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Noninvasive treatment of deep venous thrombosis using pulsed ultrasound cavitation therapy (histotripsy) in a porcine model

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Abstract

Purpose—This study evaluated histotripsy as a noninvasive, image-guided method of thrombolysis in a porcine model of deep vein thrombosis. Histotripsy therapy uses short, high-intensity, focused ultrasound pulses to cause mechanical breakdown of targeted soft tissue by acoustic cavitation, which is guided by real-time ultrasound imaging. This paper is an *in-vivo* feasibility study of histotripsy thrombolysis.

Methods and Materials—Acute thrombi were formed in the femoral vein of juvenile pigs weighing 30–40 kg by balloon occlusion with two catheters and thrombin infusion. A 10-cm diameter 1-MHz focused transducer was used for therapy. An 8 MHz ultrasound imager was used to align the clot with the therapy focus. Therapy consisted of 5 cycle pulses delivered at a rate of 1 kHz and peak negative pressure between 14–19 MPa. The focus was scanned along the long axis of the vessel to treat the entire visible clot during ultrasound exposure. The targeted region identified by a hyperechoic cavitation bubble cloud was visualized via ultrasound during treatment.

Results—Thrombus breakdown was apparent as a decrease in echogenicity within the vessel in 10 of 12 cases, and in 7 cases, improved flow through the vein as measured by color Doppler. Vessel histology showed denudation of vascular endothelium and small pockets of hemorrhage in the vessel adventitia and underlying muscle and fatty tissue, but perforation of the vessel wall was never observed.

Conclusions—The results indicate histotripsy has potential for development as a noninvasive treatment for deep vein thrombosis.

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Introduction

Deep vein thrombosis (DVT) is a widely prevalent disease with complex pathophysiology and high economic burden. DVT may begin without symptoms, but often manifests itself as pulmonary emboli, which are considered the most significant preventable cause of death in hospitalized patients (1). The standard of treatment is short-term anticoagulation with heparin, followed by long-term dosing of oral anticoagulants (3–6 months) (2,3). However, this often fails to recanalize vessels, which can result in post-thrombotic syndrome and loss of venous valve function (4). Studies suggest that faster recanalization of the vessels is beneficial in preserving valve competence (5). Thus, a technique which induces active breakdown of the occlusion is desirable to ensure long-term patency of the afflicted vessel. Thrombolytic agents (e.g. rt-PA, urokinase) are employed in more severe cases of DVT, but have an associated risk of major bleeding (e.g. intracranial, peritoneal, or pericardial hemorrhage) (6). Catheter-directed thrombolytics and mechanical thrombectomy are also available, but these can have similar side effects of bleeding, as well as risk of pulmonary embolism without proper filtration (7).

Ultrasound has been assessed extensively as a method to promote thrombolysis (8,9). In the majority of past studies, ultrasound was used in conjunction with a thrombolytic agent to enhance its efficacy or as a method of mechanical thrombectomy with a catheter-based device (10). The feasibility of these methods has been shown with *in vitro* models and *in vivo* animals (11). Additionally, clinical studies have demonstrated the efficacy of ultrasound-enhanced thrombolysis using either low-power transcutaneous or catheter-delivered ultrasound in conjunction with thrombolytic agents, particularly for treatment of ischemic stroke (12,13). Ultrasound can be focused to high intensity within internal vessels or organs from an extracorporeal position. Ultrasound-induced shear forces or cavitation bubbles can create enhanced delivery and permeation of thrombolytic agents into the clot, improving efficacy (14,15). However, the limitations of these approaches are the continued presence of significant side effects which occur with thrombolytic drugs or catheter-based mechanical thrombectomy.

