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Non-Invasive Embolus Trap (NET) using Histotripsy --An Acoustic Parameter Study

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Abstract

Free-flowing particles in a blood vessel were observed to be attracted, trapped, and eroded by a histotripsy bubble cloud. This phenomenon may be used to develop a Non-Invasive Embolus Trap (NET) to prevent embolization. This study investigates the effect of acoustic parameters on the trapping ability of the NET generated by a focused 1.063MHz transducer. The maximum trapping velocity, defined by the maximum mean fluid velocity at which a 3–4 mm particle trapped in a 6 mm diameter vessel phantom, increased linearly with peak negative pressure (P₋) and increased as the square root of pulse length and pulse repetition frequency (PRF). At 19.9 MPa P₋, 1000 Hz PRF and 10 cycle pulse length, a 3 mm clot-mimicking particle could remain trapped under a background velocity of 9.7 cm/s. Clot fragments treated by NET resulted in debris particles < 75 μm. These results will guide the appropriate selection of NET parameters.

Keywords

histotripsy; cavitation; thrombolysis; acoustic trapping; emboli; NET

Introduction

Venous thromboembolism is a serious problem, with a projected 505 out of 100,000 people in the United States estimated to be affected by 2025 (Deitelzweig et al., 2011). In addition, deep vein thrombosis (DVT) has a high incidence, with 5 out of 10,000 people in the general population being diagnosed for the first time each year. DVT typically occurs when portions of the deep veins of the legs develop blood clots. Factors that increase these odds include age, obesity, cancer, surgery, trauma and drugs (Kyrle and Eichinger, 2005). For instance, the risk is increased 8 fold for those in hospitals and 22 fold for those undergoing surgery (Fowkes et al., 2003). The most devastating complication from DVT is the risk of pulmonary embolism (PE), in which a fragment of loosely-held or wall-adhered clot breaks away and enters the vasculature of the lungs. PE causes over 300,000 deaths annually and can have an acute onset (Fowkes et al., 2003).

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Once acute DVT has been confirmed, low-risk patients can undergo treatment with low-molecular weight or unfractionated heparin to prevent further clotting. In other cases, thrombolytic drugs such as tissue plasminogen activator (tPA), urokinase, or streptokinase are used to actively break up clots (Kyrle and Eichinger, 2005; Menon et al., 2004). Catheter-based drug delivery was shown to lyse clots more effectively than systemic drug infusions, and mechanical thrombectomy was shown to have better clinical outcomes than drug delivery alone (Cohen et al., 2001; Mewissen et al., 1999). However, drug-based therapies have elevated bleeding risks. Catheter-based treatments carry risks including hemorrhage and infection and are costly due to the monitoring required (Gutt et al., 2005).

Therapeutic ultrasound has been shown to enhance and accelerate clot lysis (Parikh et al., 2008; Siegel et al., 2001). Lower intensity ultrasound in conjunction with drugs such as tPA and contrast agents increase enzymatic fibrinolysis by enhancing drug delivery into the clot (Francis and Suchkova, 2001; Hitchcock and Holland, 2010; Holland et al., 2008). High intensity ultrasound has also been used to mechanically disrupt clots into smaller fragments (Rosenschein et al., 2000).

Our previous studies have shown that histotripsy can controllably fractionate blood clots for the noninvasive treatment of thrombolysis (Maxwell et al., 2009). Guided by real-time image feedback, histotripsy utilizes cavitation to mechanically fractionate soft tissue by high-pressure, microsecond-long pulses at low duty cycles (<1%) (Parsons et al., 2006a; Roberts et al., 2006; Xu et al., 2005; Xu et al., 2004). As applied to thrombolysis, histotripsy can completely fractionate a blood clot weighing 300 mg within 5 minutes (Maxwell et al., 2009). The feasibility and safety of the histotripsy thrombolysis therapy has also been demonstrated *in-vivo* in a porcine DVT model (Maxwell et al. 2011).

Embolic protection devices (EPDs) are used in current interventional cardiovascular procedures to minimize the risk of embolization when clot fragments may be generated at the operation site. There are 3 main types of EPDs: distal occlusion balloons, distal filters, and proximal occlusion devices (Bosiers et al., 2008). These are placed within the blood vessel and prevent embolization through either size-discriminant filtering or blockage of portions of vasculature that would risk receiving embolic fragments. However, many patients are not eligible for these devices because of certain anatomical features or lesions (Webb et al., 2005). In addition, the risks from these devices range from patient intolerance, vasospasms, and filter tears and overloading (Bates and Campbell, 2011). Recently, an ultrasonic method has been proposed to apply acoustic radiation force on the ascending aorta to divert debris away from the brain during valve replacement surgeries in patients on extra-corporeal bypass. However, these embolic fragments are diverted to the descending aorta but not removed. This device is designed to use with open heart surgery (Sauren et al., 2009; Sauren et al., 2007).

