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Supplementary Materials for

Accelerating thrombolysis using a precision and clot-penetrating drug delivery strategy by nanoparticle-shelled microbubbles

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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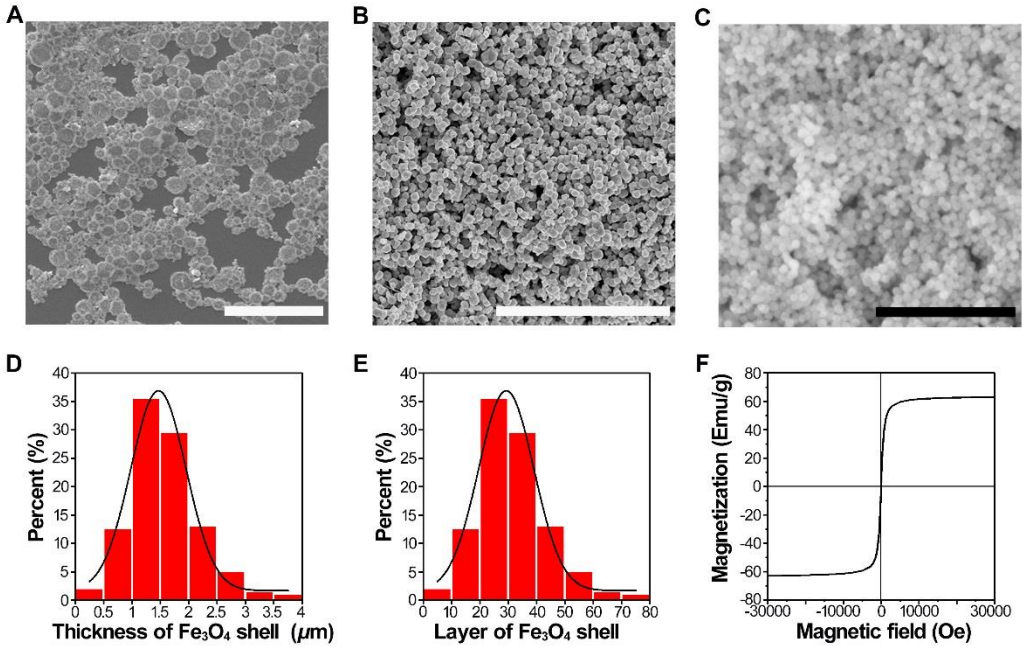

Figure S1. Characterization of the structure and the shell thickness of the MMB-SiO_2-tPA by the environmental scanning electron microscope (ESEM). (**A**) Representative ESEM image of MMB-SiO_2-tPA at SE mode. Scale bar : 100 μm. (**B**) Scanning electron microscopy (SEM) image of Fe_3O_4 nanoparticles used for the fabrication of MMB-SiO_2-tPA. Scale bar : 5 μm. (**C**) Scanning electron microscopy (SEM) image of SiO_2 nanoparticles used for the fabrication of MMB-SiO_2-tPA. Scale bar : 500 nm (**D**) The thickness distribution of the nanoparticle shell of the MMB-SiO_2-tPA, n = 200. (**E**) The calculated number of nanoparticle layers of the MMB-SiO_2-tPA, n = 200. (**F**) The corresponding magnetic hysteresis loops at T = 300 K of iron oxide nanoparticles used for fabrication of MMB-SiO_2-tPA.

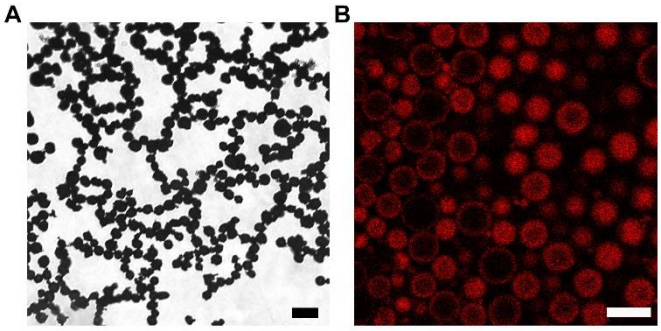

Figure S2. Bright field and confocal microscope images of MMB-SiO_2-tPA. (**A**) Representative bright-field microscopy image of MMB-SiO_2-tPA. Scale bar: 20 μm. (**B**) Representative laser scanning confocal microscopy image of MMB-SiO_2-Cy5.5. Scale bar : 10 μm.

