S6.** Photothrombosis-on-a-chip analysis of fibrin fluorescence intensity.

(a) Fibrin fluorescence intensity at the laser site with laser intensity maintained at 20 mW and varying laser durations.

(b) Fibrin fluorescence intensity at the laser site with laser duration fixed at 10 min and varying laser intensities.

(c) Semi-quantitative analysis of Cy5-fibrin fluorescence at upstream and laser sites, comparing different laser settings.

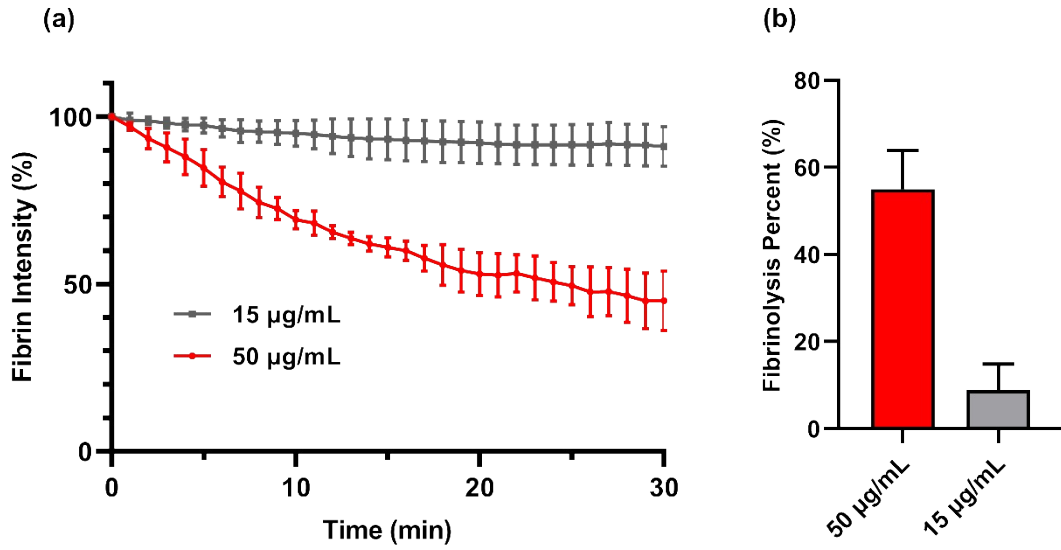


**Fig. S7.** Investigation of thrombus developing stages by CLSM images.

(a) CLSM images of Cy5-fibrin and FITC-platelets highlight areas where the thrombus formation process is particularly evident. (Scale bars: 100  $\mu\text{m}$ )

(b) 3D reconstruction images of Cy5-fibrin and FITC-platelets viewed from bottom to top. (Scale bars: 20  $\mu\text{m}$ )





**Fig. S8.** Comparison of fibrinolysis induced by different rt-PA concentrations (50, 15 µg/mL).

(a) Semi-quantitative analysis of fluorescence Cy5-fibrin intensity over time.

(b) Quantification of fibrinolysis extent.