Histotripsy also uses focused transcutaneous ultrasound, but does not incorporate thrombolytic agents. Instead, histotripsy noninvasively applies short, high-intensity ultrasound pulses to cause controlled mechanical breakdown of the thrombus by acoustic cavitation (16,17). Cavitation bubbles at the focus expand and collapse in response to the ultrasonic pressure. This motion creates high localized pressure in the thrombus, causing it to mechanically rupture. Previously, it was demonstrated that histotripsy is capable of lysing acutely formed blood clots *in vitro* (18). A cloud of cavitation bubbles forms at the focus of the transducer above a distinct pressure threshold (19), causing mechanical breakdown of the thrombus into acellular debris (20). Application of histotripsy to 300 mg thrombi *in vitro* achieved complete dissolution within 2 minutes. Almost all of the debris from the procedure was < 100 μ m diameter. The bubble cloud could be detected on ultrasound imaging as a dynamic, hyperechoic region, providing real-time feedback of therapy progression and efficacy (21).

To evaluate the *in-vivo* feasibility of treating DVT using histotripsy, we created a porcine model of acute DVT and attempted to treat thrombi transcutaneously using histotripsy. Several models for DVT have been developed previously in pigs (22,23), which have similar vascular anatomy to humans (24). For our model, acute thrombi were induced by balloon catheter occlusion of a vessel segment and procoagulant infusion. The thrombus was visualized and targeted entirely by ultrasound imaging and color Doppler. The histotripsy transducer focus was aligned with the vessel and scanned along the clot during sonication to

cause breakdown of the thrombus. Preliminary evaluation of possible tissue and vessel damage was also performed.

Methods

Animal Preparation and Thrombus Formation

The protocols described in this article have been approved by the University Committee on Use and Care of Animals at our institution. Juvenile pigs (mixed breed) weighing between 30–40 kg were used as subjects for the study. The animal was first sedated with 5 mg/kg teletamine + zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, IA, USA) and 2.5 mg/kg xylazine (Lloyd Laboratories, Shenandoah, IA, USA). The subject was then intubated and placed in a supine position. 0.5%–3.5% isoflurane (Vet-One, Meridian, ID, USA) was used as an anesthetic. Subjects did not require mechanical respiration during any of the procedures or treatment. Pulse rate, SpO₂, temperature, and respiration rate were continuously monitored during the procedure. A chemical depilatory (Nair, Church & Dwight Co., Princeton, NJ, USA) was applied to the legs for 10 minutes to ensure complete removal of hair and provide better ultrasound coupling.

Two 5F wedge occlusion balloon catheters (Arrow International, Reading, PA, USA) with 8 mm diameter balloons were introduced into the femoral vein by percutaneous venous puncture distal from the anticipated site of thrombus formation. Catheters were inserted at two adjacent access sites and positioned with balloon edges separated by 20 mm and the catheter of the proximal balloon within the formation site. Both balloons were inflated to occlude a 20 mm segment of the vessel, and 50 U (0.1 mL) thrombin (Thrombin JMI, King Pharmaceuticals, Bristol, Tennessee, USA) was infused through the distal catheter end-hole into the occluded region between the two balloons to induce thrombus formation. In general, pigs are considered hypercoagulable in comparison with humans and other species (25). To prevent thrombus formation outside the occluded vessel segment, heparin was administered systemically as a 100 U/kg bolus through an ear-vein catheter immediately after thrombin infusion. This combination allowed clots to preferentially form only in the region between the balloons. This method proved satisfactory in controlling thrombus length in the vein. Balloons were left inflated for 2 hours after thrombin injection, after which, the deflated catheters were pulled distal to the thrombus but left in the vessel to prevent bleeding. An additional bolus of heparin was delivered every hour during thrombus formation and throughout the remainder of the experiment. Anticoagulation was measured by activated clotting time (ACT) (Hemochron 400, International Technidyne Corporation, Edison, NJ, USA) or activated partial thromboplastin time (APTT). An APTT of > 70 s or ACT > 200 seconds was maintained throughout the procedure.