During an in-vitro study for histotripsy-based thrombolysis, it was found that free particles were attracted, trapped and eroded near the histotripsy-generated bubble cloud (Maxwell et al., 2009). This prompted the idea for a Non-Invasive Embolus Trap (NET) to prevent embolization caused by any escaping clot fragments. For example, the NET could be used in conjunction with the histotripsy thrombolysis as a secondary bubble cloud positioned downstream from the thrombolysis treatment site to capture and fractionate any emboli in treatment of DVT. This study investigated the feasibility of the NET to trap a clot particle in a large vessel phantom and the effect of the acoustic parameters on its trapping ability. The trapping ability was measured as the maximum background flow velocity at which a particle could remain trapped under the ultrasound field. In this paper, we show that trapping velocity up to 10 cm/s can be achieved using the parameters tested, which would allow the NET to function as a non-invasive EPD for venous applications. With further improvement

that enables the NET to function at a higher flow velocity, NET may have the potential to become a non-invasive EPD for arterial applications as well.

Materials and Methods

Vessel and Embolus Phantoms

A two-part, optically transparent vessel phantom similar to that described by Ryan and Foster (Ryan and Foster, 1997) was made to mimic a blood vessel and the surrounding soft tissue (Figure 1a). The inner vessel construct was made from a degassed 30% w/v gelatin solution. After solidification of the gelatin vessel, it was removed from the mold and placed in 10% formalin for 20 minutes. This was done to confer mechanical stiffness via cross-linking and to raise the effective melting temperature of the phantom. The mold used had a 6 mm inner diameter and 9 mm outer diameter. The inner diameter is within the range of vessels typically experiencing thrombosis in the leg, such as the popliteal and femoral veins (Hertzberg et al., 1997). Care was taken to prevent air bubbles. The vessel was held in the center of a 15×10×7 cm frame with tube fittings in opposing ends to connect the vessel phantom lumen in-line with the larger circulation system. The sides of this mold were temporarily covered with tape, and a 10% gelatin solution, made similar to the 30% solution, was poured to provide structural stability to the vessel phantom. After solidification, the tape was peeled off to reveal a gelatin block without acoustically-interfering layers. Prior to the experiments, the vessel phantom was submerged in a 60 × 30 × 36 cm water tank degassed to 50% oxygen concentration for an hour to achieve equilibrium.

To study the trapping of a clot particle with NET without the complication from particle erosion induced by histotripsy, erosion-resistant spherical clot phantoms were made. These clot phantoms were made by dissolving 4% w/v agarose powder into a 35:65 glycerol-water solution. Distilled water was microwaved until boiling occurred. Then glycerol was added in the appropriate ratio and mixed using a magnetic stirrer over a hot plate until the solution turned clear. The solution was then placed in a vacuum chamber set at 75 kPa and degassed for 15 minutes. This yielded a solution which was nearly neutrally buoyant to the blood-mimicking fluid used in the circulation system. Food coloring was added to the solution to make particles that were readily visible when passing through the optically transparent vessel phantom. Spheres were created by dropping the agarose solution into -4° C mineral oil. The hydrophobic interaction of the agarose solution droplets and mineral oil created round spheres of agarose which were quickly solidified (Figure 1b).

Circulation System

An *in-vitro* circulation system was constructed to produce steady, laminar flow of fluid to mimic blood flow in venous circulation. This system consisted of a roller pump (Masterflex, Cole-Parmer, Vernon Hills, IL, USA), a pulse dampener, and a vessel phantom as shown in Figure 2. The roller pump was modified to allow PC control of the volume flow rate. The average velocity of the flow was determined by dividing the volume flow rate by the cross-sectional area of the vessel. The pump was connected to an external power supply (V2000, Hewlett-Packard, Palo Alto, CA, USA) and controlled via general purpose interface bus (GPIB) connection using MATLAB software (Mathworks, Natick, MA, USA). A 35:65 solution of glycerol and water was used as the circulating fluid. The water was degassed to 80% before the glycerol was added. This solution had a dynamic viscosity of 3 cP, similar to human blood viscosity. A sealed container connected to the system through tube fittings was used as the pulse dampener, which smoothed out the pulsations introduced by the roller pump to produce flow mimicking venous blood flow.

