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Measurement of Thrombolysis Enhanced by EkoSonic Catheter-Based Endovascular Therapy

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***Abstract*—To date, there have been no studies that use ultrasound imaging tools to measure the spatial extent of thrombolysis from catheter-based treatments. This study uses contrast-enhanced ultrasound imaging to measure and visualize plasma clot lysis by either an EkoSonic endovascular system or a conventional side-hole drug delivery catheter. Ultrasound enhances drug-mediated thrombolytic therapy by 80-350%; the degree of enhancement depends on the drug delivery strategy, and the presence or absence of plasma flow through the system during therapy. The measurements and visualizations give a more detailed picture of the lysis process and may inform future strategies for ultrasound-assisted catheter-based intervention.**

Keywords— thrombolysis, catheter, ultrasound, clot, imaging

I. Introduction

Ultrasound (US) imaging is a valuable tool to visualize and measure the underlying anatomy and physiology indicative of vascular pathology. Imaging can identify the location and severity of thrombosis, and it can monitor interventions. It has been widely used for guidance in HIFU-based therapies [1]–[5].

While catheter-based thrombolytic intervention is widely used clinically, there have been no US imaging studies to assess its efficacy. Reported measurements to evaluate thrombolysis treatment include clot mass [6]–[8]; microscopic analysis of fibrin structure [9], [10]; right-to-left ventricle diameter ratio [11]; and fibrin degradation products such as labeled fibrinogen [12], [13] or D-dimer proteins [10], [14], [15]. Contrast-enhanced imaging can reveal the extent of thrombus occlusions, and because US-assisted treatment relies on both local thrombolytic drug delivery as well as a US field, imaging can evaluate both the impact of US on thrombolytic therapy and the uniformity of lysis throughout the volume of the clot. Imaging clots that undergo this intervention may provide a better understanding of the thrombolysis process and assist with design enhancements and strategies to improve patient outcomes, specifically with the aim of achieving greater recanalization in less time and with less thrombolytic drug.

This study employs US imaging to compare the outcomes of thrombolytic therapy using a conventional side-hole drug delivery catheter to that using the EkoSonic endovascular system.

II. Materials and Methods

A. Experimental Overview

The experiments included three sets of conditions and three types of therapy (saline, lytic, lytic + US). The experiments are outlined in Table 1, and the sets are described below. The experimental procedure is diagrammed in Fig. 1.

- Set 1: slow lytic infusion; no plasma flow. 6.7 mL of thrombolytic drug—tissue plasminogen activator (tPA)—was infused through the catheter continuously throughout the 40 minute treatment at a rate of 10 mL/hour. No additional fluid flow was introduced to the system.
- Set 2: bolus lytic infusion; no plasma flow. 0.67 mL of tPA was infused through the catheter over the first 4 minutes of the treatment period at 10 mL/hour, followed by 36 minutes of US exposure or none. The volume of tPA solution was 10 times smaller than in Set 1, but its concentration was 10 times greater. No additional fluid flow was introduced to the system.
- Set 3: slow lytic infusion; plasma flow. 6.7 mL of tPA was infused through the catheter continuously during the 40 minute treatment time at 10 mL/hour (identical to Set 1). 40 mL of heparinized plasma also flowed through the clot's central cavity at a rate of 60 mL/hour.

TABLE I. Experimental Conditions

	Control (saline)	tPA	tPA + US
Set 1: slow infusion no flow	n = 4	n = 5	n = 6
Set 2: bolus infusion no flow	n = 1	n = 5	n = 6
Set 3: slow infusion flow	n = 1	n = 5	n = 5

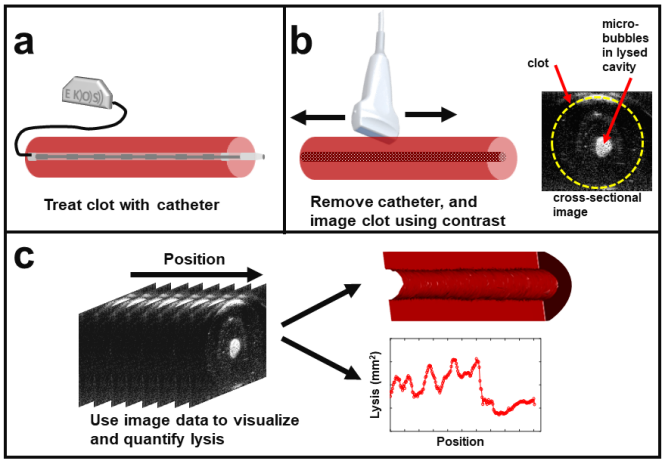

Fig. 1. Diagram of the experimental procedure for studying catheter-induced thrombolysis. (a) A clot is formed around a catheter, and the catheter is then used to treat the clot. (b) The catheter is removed, microbubble contrast is infused through the cavity created by the catheter, and a US imaging system collects a series of cross-sectional images along the length of the clot. (c) The image data is then used to measure the volume lysed by the catheter and to visualize the lysis.