Ultrasound imaging of the femoral vessels was performed to collect two-dimensional (2D) and color Doppler images of the vessel transverse and long-axis views. Images were captured before and after clot formation using a 10 MHz linear array probe (10L, Logiq 9, GE Healthcare, Fairfield, CT, USA). After clots were formed, a bath of degassed water heated to 40°C was placed over the legs of the subject. Degassed water (O₂ concentration 40% of saturation level) was used to minimize cavitation or bubble formation in the water, which can reduce ultrasound transmission into the subject. The bath consisted of a stainless steel bowl with a hole cut in the bottom and a transparent polyethylene sheet lining the bowl (Figure 1). The polyethylene was coupled to the legs using degassed ultrasound coupling gel (Lithoclear MV, Sonotech Inc., Bellingham, WA, USA). The plastic sheet was inspected to ensure that all air bubbles trapped in the gel were removed from the sonication area on the leg.

Histotripsy Therapy Apparatus

A 1-MHz 10-cm diameter spherically-focused transducer with 9 cm focal length (Imasonic, Besancon, France) was held in the water bowl and attached to a computer-controlled 3-axis motorized positioning stage (Parker Hannafin, Rohnert Park, CA, USA). The transducer had a 4 cm concentric hole, through which an 8 MHz phased array ultrasound imaging probe was placed (S8, SONOS 7500, Philips Healthcare, Andover, MA, USA). This imager was used to align the therapy focus with the clot. The transducer was driven by a custom class D amplifier developed in house connected with an FPGA timing board (Altera DE1, Terasic Technologies, Dover, Delaware, USA) to control ultrasound pulsing parameters (26). The transducer pressure output was calibrated prior to the experiments by a fiber-optic probe hydrophone which recorded pressure level vs. input voltage at the focus in degassed water (27). The focus is ellipsoidal, with dimensions at the -6dB pressure being $1.9 \text{ mm} \times 1.9 \text{ mm} \times 13.5 \text{ mm}$, the longer dimension being along the axis of ultrasound propagation. This volume defines the region over which the acoustic pressure is greater than half the pressure at the center of the focus.

Pretreatment Planning and Targeting

Alignment of the transducer focus with the thrombus was performed in three steps, guided by ultrasound imaging. First, the focus of the transducer was located on the ultrasound image by applying histotripsy pulses to an empty water bath. During this procedure, a cavitation cloud appeared at the focus as a bright (hyperechoic), percolating region on the 2D image. Since this cavitation is the primary mechanism of thrombolysis, the temporally changing hyper echoic zone on ultrasound image is used as the indication that histotripsy thrombolysis treatment is progressing normally. The center of the hyperechoic zone was marked as the focal position. Second, the focus was aligned within the vessel proximal to the end of the thrombus, with the therapy ultrasound propagation perpendicular to the vessel. A short ($< 1 \text{ s}$) exposure was performed to confirm cavitation in the vessel lumen. Third, a scanning route to move the focal position along the entire length of the thrombus was determined prior to the treatment. The focus was scanned from the proximal end of the thrombus distally along the thrombus by movement of the entire transducer without rotation. At the new positions (spaced $\sim 5 \text{ mm}$ apart), cavitation was confirmed by short exposure to ensure the cloud position overlapped the thrombus and was contained to the vessel lumen. After confirmation, this position was recorded by the computer. This process of moving, confirming, and recording the target position was repeated until the distal end of the clot was reached. A program was then used to interpolate a path for the transducer through the alignment points (Figure 2).