Ultrasound System

A bubble cloud was generated inside the center of the vessel phantom using an 8-element focused PZT transducer with a center frequency of 1.063 MHz and an elliptical aperture (17.5 cm long axis and 15.5 cm short axis) with a 10 cm focal length. The focal region was ellipsoidal, with the -6dB peak negative pressure beamwidth of the focal region being 1.2 mm transverse to the beam axis, and 6.3 mm along the beam axis. All elements were driven in phase with a class-D amplifier developed in lab, which was controlled by a field-programmable gate array (FPGA) logic board (DE1, Altera, San Jose, CA, USA) to produce histotripsy pulses (Figure 3). Pulse repetition frequencies (PRF) ranging from 30 Hz to 1000 Hz and pulse lengths from 5 to 50 cycles were tested. Acoustic pressure waveforms were measured by a fiber optic hydrophone with an active element of 100 μ m in diameter made in house (Parsons et al., 2006b). Peak rarefactional pressures (P-) ranged from -4.9 MPa to -19.9 MPa, and peak compressional pressures (P+) ranged 28 MPa to 58.6 MPa. To study the effects of pulse lengths and PRF on trapping, a fixed P-/P+ of -19.9 MPa/ 58.6 MPa was used. The transducer was aligned such that the acoustic axis was perpendicular to the direction of circulatory flow through the vessel phantom, with the focus centered in the middle of the vessel as shown in Figure 2.

Trapping Ability Evaluation

To study the effect of acoustic parameters on trapping, the trapping ability was evaluated by measuring the maximum mean fluid velocity of the circulation system for which a particle could remain trapped, termed here as the "maximum trapping velocity." This metric is physiologically relevant for cases when emboli flow downstream with an initial velocity and enters NET. To measure this, a clot-mimicking particle was allowed to flow into the focus of the transducer, and with fixed mean fluid velocity. The highest fluid velocity that the particle remained trapped for at least 20 seconds was recorded as the maximum fluid velocity. Initial tests demonstrated that the maximal trapping velocity measured was the same after 20 seconds as longer time periods. If the particle escaped before this time, the background velocity was decreased and the trial repeated until the maximum trapping velocity could be determined. Since the phantom was optically transparent, it was visually apparent when the particle escaped. With each parameter combination, four measurements were taken, and the averages and standard deviations were calculated.

Cavitation Monitoring

Acoustic backscatter measurements were taken to monitor the cavitation activity in the vessel phantom for each acoustic pressure. A passive 5 MHz single element transducer (2.5 cm aperture, 10 cm focal length, Valpey Fisher Corp., Hopkinton, MA) was placed in the center hole of the therapy transducer. The backscatter signals of the therapy pulses from the bubble cloud were range-gated and collected by a digital oscilloscope and analyzed using MATLAB. The moving standard deviation of the normalized acoustic backscatter energy was used to determine the initiation of the bubble cloud using a method previously described by Xu et. al (2005). This method detects initiation based on significantly increased temporal variability of the backscatter energy. The initiation threshold was when the moving standard deviations of normalized backscatter energy exceed a threshold based on the uninitiated normalized backscatter energy. The percentage of initiated time was calculated as the percentage of time period when the backscatter energy moving standard deviation was above the threshold over the entire time of histotripsy application at each trial (a total of 2000 pulses).

Debris Size Measurements

Blood clots were made from whole canine blood as previously described by Maxwell et. al (2009) and formed into cylindrical clots with 5 mm length sections and 4 mm diameters (see Figure 1c). Six clots were introduced to the flow system and the NET using 1000 Hz PRF, 10 cycle pulse length, and 19.9 MPa P_- . These initial parameters were chosen, because experimental observation suggests this parameter set can trap small particles (<1 mm) better than other parameter sets with lower PRF or pressure. The mean background flow velocity was 2 cm/s. Clots were treated in circulating saline solution, and the suspended debris was collected at the outlet of the circulation system. The debris experiment was conducted in saline rather than glycerol-water solution, due to the concern of cell lysis in the hypotonic glycerol solution. The saline was degassed to 80% to be consistent with the gas concentration of the glycerol-water solution. Debris size was measured using a Coulter counter with 100 μm and 560 μm aperture tubes (Multisizer 3, Beckman Coulter, CA, USA). This allowed measurements of particles between 2 and 300 microns. Any larger debris particles were removed and assessed under microscope for measurement prior to Coulter counter measurement. The Coulter counter counts and sizes particles suspended in an electrolyte by measuring the relative impedance change introduced by the particles.

Results

Figure 4 shows a time-series of images in which a 3–4 mm diameter clot particle was introduced into a 6 mm vessel phantom, pulled into the ultrasound focus, and completely fractionated over 25 seconds. The 6 mm vessel phantom and circulation system mimic the femoral vein in the leg. To study the effect of acoustic parameters on the trapping induced by histotripsy, 3–4 mm diameter agarose particles were introduced in a 6 mm diameter vessel phantom and their trapping under various acoustic parameters was studied. The maximum trapping velocity was measured as a function of the peak negative pressure, PRF and pulse length.