Each experiment involved three stages: clot formation, thrombolysis treatment (Fig. 1a), and imaging. After treatment, a solution of microbubble US contrast agent was infused through the cavity created by the catheter (Fig. 1b). A series of US images was collected of each clot, and lysis efficacy was assessed by measuring the area through which contrast flowed (Fig. 1c).

B. Test Fixture Preparation

A test fixture was assembled using: rubber stoppers, Luer T-connectors, heat shrink tubing (0.74", 0.003" wall), Tuohy Borst valves, 2.5 cm stainless steel hypodermic tubing, and Tygon tubing (17.7 mm O.D., 12.7 mm I.D.).

Rubber stoppers were cut to 1 cm lengths, and 1 mm holes were drilled through the center. Each stopper was inserted into a 1 cm length piece of Tygon tubing. The steel tubing was inserted and glued into one side of a T-connector, and the tubing was inserted through one rubber stopper so that it protruded 1.5 cm out of the narrow end of the stopper. The stoppers with Tygon tubing were glued to the inside of each end of the heat shrink tubing. Tuohy Borst valves were attached to the proximal and distal ends of the fixture's T-connectors. The stoppers were removable from the Tygon tubing. The total length of the heat shrink was 29 cm, allowing room for the full catheter treatment zone (TZ), extra clot proximal and distal, and extra space at the distal end of the clot to avoid clogging the exit port of the fixture.

An acrylic tank (43 cm deep, 24 cm wide, 41 cm long) was filled with water and heated to 37°C. T-framing on the outside allowed fixing a stepper motor for moving the imaging probe and a clamp to hold the clot fixture.

C. Materials

Pooled normal human plasma was obtained from Innovative Research (Novi, MI). Bovine thrombin was obtained from VWR. tPA was obtained from Genentech (San Francisco, CA). Microbubbles were purchased from Bracco Diagnostics (Monroe Township, NJ). US-enhanced catheters with 18 cm treatment zones were acquired from EKOS (Bothell, WA). Side-hole Cragg-McNamara infusion catheters with 20 cm treatment zones were acquired from Medtronic (Minneapolis, MN).

D. Clot Formation

A 15 cm long (1.78 mm O.D.) polyether block amide extrusion (Putnam Plastics Corp) was added to each EkoSonic or side-hole catheter, allowing it to extend through the distal Tuohy Borst valve when the TZ was centered in the fixture. The extension also provided a channel for coolant to flow through the EkoSonic catheter that would not dilute the tPA within the clot fixture.

A catheter was inserted into the heat shrink fixture so that the proximal TZ marker band was 1 cm past the steel tubing. Because the steel tubing is visible from the US images, it can be used to determine the catheter position with respect to the images. A waterproof marker was used to mark the catheter in this position.

The catheter was further inserted through the fixture so the entire TZ was distal to the fixture, ensuring that the drug holes did not clog while the clot was forming. The distal Tuohy Borst valve was shut around the catheter. The catheter was pulled taut, and the proximal Tuohy Borst valve was also shut, centering the catheter radially within the fixture.

1.35 mL of 2 M $CaCl_2$ was added to 78 mL of plasma at room temperature. The mixture was degassed by aspirating into a large syringe, capping it, pulling the plunger to create negative pressure, and tapping the syringe. Removing bubbles ensured that the clot was hypoechoic, allowing lysis to be measured by flowing contrast agent. The degassed plasma mixture was then injected into a beaker.

0.5 mL of 8.1 µg/mL thrombin in PBS was added to the plasma mixture. A motorized pipette was fitted with a large tip, polymer tubing, and male Luer tip; this was used to aspirate the plasma mixture. The Luer tip was connected to the proximal T-connector of the clot fixture, and the plasma solution was injected into the heat shrink fixture. Pinch clamps attached to the polymer tubing prevented back flow of plasma. The fixture was placed vertically in the water bath and left to form for 60 minutes. The catheter was primed with coolant and/or drug during the incubation.

