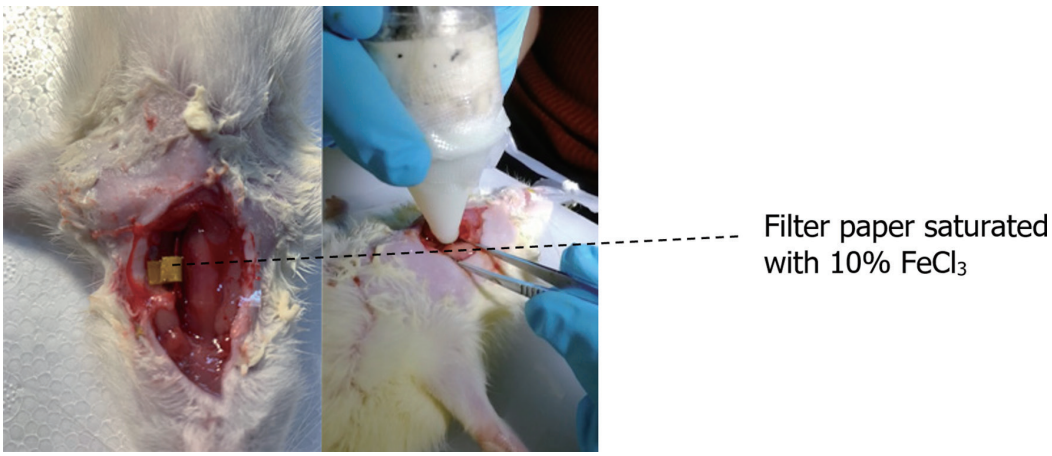

Figure S1 The establishment of a rat model of platelet-rich thrombi. The rat carotid artery was exposed to 10% ferric chloride ($FeCl_3$) for 10 minutes. A piece of filter paper (1 cm × 1 cm) saturated with 10% $FeCl_3$ solution was placed between the carotid artery and a plastic sheeting to form thrombus. 1 MHz therapeutic ultrasound was delivered from a single-element transducer (A302S) for 20 minutes.

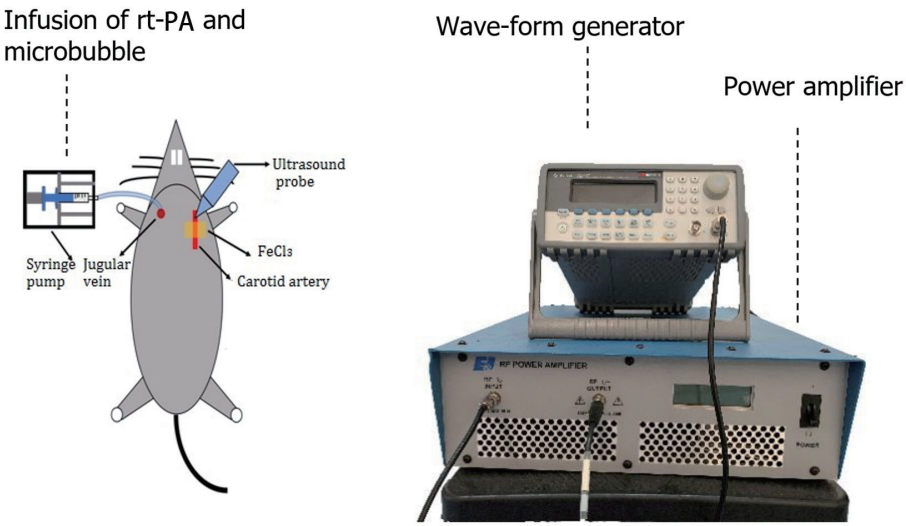

Figure S2 Long-pulsed ultrasound enhanced microbubble mediated recombinant tissue plasminogen activator (rt-PA) thrombolysis. Recombinant tissue plasminogen activator and microbubble were infused through the right external jugular vein of the rats. Therapeutic ultrasound (1 MHz, 0.6 MPa) with 1,000 μs pulse-length was delivered from an arbitrary function generator (33250A) and intensified by a power amplifier (EI 2200L). $FeCl_3$, ferric chloride.

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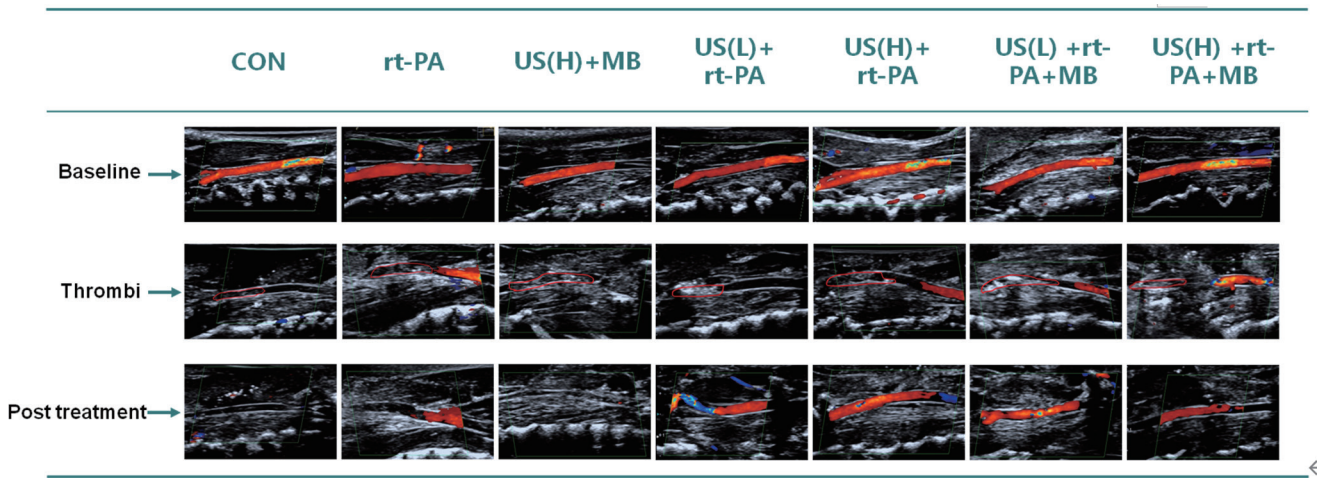