Effect of acoustic pressure

Trapping of the 3–4 mm clot-mimicking particle in the presence of a background flow (> 1 cm/s) was only observed when the pressure exceeded a threshold (Figure 5). For this pressure study, pulse length of 10 cycles were used at 500 Hz PRF. When the peak negative pressure (P_-) was below 15 MPa, the clot-mimicking particle was never trapped by the ultrasound field. Correspondingly, the bubble cloud was never initiated below 10 MPa P_- based on the acoustic backscatter measurement. At P_- of 11.9 and 14.2 MPa, the bubble cloud was intermittent and extinguished, with the initiated time occupying 6% and 78% of measured time. The intermittent bubble cloud did not create trapping at any background flow velocity. At P_- exceeding 15 MPa, the bubble cloud was always initiated and maintained without extinction (Figure 5a). At above 15 MPa P_- , trapping of the clot mimicking particles was observed in the presence of the background flow >1 cm/s. The maximum trapping velocity increased linearly with the P_- ($R^2 = .96$), from 0.5 cm/s for P_- of 15 MPa, to 7.3 cm/s for P_- of 19.9 MPa.

Effect of pulse length and PRF

The maximum trapping velocities for a 3–4 mm agarose particle under different pulse lengths and PRFs are plotted in Figures 6 a) and 6b). To study the effect of pulse length, 100 Hz PRF and 19.9 MPa P_- was used. The maximum trapping velocity for the 3–4 mm particle increased as the pulse length increased. For example, the maximum trapping velocity was 2.6 cm/s, 4.0 cm/s, and 5.6 cm/s for pulse lengths of 10, 20, and 40 cycles, respectively. However, the trend was not linear as in the case of acoustic pressure. A fit to the square root of pulse length showed that $R^2 = .93$.

The maximum trapping velocities also increased with increasing PRF. To study the effect of PRF, 10-cycle pulse length was used at 19.9 MPa P₋. The maximum trapping velocities were 2.7 cm/s, 7.5 cm/s, and 9.6 cm/s for PRFs of 100, 500 and 1000 Hz, respectively. A fit to the square root of PRF showed $R^2 = .95$. Since the maximum trapping velocity increased linearly with acoustic pressure (Figure 5), the maximum trapping velocity was fitted to the square root fit of pulse length and PRF (which vary linearly with acoustic intensity).

Debris size measurements

Six 5 mm length clots were treated by the NET using 1000 Hz PRF, 10-cycle pulse length and 19.9 MPa (P₋) under 2 cm/s background flow. The clots flowed into the transducer focus and were treated for 10–15 seconds before no visible clot particles remained at the focus. The solution containing all the clot debris fractionated by the NET was collected from the circulating system after treatment. The size distribution of debris from the NET was measured. First, the suspension with the debris particles was examined under visual observation. In 5 of the 6 treatments, there were no visible debris particles. Then, the five suspension samples and the sixth sample with the visible debris particles removed were run through the Coulter counter to characterize the debris size distribution. The Coulter counter tubes used here allow measurement of particles up to 300 μm . For each of the six samples, 2 measurements were made. No invalid measurements occurred, indicating that no particles above 300 μm in diameter were in the samples. The largest debris particle counted was 79 μm . The Coulter counter measurements showed that 99% of the total number of particles were smaller than 10 μm in diameter (Figure 7a), and occupied 39.2% of the total volume. Approximately 99% of the total volume of particles was smaller than 35 μm (Figure 7b).

In 1 of 6 treatments, 5 pieces of visible debris particles were found and removed for microscopic evaluation. Measurements with a microscope were made and the long and short axis of each debris particle was quantified using image analysis software. The 5 pieces of debris ranged from 583 μm long axis diameter $\times$ 465 μm short axis diameter to 1616 μm $\times$ 945 μm . When a cylindrical body is assumed, the volume from these larger fragments occupies approximately 4% of the volume of the original clot with dimensions of 4 mm diameter by 5 mm length.

Discussion

Our results show that a histotripsy-induced cavitation bubble cloud can trap a free-flowing clot particle. The maximum trapping velocity increased with increasing pressure, pulse length, and PRF. In this work, a 6 mm vessel could trap a 3–4 mm particle under an average blood velocity of up to 9.6 cm/s. In the upper portion of the leg, the common femoral vein (CFV) of acute DVT patients can vary from 5.00–21.70 mm in diameter, while the popliteal vein varies from 3.80–14.40 mm (Hertzberg et al., 1997). Blood flow velocities in the CFV varies widely from 6 cm/s to 40 cm/s, as found from a study of young healthy adults (Fromy et al., 1997). In DVT patients, the blood flow downstream from the occluded or partially occluded vein where the treatment is needed would be slower. Thus, the highest maximum trapping velocity of 9.6 cm/s achieved in our work could be sufficient for using NET in most venous applications. Further system optimization may improve these numbers to accommodate a greater range of vessel diameters at higher fluid velocities.