E. Thrombolysis treatment

After incubation, the fixture was removed from the bath. The Tuohy Borst valves were opened enough for the catheter to move freely. The catheter was positioned so that the TZ was positioned appropriately within the fixture (using the marking). The Tuohy Borst valves were shut, pulling the catheter taut to ensure the catheter was centered radially; US imaging was used to confirm this. In experiments with plasma flow, the proximal tube (clamped) was removed, and the tube was primed with plasma via a tube attached to the proximal T-connector.

The fixture was placed in the water bath with the distal portion of the catheter facing up. The entire clot was submerged but the distal T-connector was above the water's surface. Exit tubing directed lysate from the fixture to a waste bucket.

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The infusions of tPA, coolant, and/or plasma were started using a roller pump. In US-treated samples, an EKOS Control Unit (CU4.0, EKOS Corp., Bothell, WA) was powered on to deliver US pulses (MACH4e protocol). Every clot treatment was 40 minutes long. The US was emitted at 2.2 MHz, with a maximum peak negative pressure of 1.5 MPa, 10-17% duty cycle and 21-27 Hz pulse repetition frequency.

F. Clot imaging

After treatment, the flow of drug, coolant and/or plasma was stopped, and the control unit was powered off. The fixture was removed from the bath, the catheter was removed from the clot fixture, and the Tuohy Borst valves were shut completely. Tubing delivering microbubble contrast was attached to the proximal T-connector. 100 µL of Lumason microbubbles was added to 50 mL of PBS, and the solution was infused at 3.5 mL/min to enter the channel created by the catheter. Effluent was emptied into a waste bucket. The bubble solution was replenished as necessary.

An L22-8v probe connected to a Verasonics US imaging system (Vantage 128, Verasonics, Kirkland, WA) was aligned to capture image data of the clot fixture. The step size of the motor was 0.5 mm. The maximum range of the motor is 10 cm, so each clot was imaged in three scans (200 images per scan), with the probe repositioned after each scan. 600 images were collected of each clot.

From the images, the clot's lysed volume LV was calculated by applying a threshold to each image, summing the pixels, and multiplying by the pixel area and step size. A lysis enhancement factor LEF (%) was calculated using (1).

$$LEF\ (\%) = \left(\frac{LV_{US+tPA} - LV_{tPA}}{LV_{tPA} - LV_{saline}}\right) \times 100 \qquad (1)$$

In addition, the lysed volume measured from each image slice was plotted against clot position. A Student's t-test with two-tailed distribution and unequal variance was performed to evaluate the statistical significance between groups. A p-value < 0.05 was considered significant.

III. Results and Discussion

Images of the channels created by thrombolysis are shown in Fig. 2. For control conditions (saline), the lysed area approximately matched the cross-sectional area of the catheter (left column). Lysis from treatment with tPA alone (center column) was greater than that from control treatments, to a different degree based on infusion type (slow vs bolus). In each condition, the lysis from US-assisted treatment was greater than that from treatment with tPA alone. The lysis enhancement by US depended on infusion type and on the presence of plasma flow.

Fig. 3 quantifies the average lysis under each condition. The bar plots (Fig. 3 a, c, e) display the average of n clots and m images over the TZ (m = 400-420 images). The error bars denote the variability among the n clots that were treated in each condition. The line plots (Fig. 3 b, d, f) display the average volume of lysis as a function of clot position (proximal to distal). Each line (except for saline treatment) is the average of n clots.

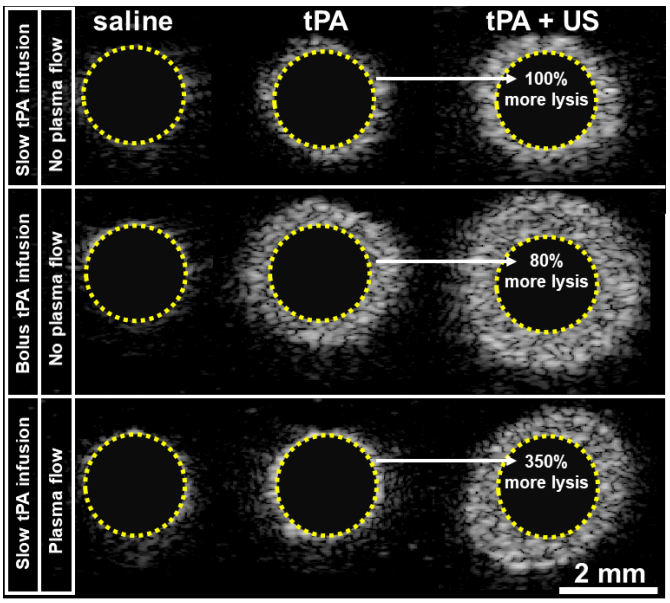

Fig. 2. Ultrasound images of microbubbles inside the cavity created by thrombolysis treatment, indicating the average cross-sectional lysed volume using saline (left), tPA (middle), and tPA with ultrasound (right). Experimental conditions are separated by row. Catheter circumference denoted by dotted line.