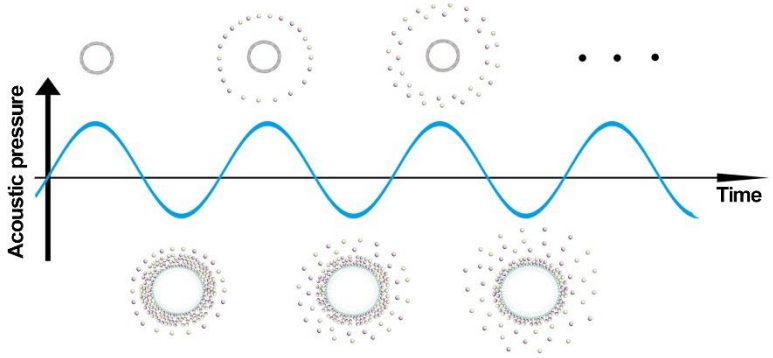

Figure. S3. Schematic illustration of nanoparticle re-assembling and release process under stable microbubble oscillations.

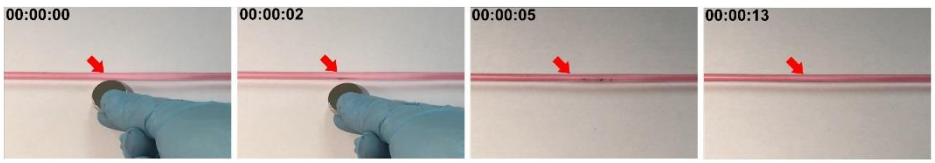

Figure S4. Magnetic responsiveness and enrichment capacity of MMB-SiO_2-tPA in an *in vitro* vessel system that mimicking blood flow condition.

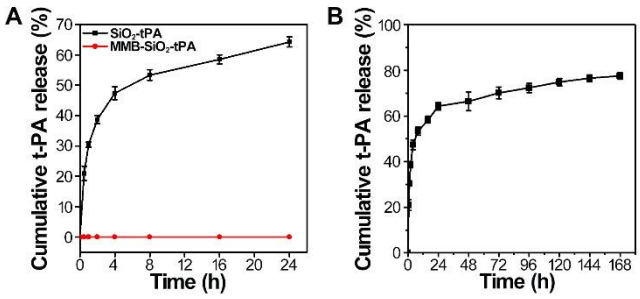

Figure S5. ***In vitro*** **cumulative release profile of tPA from SiO_2-tPA nanoparticles.** (**A**) tPA release profile from SiO_2-tPA nanoparticle and MMB-SiO_2-tPA (without ultrasound) in 24 h. (**B**) tPA release profile from SiO_2-tPA nanoparticles in 7 days.

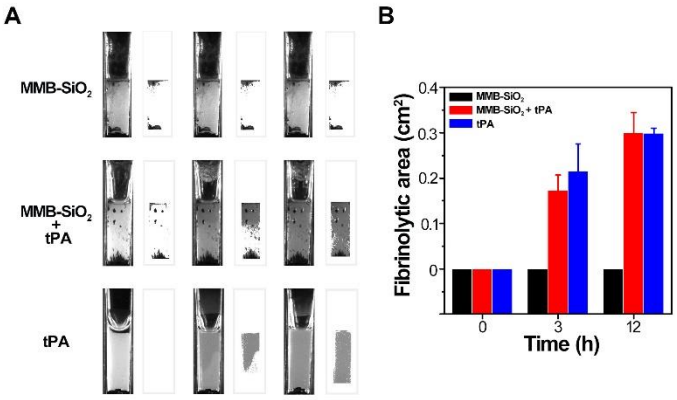

Figure S6. ***In vitro*** **fibrin-lysis assay by a vertical-channel gel system composed of agarose-fibrin.** (**A**) Representative photographs of the fibrinolytic process of the agarose-fibrin gel incubated with MMB-SiO_2, MMB-SiO_2 + tPA, native tPA at different thrombolysis times, respectively. **(B)** Quantification of fibrinolytic area of fibrin over time incubated with MMB-SiO_2, MMB-SiO_2 + tPA, native tPA.