In many acoustic trapping methods, standing waves are responsible for particle attraction. Particles are drawn to the nodes and anti-nodes of standing waves through primary and secondary acoustic radiation forces (Nilsson et al., 2009). Our results show that trapping only occurs when the cavitation cloud is initiated. Microstreaming eddy currents set up by single bubble oscillations is a well-known phenomenon (Elder, 1958; Rogers and Neild, 2011). In the case of histotripsy, we hypothesize that the trapping is due to cavitation cloud

enhanced streaming. The acoustic radiation force applied by the histotripsy pulse combined with the highly attenuative bubble cloud produce significant streaming in the blood. Models predict that under high flow, the streaming will be proportionate to acoustic pressure (Mitome et al., 1995). Similarly, in this article we found that the maximum trapping crossflow velocity was a linear function of acoustic pressure. The fluid flow confined to the boundaries of the vessel wall produce recirculation streamlines with local pressure gradients such that the clot particles can be trapped in the lower pressure locations. This hypothesized NET mechanism was investigated separately using particle image velocimetry (PIV) to follow the fluid streaming induced by histotripsy in a vessel (Figure 8). With the histotripsy-induced cavitation bubble cloud, the representative PIV image shows two vortices produced on either side of the bubble cloud, similar to that observed by Marten et al. in fish capillaries under microstreaming conditions (Marten et al., 1982). The clot particle was observed to be trapped in either of the vortices. Research to further understand the mechanism behind NET is currently underway. To improve the trapping ability, in addition to parameter adjustments, it is also possible to generate more than one cavitation cloud to alter the streaming patterns with multiple vortices favoring trapping.

In a previous work studying the size distribution of histotripsy-treated debris in bulk tissue, >99% of treated debris were < 6 μm (Xu et al., 2008). In this study, in 1 of 6 clots, few larger fragments of several hundred microns were observed. Other than these larger fragments, the largest clot debris particle size was measured to be 79 μm . Such particles are unlikely to form hazardous emboli, especially in the venous circulation. Currently, 100- μm pore mechanical filters are used for preventing embolism in the carotid or coronary arterial circulation (circulatory beds with the least tolerance for obstruction) during catheter-based arterial procedures. (Eskandari, 2005; Yadav, 2004). It was unclear what resulted in the larger fragments in that one particular measurement. To completely eliminate any fragments larger than 100 μm using NET, a future study examining NET parameters to minimize the debris clot particles will be conducted.

The debris experiment was conducted in the saline instead of glycerol-water solution, due to the concern of cell lysis from the hypotonic glycerol solution. The glycerol-water based solution was formulated to mimic the viscosity of blood at high shear rates. Despite the viscosity difference between the saline and the glycerol-water solution, there was no significant difference in the threshold to generating cavitation bubble cloud and in the maximum trapping ability between trials using the glycerol-water solution and regular water. We expect differences when transferring this idea to an *in-vivo* model and we are working to set up an animal model to test these differences. It is worth noting that parameters similar to those used in this NET study have been used to generate bubble cloud non-invasive *in-vivo* in the blood in the femoral vein (Maxwell et al. 2011) and in the heart chamber (Xu et al. 2010; Owens et al. 2011a; Owens et al. 2011b). The similar parameters have also been used to break up blood clots *in vivo* (Maxwell et al. 2011).

Since we are proposing to generate a cavitation bubble cloud into a vessel, safety concerns such as hemolysis and vessel damage should be addressed. Studies involving rotating exposure chambers have examined the effects of microbubbles on hemolysis and concluded that microbubble activity increases the likelihood of hemolysis (Poliachik et al., 1999). However, the extent of hemolysis may have been overestimated through non-acoustic factors such as bubble recycling (Williams and Miller, 1989). As our bubble cloud introduces a self-made rotation mechanism, namely the vortices, we do expect some degree of bubble recycling as vortices by nature introduce return flow. In our recent *in vivo* preliminary experiments exposing free flowing porcine venous blood to histotripsy-induced cavitation bubbles, we observed increases in free hemoglobin (from 8 to 400 mg/dl) and lactate dehydrogenase (315 to 715 IU/L) with no change in hematocrit after 30 minutes of

therapy. This suggests that some hemolysis is occurring but that the erythrocyte count is unaltered. Maxwell et al. (2011) has studied histotripsy-based thrombolysis *in-vivo* in a porcine model and no major immediate complications that might occur with major hemolytic effects were observed. Likewise, Xu et al. has used histotripsy to perforate the atrial and ventricular septum in the heart of adult dogs and neonatal pigs, and no major complications were observed immediately or within a month after the treatment (Owens et al., 2011a; Owens et al., 2011b; Xu et al., 2010). In both these cases, a cavitating bubble cloud was exposed directly to blood either in the femoral vein or in the heart chambers, and did not produce any immediate side effects. Thus, a safe non-invasive embolus trap may be feasible for trapping and erosion. Vessel damage was also studied previously for histotripsy-based thrombolysis. In an *in-vivo* study, vessel damage was limited to focal endothelial damage and minor hemorrhage if cavitation was confined to the vessel lumen (Maxwell et al., 2011). Further animal studies are necessary to determine the in-vivo feasibility and safety of NET.