Treatment with saline did not produce significant lysis, in either the presence or absence of plasma flow. For clots with no plasma flow, a tPA infusion with or without US induced more lysis when infused as a bolus than as a continuous infusion (Fig. 3 a, c). In these cases, the dose (mg) of tPA delivered to the clots was equivalent. However, in the bolus case, the clot was exposed to the full dose of tPA for a longer period.

For cases of slow tPA infusion, in the absence of US, the lysis was the same in the presence and absence of plasma flow (Fig. 3 a, e). The plasma flow has two likely effects on lysis. First, the plasma (and plasminogen) is always present at the clot-lytic interface. The result is that tPA has a continuous supply of plasminogen that can be converted to plasmin, which then lyses the fibrin structure. Hence, plasma flow may increase lysis compared to a model in which 6.7 mL of saline-diluted tPA washed out any plasma present at the clot surface. Alternatively, flowing plasma dilutes the local concentration of tPA by six times, which may reduce lysis compared to treatment where the local tPA was not diluted by flowing plasma.

The US-assisted EkoSonic treatment resulted in more lysis when plasma was present (Fig. 3e, right) than when no plasma was flowing through the clot (Fig. 3a, right). The tPA concentration, volume, and infusion time were identical in these two conditions. Despite the tPA dilution by plasma flow, the clots underwent more lysis. This is evidence that the washout of plasma inhibited the lytic effect, and that under these conditions, the presence of plasma aids in lysis more than the dilution of tPA diminishes it.