Upon completion of targeting, therapy pulses were applied to the thrombus following the predetermined scanning route at a set velocity. A velocity of 0.1 mm/s was used, covering 1 cm of the thrombus in 100 seconds. The histotripsy transducer was driven to emit 5 cycle ultrasound pulses at a rate of 1 kHz . The peak negative pressure (the maximum tensile pressure at the focal maximum reached during a pulse) was between $14\text{--}19 \text{ MPa}$ as measured in a water bath (free field). The transducer output necessary to generate a cavitation cloud varied between subjects, likely due to anatomical variation of the overlying tissue. In general, the lowest output level which induced a bubble cloud consistently in the vessel was used for each treatment. The measured range of pressure levels used corresponds with spatial peak pulse average intensity ($I_{\text{sppa}} = 9800\text{--}21000 \text{ W/cm}^2$) and spatial peak temporal average ($I_{\text{spta}} = 49\text{--}105 \text{ W/cm}^2$) in water at the focus. I_{sppa} is the average intensity over the duration of a pulse at the focal maximum (acoustic power per unit area), while I_{spta} is the average intensity at the focal maximum averaged over the time during and between pulses. Peak negative pressure and I_{sppa} can be considered measures of potential to generate cavitation, while I_{spta} is a measure of potential for thermal heating from the ultrasound.

Because the pulses are only being applied 0.5% of the time, the I_{spta} is much lower than I_{sppa} , and thus heating is minimized. After completion of each scan, the therapy was terminated and the transducer was removed from the water bath. The 10 MHz linear array transducer was applied to image the vein and evaluate echogenicity and flow through the vessel as indications of thrombolysis. If a substantial portion of the thrombus was still visible after treatment, another treatment was applied. For each thrombus treatment, up to 3 additional scans were performed, with the spatial path of the focus modified to target the remaining thrombus in the vein each time.

Vessel Damage Evaluation

Histological samples where clots were formed but not treated showed that catheter insertion and balloon inflation caused damage to the vessel prior to treatment. To evaluate the vessel damage caused by histotripsy alone, vessels without clots were treated. Untreated vessels without clots formed were used as controls. A 15 mm segment of the femoral vessel was treated for 300 seconds using the same ultrasound parameters as those used to treat thrombi with peak negative pressure of 19 MPa, scanning the vessel twice at a rate of 0.1 mm/s.

Post Treatment

All animals were euthanized within 30 minutes from end of treatment by intravenous delivery of 150 mg/kg sodium thiopental. The femoral bundle and surrounding muscle tissue were extracted from each leg, and rinsed thrice with 0.9% saline. Afterwards, the tissue was placed in 10% formalin solution for fixation. H&E slides were then prepared as cross sections of the femoral bundle and examined by light microscopy for signs of injury. The lungs of several animals were grossly visualized within 5 minutes after euthanasia for indications of injury due to embolic events.

Results

Thrombus Formation

Consistent-length clots were formed using the method outlined above in 12 subjects. Without introduction of heparin, clot formation would continue beyond the intended site down the length of the vessel once the balloons were deflated. Additionally, some thrombus would also form on the catheters while balloons were inflated. Heparin was necessary to prevent this action. Using the combined thrombin/heparin approach, thrombi formed in the 12 subjects were 20.1 ± 6 mm length (mean $\pm$ standard dev.). The diameter of the vein was 5.5 ± 1.0 mm along the formation site, at a depth of 13–17 mm. In 6 of 12 cases, the clots were occlusive, as indicated by lack of flow through the hyperechoic clotted region on color flow Doppler (Fig 3a,b). In the other 6 cases, the clots were partially occlusive with flow indicated as a narrowed channel through the lumen after the clot was formed (Fig 3c,d). The vessel occlusion or partial occlusion and thrombus location were identified using ultrasound imaging with the 10 MHz linear array placed on the skin.

Treatment Outcome

The histotripsy treatment applied was guided by ultrasound imaging using the 8 MHz phased array inserted in the center hole of the therapy transducer, which was 8 cm distance from the thrombus. The thrombus was distinguished in the vessel lumen by an area of increased echogenicity on the therapy guidance imager (Figure 4a). The bubble cloud generated at the focus of the therapy transducer was confined to the vessel lumen as it appeared on a 2D image as a dynamic echogenic region on the surface of or within the thrombus (Figure 4b,c). As the focus was scanned over the clot following the predetermined route, the treated area underwent a reduction in echogenicity, indicating thrombolysis was

occurring in that region (21). After treatment, the 10 MHz linear array imager was used to confirm the treatment result with higher quality imaging. When echogenicity reduction was observed by the therapy guidance transducer, it was always confirmed by the linear array imager. Usually, the bubble cloud did not occupy the entire width of the vessel, and thus the entire thrombus was not lysed within a single scan of the vein. Under these circumstances, another scan was performed adjusting the focus to a new position laterally within the vessel and scanning the same longitudinal section.