Conclusions

The effect of acoustic parameters on the trapping ability of histotripsy-induced NET was studied. The trapping only occurred when a cavitation bubble cloud was initiated. Using the maximum trapping velocity as a metric to evaluate trapping ability, the maximum trapping velocity increased linearly with acoustic pressure amplitude and as the square root of pulse length and PRF. These findings will help us select NET parameters that are energy efficient. Future work will focus on finding optimal parameters to minimize the debris size of NET-treated emboli and improve the overall safety of NET.

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References

- Bates MC, Campbell JE. Pitfalls of embolic protection. *Techniques in Vascular and Interventional Radiology*. 2011; 14:101–107. [PubMed: 21550513]
- Bosiers M, Deloose K, Verbist J, Peeters P. The impact of embolic protection device and stent design on the outcome cas. *Perspectives in Vascular Surgery and Endovascular Therapy*. 2008; 20:272–279. [PubMed: 18930936]
- Cohen DJ, Ramee S, Baim DS, Sharma S, Carrozza JP, Cosgrove R, Jones N, Berezin RH, Cutlip DE, Ho KKL, Kuntz RE. Economic assessment of rheolytic thrombectomy versus intracoronary urokinase for treatment of extensive intracoronary thrombus: Results from a randomized clinical trial. *American Heart Journal*. 2001; 142:648–656. [PubMed: 11579355]
- Deitelzweig SB, Johnson BH, Lin J, Schulman KL. Prevalence of clinical venous thromboembolism in the USA: Current trends and future projections. *American Journal of Hematology*. 2011; 86:217–220. [PubMed: 21264912]
- Elder SA. Cavitation microstreaming. *Journal of the Acoustical Society of America*. 1958; 31:54–64.
- Eskandari MK. Cerebral embolic protection. *Seminars in Vascular Surgery*. 2005; 18:95–100. [PubMed: 15986327]
- Fowkes FJI, Price JF, Fowkes FGR. Incidence of diagnosed deep veing thrombosis in the general population: Systematic review. *Eur J Vasc Endovasc Surg*. 2003; 25:1–5. [PubMed: 12525804]
- Francis CW, Suchkova VN. Ultrasound and thrombolysis. *Vascular Medicine*. 2001; 6:181–187. [PubMed: 11789973]
- Fromy B, Legrand MS, Abraham P, Leftheriotis G, Cales P, Saumet JL. Effects of positive pressure on both femoral venous and arterial blood velocities and the cutaneous microcirculation of the forefoot. *Cardiovascular Research*. 1997; 36:372–376. [PubMed: 9534858]