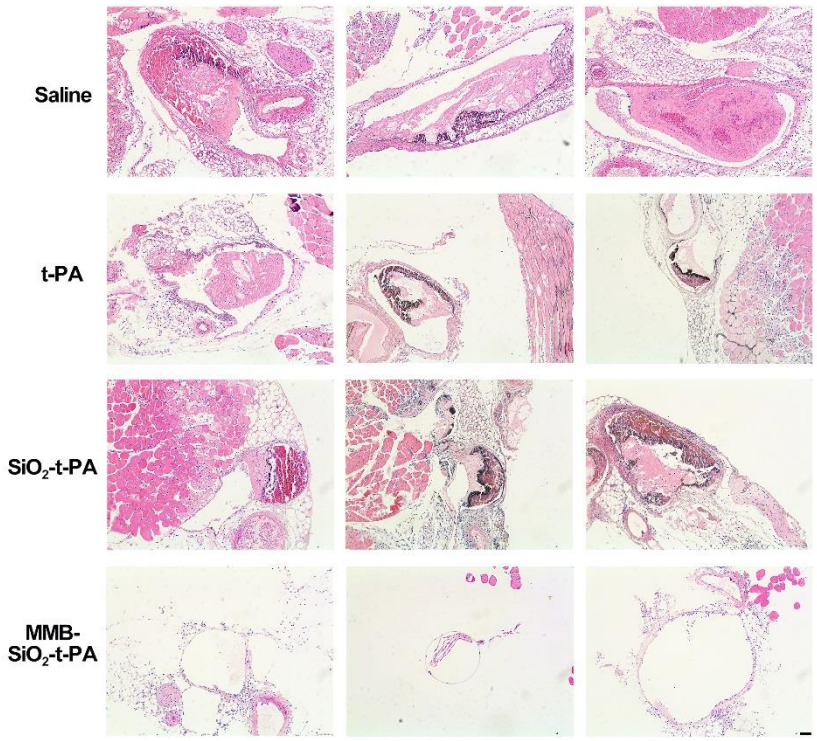

Figure S7. Histological analysis of the femoral vein after treatment with saline, native tPA, SiO_2-tPA, and MMB-SiO_2-tPA for 12 h, respectively. The scale bar represents 50 μm.