In 10 of 12 cases, the treatment resulted in a reduction in thrombus volume as indicated on 2D ultrasound. In 7 of 12 instances, vessels showed improved signs of flow. For the 6 fully occlusive cases, 4 had flow at least partially restored through the vein according to color Doppler imaging, visualized as creation of a flow channel through the lumen (Figure 5). The average flow channel diameter (measured at the narrowest point with color Doppler) was 3.2 ± 0.5 mm for these cases. For the 6 partially occlusive cases, 3 showed flow improvement as an increase in dimensions of the flow channel in the occluded region. The average increase in flow channel diameter was 2.6 ± 1.0 mm for these treatments. In 2 of 12 cases, no apparent echogenicity reduction or flow improvement was observed. In these two experiments, a bubble cloud could not be generated in the vessel or thrombus. For all treatments which a bubble cloud was generated in the vessel, at least partial breakdown was achieved. The mean ultrasound exposure time for a treatment was 10.5 ± 5.5 minutes (range 2.6 to 18.0 minutes). The outcome of 12 treated thrombi is presented in Table 1.

As an acute study, assessment of vessel damage in the 12 vessels treated with clots was confounded by tissue injury from percutaneous catheter insertion and balloon dilation. Significant hemorrhage was apparent, likely attributed to the thrombus formation process. However, in these cases, no vessel perforations attributed to histotripsy were identified.

To more accurately assess the damage incurred due to histotripsy alone, 4 femoral vein segments without formed thrombi were treated, eliminating the factor of catheter-induced damage. These vessels were all intact upon histological examination. In 3 of the 4 subjects, minor zones of hemorrhage were present in the venous adventitia and fat or muscle tissue below the femoral vein (the region overlapping the distal part of the ultrasound focus) (Figure 6a,b,c). Regional denudation of endothelium lining the vein was observed as well. In one specimen, significant intramural hemorrhage was visible in part of the vessel wall and surrounding tissue, although no perforation of the vessel wall occurred (Figure 6d,e,f). During this treatment, some small echogenicities indicating cavitation were observed beneath the vessel in addition to the cavitation cloud in the lumen. The femoral artery did not show signs of damage or endothelial disruption. Thus, damage was confined to the area near the focal zone of the transducer. Control samples (untreated vessels without clots) did not show any signs of vessel injury or hemorrhage. No macroscopic indications of injury due to embolism were observed in the lungs of 4 of the subjects.

Discussion

This study demonstrates the feasibility of employing histotripsy as a noninvasive thrombolytic therapy. The clots formed in the femoral vein could be targeted using a combination of 2D and Doppler ultrasonography. Furthermore, the bubble cloud generated during therapy provided feedback for where thrombolysis was occurring. In most cases, cavitation was confined to the vessel lumen, and the corresponding decrease in echogenicity of the thrombus only occurred in this region. However, the large therapy transducer used in this work placed the therapy guidance imager 8 cm from the target vessel, providing low resolution images of the clot for targeting. Although histotripsy has been previously performed using such large transducers (9–15 cm aperture and 9–10 cm focal length)

(19,28), this is unnecessary for treatment of DVT, as the typically afflicted veins lie only 1–3 cm beneath the skin surface (29). An improved transducer with shorter focal distance would allow imaging to be directly at the skin surface, providing image quality similar to the linear array probe used in this study.