- Gutt CN, Oniu T, Wolkner F, Mehrabi A, Mistry S, Buchler MW. Prophylaxis and treatment of deep vein thrombosis in general surgery. *The American Journal of Surgery*. 2005; 189:14–22.
- Hertzberg BS, Kliewer MA, DeLong DM, Lalouche KJ, Paulson EK, Frederick MG, Carroll BA. Sonographic assessment of lower limb vein diameters: Implications for the diagnosis and characterization of deep vein thrombosis. *American Journal of Roentgenology*. 1997; 168:1253–1257. [PubMed: 9129422]
- Hitchcock KE, Holland CK. Ultrasound-assisted thrombolysis for stroke therapy. *Stroke*. 2010; 41:S50–S53. [PubMed: 20876505]
- Holland CK, Vaidya SS, Datta S, Coussios C-C, Shaw GJ. Ultrasound-enhanced tissue plasminogen activator thrombolysis in an in vitro porcine clot model. *Thrombosis Research*. 2008; 121:663–673. [PubMed: 17854867]
- Kyrle PA, Eichinger S. Deep vein thrombosis. *Lancet*. 2005; 365:1163–1174. [PubMed: 15794972]
- Marten CJ, Pratt BM, Watmough DJ. A study of ultrasound-induced microstreaming in blood vessels of tropical fish. *British Journal of Cancer*. 1982; 45:161–164.
- Maxwell AD, Cain CA, Duryea AP, Yuan L, Gurm HS, Xu Z. Noninvasive thrombolysis using pulsed ultrasound cavitation therapy-histotripsy. *Ultrasound in Medicine and Biology*. 2009; 35:1982–1994. [PubMed: 19854563]
- Maxwell AD, Owens G, Gurm HS, Xu Z. Noninvasive treatment of deep venous thrombosis using pulsed ultrasound cavitation therapy (histotripsy) in a porcine model. *J Vasc Interv Radiol*. 2011; 22:369–377. [PubMed: 21194969]
- Menon J, Salman MM, Hamilton G. Venous thrombolysis: Current perspectives. *Current Treatment Options in Cardiovascular Medicine*. 2004; 6:159–168. [PubMed: 15066245]
- Mewissen MW, Seabrook GR, Meissner MH, Cynamon J, Labropoulos N, Haughton SH. Catheter-directed thrombolysis for lower extremity deep venous thrombosis: Report of a national multicenter registry. *Radiology*. 1999; 211:39–49. [PubMed: 10189452]
- Mitome H, Kozuka T, Tuziuti T. Effects of nonlinearity in development of acoustic streaming. *Jpn J Appl Phys*. 1995; 34:2584–2589.
- Nilsson J, Evander M, Hammarstrom B, Laurell T. Review of cell and particle trapping in microfluidic systems. *Analytica Chimica Acta*. 2009; 649:141–157. [PubMed: 19699390]
- Owens GE, Miller RM, Ensing G, Ives K, Gordon D, Ludomirsky A, Xu Z. Therapeutic ultrasound to non-invasively create intracardiac communications in an intact animal model. *Catheter Cardiovasc Interv*. 2011a; 77:580–588. [PubMed: 20853366]
- Owens GE, Miller RM, Owens ST, Swanson SD, Ives K, Ensing G, Gordon D, Xu Z. Intermediate-term effects of intracardiac communications created non-invasively by histotripsy. *Pediatr Cardiol*. 2011b; 11:11.
- Parikh S, Motarjeme A, McNamara T, Raabe R, Hagspiel K, Benenati JF, Sterling K, Comerota A. Ultrasound-accelerated thrombolysis for the treatment of deep vein thrombosis: Initial clinical experience. *J Vasc Interv Radiol*. 2008; 19:521–528. [PubMed: 18375296]
- Parsons JE, Cain CA, Abrams GD, Fowlkes JB. Pulsed cavitation ultrasound therapy for controlled tissue homogenization. *Ultrasound in Medicine and Biology*. 2006a; 32:115–129. [PubMed: 16364803]
- Parsons JE, Cain CA, Fowlkes JB. Cost-effective assembly of a basic fiber-optic hydrophone for measurement of high-amplitude therapeutic ultrasound fields. *J Acoust Soc Am*. 2006b; 119:1432–1440. [PubMed: 16583887]
- Poliachik SL, Chandler WL, Mourad PD, Bailey MR, Bloch S, Cleveland RO, Kaczowski P, Keilman G, Porter T, Crum LA. Effect of high-intensity focused ultrasound on whole blood with and without microbubble contrast agent. *Ultrasound in Medicine and Biology*. 1999; 25:991–998. [PubMed: 10461729]
- Roberts WW, Hall TL, Ives K, Wolf JS, Fowlkes JB, ACC. Pulsed cavitation ultrasound: A noninvasive technology for controlled tissue ablation (histotripsy) in the rabbit kidney. *Journal of Urology*. 2006; 175:734–738. [PubMed: 16407041]
- Rogers P, Neild A. Selective particle trapping using an oscillating microbubble. *Lab on a Chip*. 2011; 11:3710–3715. [PubMed: 21947131]

- Rosenschein U, Furman V, Kerner E, Fabian I, Bernheim J, Eshel Y. Ultrasound imaging-guided noninvasive ultrasound thrombolysis: Preclinical results. *Circulation*. 2000; 102:238–245. [PubMed: 10889137]
- Ryan LK, Foster FS. Tissue equivalent vessel phantom for intravascular ultrasound. *Ultrasound in Medicine and Biology*. 1997; 23:261–273. [PubMed: 9140183]
- Sauren LD, la Meir M, Bolotin G, van der Veen FH, Heijmans JH, Mess WH, Maessen JG. The emblocker: Efficiency of a new ultrasonic embolic protection device adjunctive to heart valve surgery. *Ann Thorac Surg*. 2009; 88:253–257. [PubMed: 19559235]
- Sauren LDC, la Meir M, Palmen M, Severdija E, van der Veen FH, Mess WH, Maessen JG. New ultrasonic radiation reduces cerebral emboli during extracorporeal circulation. *European Journal of Cardio-thoracic Surgery*. 2007;32. [PubMed: 17980613]
- Siegel RJ, Atar S, Fishbein C, Brash AV, Peterson TM, Nagai T, Pal D, Nishioka T, Chae J, Birnbaum Y, Zaneli C, Luo H. Noninvasive transcutaneous low frequency ultrasound enhances thrombolysis in peripheral and coronary arteries. *Echocardiography*. 2001; 18:247–257. [PubMed: 11322908]
- Webb LA, Dixon SR, Safian RD, O'Neill WW. Usefulness of embolic protection devices during saphenous vein graft intervention in a nonselected population. *Journal of Interventional Cardiology*. 2005; 18:73–75. [PubMed: 15882150]
- Williams AR, Miller DL. The role of non-acoustic factors in the induction and proliferation of cavitation activity *in vitro*. *Phys Med Biol*. 1989; 34:1561–1569. [PubMed: 2587627]
- Xu Z, Fan Z, Hall TL, Winterroth F, Fowlkes JB, Cain CA. Size measurement of tissue debris particles generated from pulsed ultrasound cavitation therapy-histotripsy. *Ultrasound in Medicine and Biology*. 2008; 35:245–255. [PubMed: 19027218]
- Xu Z, Fowlkes JB, Rothman ED, Levin AM, Cain CA. Controlled ultrasound tissue erosion: The role of dynamic interaction between insonation and microbubble activity. *Journal of the Acoustical Society of America*. 2005; 117:424–435. [PubMed: 15704435]
- Xu Z, Ludmirsky A, Eun LY, Hall TL, Tran BC, Fowlkes JB, Cain CA. Controlled ultrasound tissue erosion. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*. 2004; 51:726–736.
- Xu Z, Owens G, Gordon D, Cain CA, Ludmirsky A. Noninvasive creation of an atrial septal defect by histotripsy in a canine model. *Circulation*. 2010; 121:742–749. [PubMed: 20124126]
- Yadav JS. Embolic protection devices: Methods, techniques, and data. *Techniques in vascular and interventional radiology*. 2004; 7:190–193. [PubMed: 16084443]