Figure S3 Pulse-wave Doppler images of rats' carotid artery at three different stages (baseline, thrombi, thrombolysis). n=6 for each group. CON, control; rt-PA, recombinant tissue plasminogen activator; US, ultrasound; US(H), high duty-cycle (10%) ultrasound; US(L), low duty-cycle (0.03%) ultrasound; MB, microbubble.

Table S1 The peak systolic velocity (PSV) and resistance index (RI) at baseline stage in different groups before thrombi formation. Data are expressed as the mean ± standard deviation (n=6 for each group)

Index/Group	CON	rt-PA	US(H) + MB	US(L) + rt-PA	US(H) + rt-PA	US(L) + rt-PA + MB	US(H) + rt-PA + MB	P value
Peak systolic velocity (cm/s)	59.08±13.70	56.77±2.90	47.38±6.57	57.88±17.32	57.08±12.15	41.73±9.23	48.06±15.77	0.171
Resistance index	0.80±0.04	0.80±0.04	0.89±0.09	0.83±0.04	0.86±0.04	0.81±0.06	0.83± 0.06	0.053

CON, control; rt-PA, recombinant tissue plasminogen activator; US, ultrasound; US(H), high duty-cycle (10%) ultrasound; US(L), low duty-cycle (0.03%) ultrasound; MB, microbubble.

Table S2 Recanalization grading after thrombolytic therapies

Recanalization grading	CON	rt-PA	US(H) + MB	US(L) + rt-PA	US(H) + rt-PA	US(L) + rt-PA + MB	US(H) + rt-PA + MB
0	6	6	6	2	2	1	–
I	–	–	–	2	–	1	–
II	–	–	–	2	4	4	6

No recanalization was observed in control, recombinant tissue plasminogen activator and ultrasound+ microbubble groups. Using long pulsed ultrasound (1,000 μs) and recombinant tissue plasminogen activator (1 mg/mL), high duty-cycle ultrasound caused 4/6 grade II for recanalization. When microbubble was added, high duty-cycle ultrasound with recombinant tissue plasminogen activator successfully achieved all grade II of recanalization. CON, control; rt-PA, recombinant tissue plasminogen activator; US, ultrasound; US(H), high duty-cycle (10%) ultrasound; US(L), low duty-cycle (0.03%) ultrasound; MB, microbubble.

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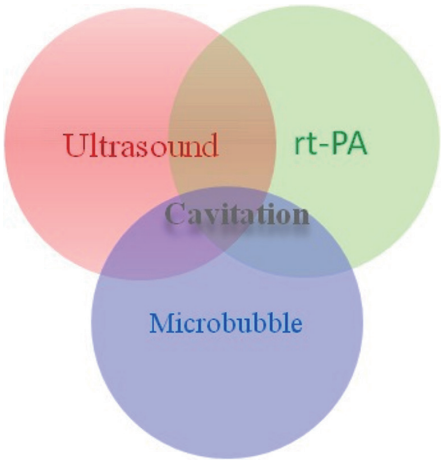

Figure S4 Synergistic effects of ultrasound, recombinant tissue plasminogen activator (rt-PA) and microbubbles through the cavitation mechanism.

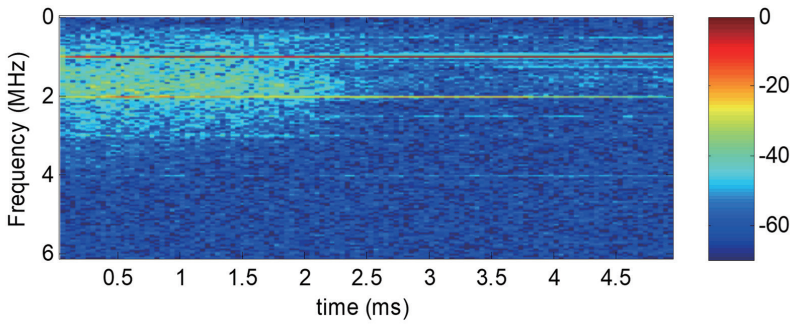

Figure S5 The passive detection of microbubble cavitation during the sonothrombolysis. A 2.25 MHz transducer, confocally aligned with the treatment transducer focal territory, was used to passively detect scattered energy. The signal was amplified by 10 dB and digitized by an oscilloscope at 50 MHz sampling frequency. A time frequency analysis was performed over the pulse duration, using a 100 μs sliding window with 60% overlapping. And the frequency content of the received signal at each time point is displayed as a function of time.

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