In over 50% of the treatments, histotripsy improved the flow through the vessel. In 83% of cases, a reduction in thrombus volume and burden was observed, indicating homogenization of the treatment zone. In the 2 instances where histotripsy could not successfully treat any part of the thrombus, a bubble cloud could not be generated within the vessel lumen. The likely cause of this phenomenon was air trapped either at the skin surface or within the vessel. During catheterization, air may have entered the insertion site. Air was visible in one of the cases within the vessel, which likely prevented transmission of ultrasound to the clot. In the other case, cavitation formed in the water bath on the plastic bag at the bottom of the bowl, indicating possible poor coupling to the animal. These problems, however, are unrelated to the actual treatment. In all cases where a bubble cloud was initiated in the vessel, at least partial breakdown of the thrombus was achieved. Flow restoration was not always achieved, in part because of limited resolution of the therapy guidance transducer. In several instances, the full extent of the clot was not clearly delineated, and thus could not be treated. This problem may also be addressed by improved imaging, which will allow high-resolution imaging of the entire clot extent and thus improved accuracy and targeting.

Vessels which were treated without forming clots contained hemorrhage localized to the area around the treatment zone. While vessel perforation was not observed in any of the treatments, small areas of medial and adventitial damage were present in the part of the vessel where the bubble cloud was seen during imaging. Local pockets of hemorrhage have also been observed in soft tissues such as prostate or kidney treated by histotripsy (30,31). The collateral damage was inferred in these cases to be caused by cavitation bubbles. Injury to surrounding tissue during histotripsy thrombolysis may resolve as fibrous growth and scarring, while damage to the vessel wall can cause neointimal growth and reocclusion if severe (32). In 3 of the 4 cases, the damage to the actual vessel was minimal, during which cavitation was confined to the vessel lumen. In the other case where cavitation was observed outside the vessel, significant damage to surrounding tissue was found. Unfortunately, the focal length of the therapy transducer (13 mm) was nearly double the mean vessel diameter (6 mm). Design of a smaller focal size transducer is achievable using higher frequency or increasing transducer curvature, which could constrain cavitation activity to an area entirely within the vessel. Additionally, choice of acoustic parameters in histotripsy plays a large role in the size and extent of cavitation clouds generated, as well as the tissue erosion rate (33). Smaller focal size and higher resolution imaging may improve the precision such that histotripsy could be extended to treat smaller and more tortuous vessels. Despite the range of observed effects, the vessels with minimal injury suggest that the procedure is possible without incurring significant damage.

Other side effects may occur during histotripsy which were not evaluated in this initial study, including risk of embolism and hemolysis. In a previous *in vitro* study of histotripsy thrombolysis, the debris generated by clot breakdown was measured (18). In all cases, > 96% of the clot weight was reduced to particles smaller than 8 μm , with no particles larger than 100 μm found. Upon preliminary gross examination of the lungs in this study, no injury due to embolism was observed. While these results suggest that the debris generated by histotripsy consists of clinically insignificant fragments, a full evaluation of the clot particles generated by the procedure *in vivo* is needed to confirm this assertion. Hemolysis is another effect which warrants investigation, as acoustic cavitation is known to cause lysis of red blood cells in suspension (34,35). Elevated potassium levels due to hemolysis can lead to hyperkalemic cardiac arrhythmia (36). While no signs of arrhythmia were observed during

or after treatment in any subjects for the present study, the typical level of hemolysis which occurs during histotripsy should be evaluated.

Acute thrombi formed over 3 hours were treatable in this study, but DVT usually presents itself clinically over longer terms. While DVT begins as a red thrombus, it rapidly organizes to fibrous and then collagenous architecture over days or weeks, with small recanalized channels within the thrombus (37). As the clot grows older and stiffer, it may be more difficult to induce thrombolysis by histotripsy. The relative proportions of materials that make up the clot (e.g. red blood cells, platelets, fibrous tissue) will also contribute to changes in mechanical properties and the threshold for generating cavitation. Previous work in histotripsy has shown that materials with higher Young's modulus do not erode as readily as soft tissues normally treated (38). DVT maturity can be determined through ultrasound 2D echogenicity or elastography (39,40). It may be important to identify thrombus age and modify acoustic treatment parameters of histotripsy to effectively treat acute vs. chronic DVTs. Future work will evaluate the ability of histotripsy to treat a range of DVT ages.