Figure 1.

Phantoms. A) The two-part, optically transparent gelatin vessel phantom used in line with the circulation system. The clot mimicking particles were visible within the phantom; B) Clot mimicking particles made from an agarose solution mixed with food dye for contrast, and spherically shaped by dropping into cold mineral oil; C) Blood clot model treated with NET and used to find distribution of treated debris.

Figure 2.

Experimental setup showing the closed loop circulation system, including the gelatin vessel phantom. The transducer was aligned perpendicular to the vessel phantom, and the focus was centered to the vessel. The phantom and transducer were kept in a water tank, while the rest of the circulation system was kept at the same height but outside the tank.

Figure 3.

A representative 10-cycle pulse pressure waveform recorded at the focus of the transducer with a fiber-optic hydrophone with peak negative pressure $P_- = 19.9$ MPa.

Figure 4.

Time-series of a blood clot treatment by NET. Clot flew into focus and was eroded to small debris within 25 seconds. The particle was flowing from left to right at 2.3 cm/s background flow velocity. PRF of 500 Hz, 10 cycle pulse length, and 19.9 MPa P- were used for NET treatment. The bubble cloud was present but not visible in the pictures. The large bubbles visible in all the frames were part of the gel vessel phantom.

Figure 5.

A) Peak negative pressure vs. maximum trapping velocity. Effective trapping was observed at >15MPa, and the maximum trapping velocity increases linearly with pressure at >15 MPa. B) The bubble cloud initiated time vs. pressure. At >15 MPa, the bubble cloud was initiated >80% of the time based on acoustic backscatter measurement. NET acoustic parameters were 500 Hz PRF and 10-cycle pulse length. The error bars indicate the standard deviation from the 4 trials at each pressure.

Figure 6.

A) The maximum trapping velocity as a function of the pulse length, and B) as a function of the pulse repetition frequency (PRF). The maximum trapping velocity increased nonlinearly with pulse length and PRF. Note the non-zero intercept, which results from the fact that trapping only occurs when cavitation is initiated and sustained. For low pulse length or PRF, cavitation could not be consistently sustained. For pulse length study, 100 Hz PRF was used at 19.9 MPa P-. For PRF study, 10-cycle pulse length was used at 19.9 MPa P-. The error bars indicate the standard deviation from the 4 trials at pulse length or PRF.

Figure 7.

Coulter counter measurements for debris particle sizes between 2 and 300 microns. A) Number % of debris particles between 2 and 300 microns. There were 60 bins with bin size at 5 μm . > 99% of particles were < 5 μm . B) Volume % of debris particles between 2 and 300 μm . Approximately 99% of the volume was occupied by particles < 35 μm . The ultrasound parameters were 1000 Hz PRF, 10-cycle pulse length, and 19.9 MPa P-. The background flow velocity was 2 cm/s.

Figure 8.

Representative Particle Image Velocimetry map of streamlines when therapy transducer is on. Note the vortices formed on either side of the focus, and the intense fluid flow velocity at the focus. This particular analysis used data from a treatment using 19.9 MPa P-, 10-cycle pulse length and 100 Hz PRF. There was no background fluid flow, and the focus of the therapy transducer was centered in the vessel. The peak streaming velocity was 12 cm/s, comparable to the *in-vivo* vessel blood flow.