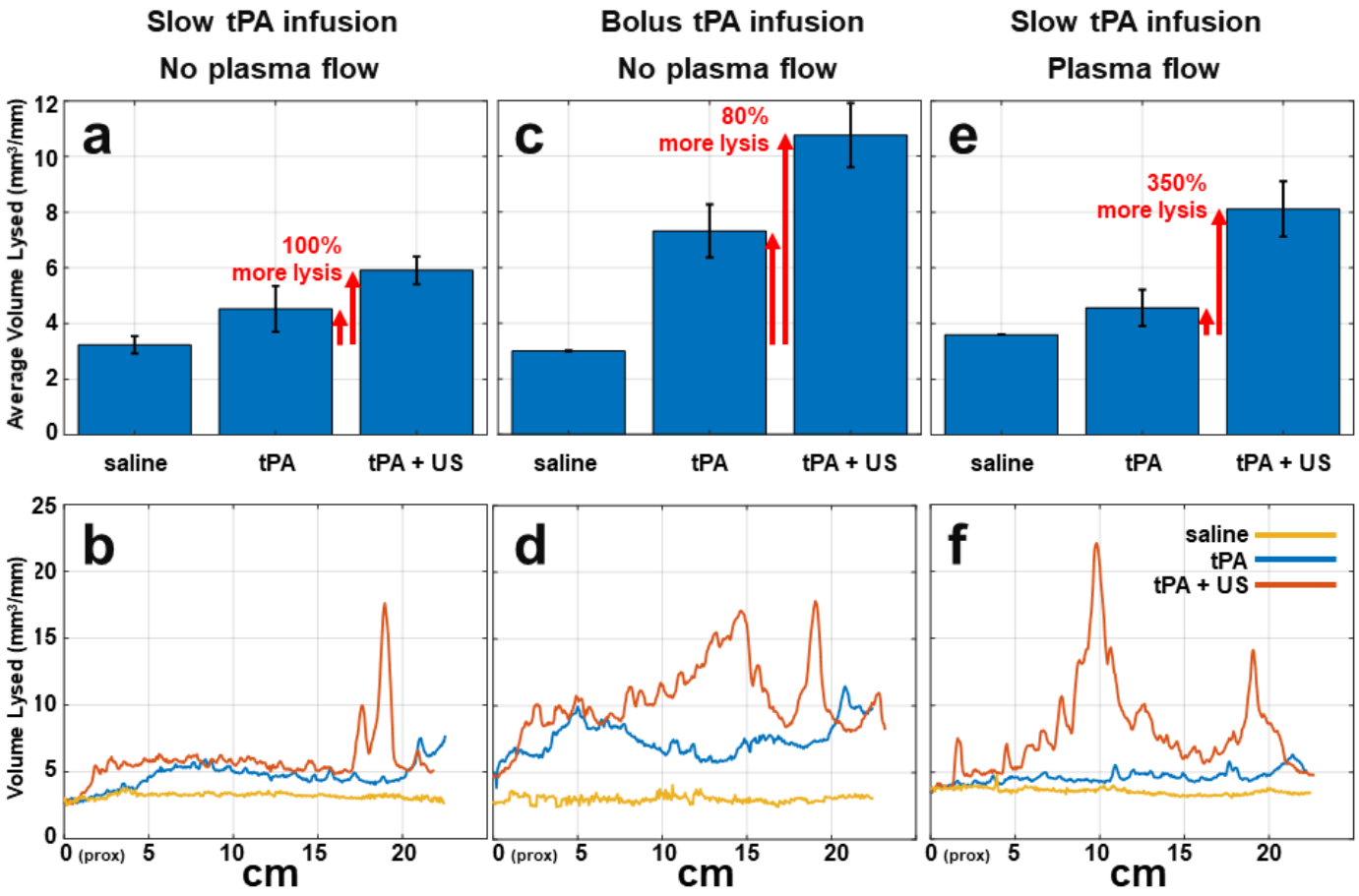

Fig. 3. Measurements of average volume lysed under each condition (top), and average volume lysed as a function of clot position (bottom). (a) tPA infused continuously throughout treatment, and without additional plasma flow. Lysis was enhanced by 100% by EkoSonic treatment over a conventional side hole catheter ($p < 0.001$). (b) Lysis enhancement was most pronounced at the distal end of the clot. (c) Infusing tPA as a bolus at the beginning resulted in greater lysis overall, with an 80% enhancement using EkoSonic ($p < 0.001$). (d) Lysis occurred more evenly throughout the clot. (e) When plasma flow was introduced, the lysis enhancement was 350% ($p < 0.001$). (f) Lysis occurred at all clot positions, though not evenly.

Because plasma flow did not affect lysis in the tPA (no US) condition (Fig. 3 a, e, center), but plasma flow increased lysis in the (tPA + US) condition (Fig. 3 a, e, right), the effect of US was much greater in experiments with plasma flow. This suggests that in environments in which a locally delivered lytic drug may be washed away by blood flow, the inclusion of US therapy may increase thrombolysis over a conventional drug delivery system by 350%. This lysis enhancement by US can be seen in the surface rendered image data shown in Fig. 4

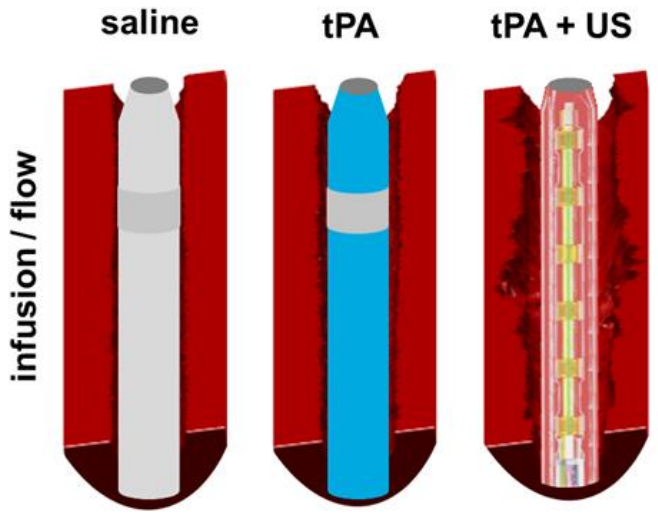

Fig. 4. Three-dimensional surface rendering of the image data from treatments with saline (left), tPA using a side-hole catheter (center), and EkoSonic (right). The experiment included plasma flow and a continuous infusion of tPA.

IV. Conclusion

Image-based measurements of tPA-mediated thrombolysis showed an enhancement when US was present in the therapy.

Therapy with US showed the greatest enhancement in the most clinically representative model, that in which plasma flowed through the system, suggesting that US from EkoSonic thrombolytic therapy is a valuable tool in achieving more lysis than a conventional side-hole catheter, and while using a lower drug dose. Imaging the extent and patterns of thrombolysis may inform design decisions to optimize the delivery of drug and US energy to achieve fast, safe, and thorough thrombolysis.

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