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Figure 1.

Experimental treatment apparatus used for histotripsy thrombolysis. A focused therapy transducer is coupled to the legs by a water bath with an acoustic window of polyethylene at the bottom. The transducer is focused on the femoral vein, guided by a phased array ultrasound imager. A motorized positioner is used to scan along the clot during treatment.

Figure 2.

(a) Thrombus formed in the femoral vein (FV) of a subject, as visualized by the 8 MHz therapy guidance imager from a distance of approximately 8 cm. (b) A scanning route determined during pretreatment planning, with the approximate focus positions (dashed ellipses) at the start and end of treatment. Each small circle along the path indicates a position of the focus during the treatment.

Figure 3.
Example ultrasound images captured with a 10 MHz linear array probe of fully occlusive thrombus in 2D (a) and color Doppler (b). 2D and color Doppler images of a partially occlusive thrombus are shown in (c) and (d).

Figure 4.

Progression of a single treatment scan. (a) The echogenic thrombus (white arrow) is differentiated from the darker empty lumen (black arrow) of the vein prior to treatment. During sonication (b,c), the bubble cloud (small arrows) appears as a dynamic hyperechoic zone on or in the thrombus, indicating the focal position of the transducer. After the 300-second treatment (d), the echogenicity of the thrombus is reduced compared with (a), indicating where the clot has been broken down. Treatment time from start of sonication is listed above each image.

Figure 5.

Ultrasound images of the femoral vein captured by a linear array imaging probe between treatments of a thrombus. (a) and (b) show the original appearance of the vessel on 2D imaging and with color Doppler. (c) and (d) show the thrombus after 240 seconds of treatment (one scan). (e) and (f) show the final condition of the clot after 720 seconds (three scans). Note the decreased echogenicity in the lumen on (c) and (e) compared with (a). Also, a flow channel is clearly visible on (d) and (f), while none was present before treatment in (b).

Figure 6.

Gross images and vessel histology of veins treated by histotripsy. (a) Gross image of femoral bundle showing minimal damage, as seen in 3 of 4 treated vessels. Histology of the vein is shown in (b), with a higher magnification displayed in (c). Minor intramural hemorrhage can be seen within the adventitia. (d) Gross image of femoral bundle shown hemorrhage below the vessel treated by histotripsy. (e) shows histology of the vein, with higher magnification displayed in (f). Greater hemorrhage was present in this specimen and the underlying muscle tissue, although the vessel remained intact.

Table 1

Summary of histotripsy therapy outcomes.

	Partial	Occlusive	All
N	6	6	12
Clot Length (mm)	21.3 $\pm$ 0.41	18.8 $\pm$ 0.6	20.1 $\pm$ 0.6
Vessel Diameter (mm)	5.3 $\pm$ 0.6	5.7 $\pm$ 0.6	5.5 $\pm$ 0.6
Percent Occlusion[†]	75.1 $\pm$ 14.2	100	87.6 $\pm$ 16.2
Treatment Time (s)	615 $\pm$ 283	636 $\pm$ 414	625 $\pm$ 334
Clot Echogenicity Decreased	5	5	10
Flow Improved on Doppler	3	4	7
Increase in Channel Diameter (mm)[‡]	2.6 $\pm$ 1.0	3.2 $\pm$ 0.5	3.0 $\pm$ 0.8

* Clot length, vessel diameter, percent occlusion, and treatment time are given as (mean $\pm$ standard deviation)

[†] 'Percent Occlusion' is defined as the percentage of the vessel cross sectional area occupied by thrombus before treatment at the section of maximum occlusion.

[‡] Increase in channel diameter lists only the cases where flow improvement was detected on color Doppler.