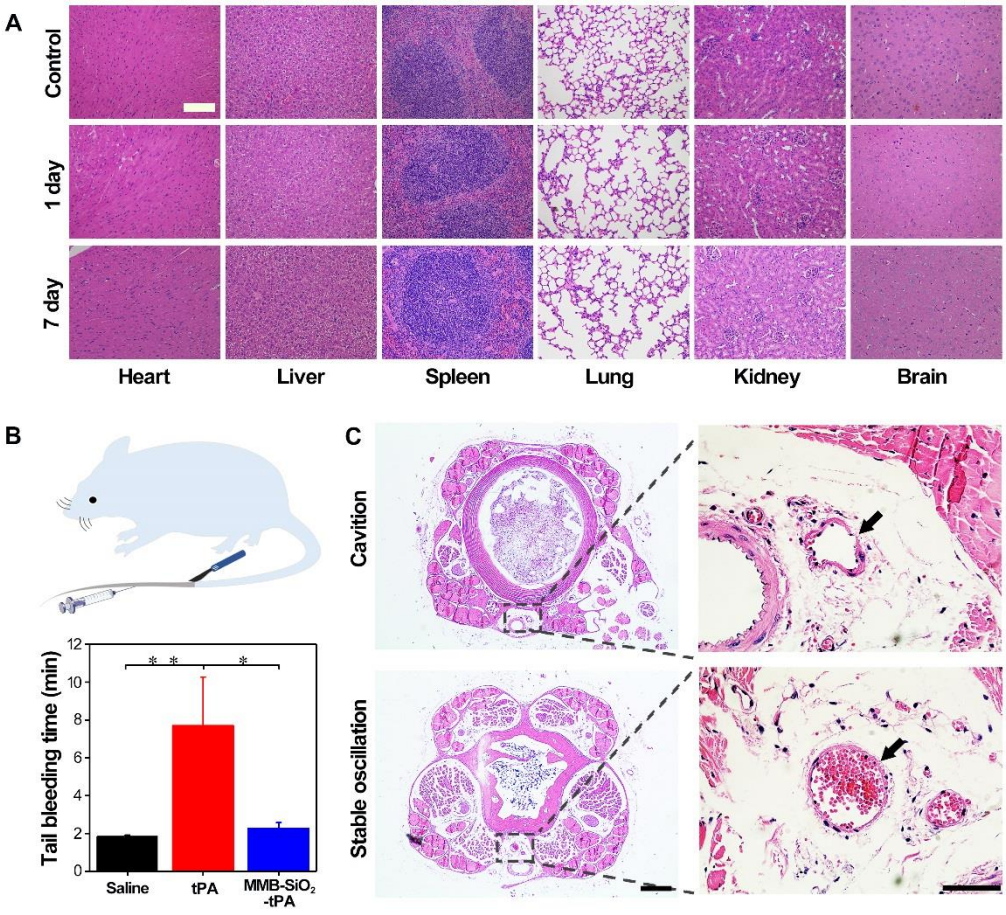

Figure S8. Safety assessment of the precision delivery strategy. (**A**) Histological analysis of major organs (heart, liver, spleen, lung, kidney, and brain) after intravenous injection of MMB-SiO_2-tPA for 1 and 7 days. Scale bar: 100 μm. (**B**) Schematic illustration of the tail bleeding time experiment (top). To assess the systemic off-target effect on the hemostatic capability of injected tPA, the tail bleeding time (bottom) after injection with saline, native tPA, MMB-SiO_2-tPA were quantified (n = 3, *P < 0.05, **P<0.01). (**C**) Representative histological analysis of the integrity of the tail vein treated by MMB-SiO_2-tPA injection and followed by low-intensity or high-intensity ultrasound. Black dotted frames indicate the enlarged area of the tail vein and black arrow indicate the vascular wall. Left scale bar: 100 μm; right scale bar: 50 μm.

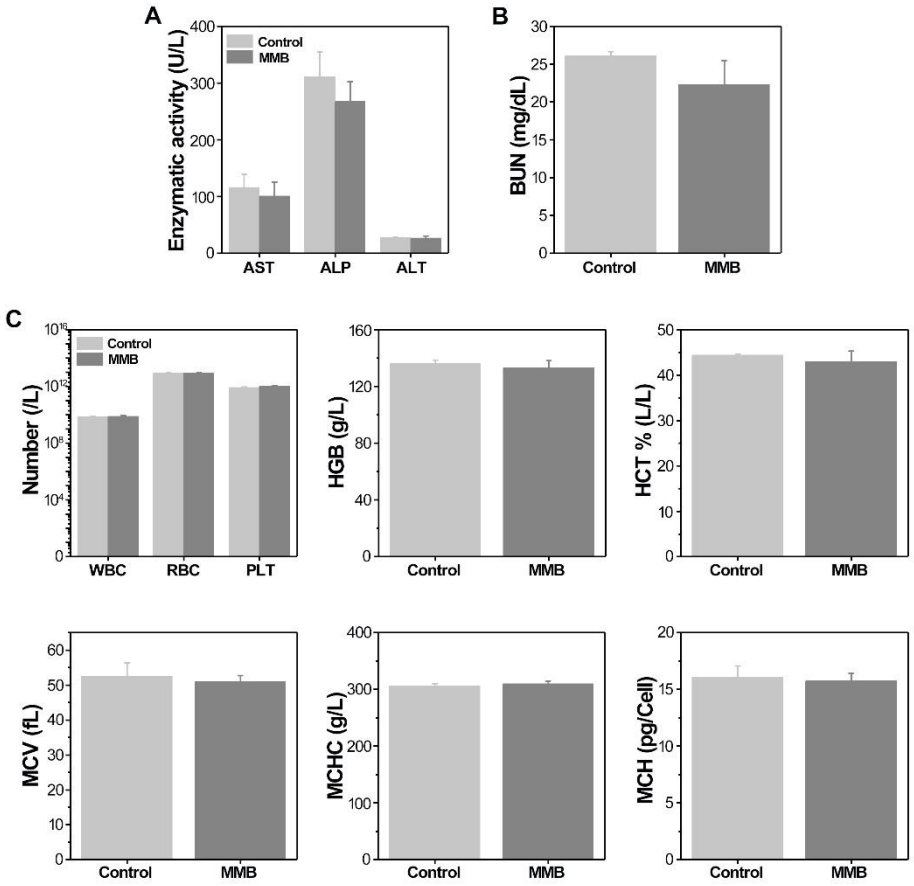

Figure S9. **Serum biochemistry assay and complete blood panel test.** Healthy C57/BL6J mice with intravenous injection of 100 μL of MMB-SiO_2-tPA were sacrificed at 7 days post-injection for blood collection. Untreated healthy mice were used as the control group. (**A**) Liver function markers: alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST). (**B**) Blood urea nitrogen (BUN) levels. (**C**) Blood levels of white blood cells (WBC), red blood cells (RBC), platelets (PLT) hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) of MMB-SiO_2-tPA treated mice (MMB) and untreated mice (control). Statistic was based on triplicate samples.

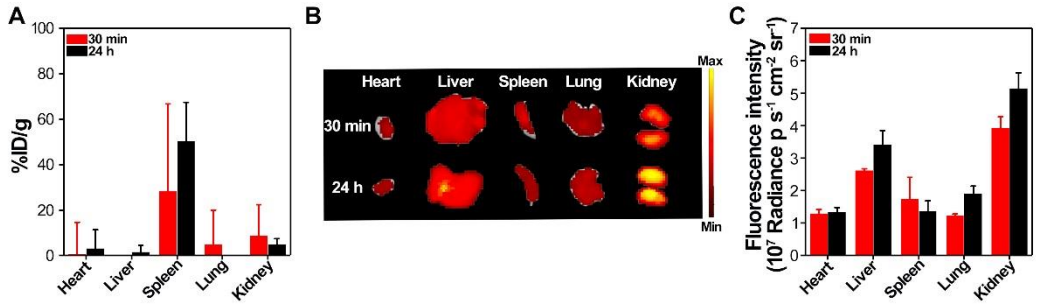

Figure S10. Distribution and clearance of the MMB-SiO_2-tPA in vivo. (**A**) Quantitation analysis of the distribution of iron oxide nanoparticles in the major organs (including heart, liver, spleen, lung, and kidney) of healthy C57BL/6J mice at 30 min and 24 hours after intravenous injection by ICP-OES (Fe). (**B**) Distribution of fluorescence labeled SiO_2-tPA nanoparticle in the major organs (including heart, liver, spleen, lung, and kidney) of healthy C57BL/6J mice at the 30 min and 24 hours after intravenous injection. (C) Quantification of the fluorescence intensity of the labeled SiO_2 nanoparticles in major organs at the 30 min and 24 hours post injection, respectively.

SUPPLEMENTARY MATERIALS AND METHODS

Observation of re-assembling of nanoparticles at the interface of a microbubble under stable oscillation

To visualize the re-assembling process of shelled nanoparticles at the interface, a microbubble (80 μm) injected within a microfluidic channel was activated by a driving frequency swept from 40-50 kHz (1.5 V and 10% gain) for 100 ms exposure time per cycle. The ultrasound was applied through a piezo transducer attached to the glass slide of the microfluidic channel. The movement of the shelled fluorescent silica nanoparticles was recorded using a CCD camera (Sensicam QE, Kelheim, Germany).

Thrombolytic drug release from SiO_2-tPA

In order to reduce the experimental analysis error, the experiment had 3 parallel samples. 2 mg SiO_2-tPA was incubated with 1 mL PBS in tube. Then, the tube was placed in a shaking bath with a speed of 250 shakes per min at 37 °C for *in vitro* drug release. At different time points, samples were centrifuged at 8000 rpm for 5 min, followed by replacement of 1 mL of supernatants with fresh pre-warmed (37 °C) PBS. The amount of released tPA was determined by the BCA protein assay kit.

Magnetic targeting

MMB-SiO_2-tPA was dissolved in DMEM solution. Then the resulted solution was injected into the vessel from a syringe at the speed of 1.2 cm/s. A magnet was placed adjacent to the vessel to enrich the MMB-SiO_2-tPA and video was recorded.

In vivo toxicity assessment of MMB-SiO_2-tPA

Six male C57/BL6J mice (8 weeks old were treated with 100 μL MMB-SiO_2-tPA and saline, respectively. After 7 days, the mice were euthanatized and the blood was collected for serum biochemistry analysis and whole blood cell test, and measurements of renal and liver function, respectively.

In vivo distribution and clearance of MMB-SiO_2-tPA

To study the biodistribution and clearance of MMB-SiO_2-tPA in the mouse model, 100 μL of MMB-SiO_2-tPA (microbubble number of 1.5×10^5) were intravenous injected into C57/BL6J mice. After 30 min and 24 hours, the main organs were collected. The fluorescence labeled SiO_2-tPA was quantified by fluorescence imaging, while the iron oxide were quantified by ICP-OES (Fe).