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Pulsed Focused Ultrasound Induced Displacements in Confined *In Vitro* Blood Clots

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Abstract

Ultrasound has been shown to potentiate the effects of tissue plasminogen activator (tPA) to improve clot lysis in a range of *in vitro* and *in vivo* studies as well as in clinical trials. One possible mechanism of action is acoustic radiation force induced clot displacements. In this study we investigate the temporal and spatial dynamics of clot displacements and strain initiated by focused ultrasound pulses. Displacements were produced by a 1.51 MHz f-number 1 transducer over a range of acoustic powers (1–85 W) in clots constrained within an agar vessel phantom channel. Displacements were tracked during and after a 5.45 ms therapy pulse using a 20 MHz high frequency ultrasound imaging probe. Peak thrombus displacements were found to be linear as a function of acoustic power up to 60 W before leveling off near 128 μm for the highest transmit powers. The time to peak displacement and recovery time of blood clots were largely independent of acoustic powers with measured values near 2 ms. A linear relationship between peak axial strain and transmit power was observed, reaching a peak value of 11% at 35 W. The peak strain occurred ~ 0.75 mm from the focal zone for all powers investigated in both lateral and axial directions. These results indicate that substantial displacements can be induced by focused ultrasound in confined blood clots, and that the spatial and temporal displacement patterns are complex and highly dependant on exposure conditions, which has implications for future work investigating their link to clot lysis and for developing approaches to exploit these effects.

Index Terms

Ultrasound; thrombolysis; tissue plasminogen activator; ischemic stroke

I. Introduction

Sonothrombolysis is under investigation as a means of enhancing blood clot degradation and holds considerable potential for applications such as myocardial infarction, deep vein thrombosis and acute ischemic stroke. While some work has concentrated upon the use of microbubbles or cavitation [1]–[5] the majority of sonothrombolysis research has investigated ultrasound in conjunction with lytic agents [6], [7], where there is now

extensive *in vitro*, *in vivo* and initial clinical evidence that ultrasound can increase the effectiveness of lytic agents to improve thrombolysis.

Sonothrombolysis effects have been achieved at a wide range of frequencies, pressures and pulsing schemes, however the majority of studies have employed relatively low intensities, with either clinical diagnostic systems or weakly focused transducers [8]–[13]. In clinical work with acute stroke for example, derated acoustic pressures were estimated to be approximately 70 kPa [14]. With the advent of improved transcranial delivery systems there is the possibility of transmitting higher intensity pulses through the skull [15]–[18], which may offer the potential to improve thrombolysis rates further. Of particular interest to the present study is initial work exploring the effects of high intensity focused ultrasound (HIFU) thrombolysis with tPA, operating at intensities above typical diagnostic levels but below the cavitation threshold. Frenkel et al. [19] demonstrated increased *in vitro* clot weight loss with the combination of 1 MHz pulsed focused ultrasound and tPA over tPA alone (62% to 50%, respectively). Stone et al. [20] demonstrated more than four times increase in clot degradation *in vivo* in a rabbit ear vein thrombus model five hours after treatment for focused ultrasound and tPA over tPA alone (96% vs. 22%, respectively) with nearly identical ultrasound exposures. Neither study demonstrated significant clot reduction through the use of focused ultrasound alone under the same conditions.

The determination of optimal ultrasound conditions to employ for a particular sonothrombolysis application is impeded to a significant extent by the lack of knowledge about the mechanisms of action. It is likely that the mechanisms will be dependant upon the particular operating conditions employed and of interest in this paper are sub-cavitation threshold exposure conditions in the absence of injected microbubbles. In this context, several mechanisms have been proposed to be involved in ultrasound mediated clot degradation with tPA, including heating and acoustic radiation forces. The absorption of ultrasound can lead to tissue heating, to an extent that will be related to the ultrasound intensity, duty cycle and exposure duration as well as the tissue absorption properties. For example, the continuous wave ultrasound study of Sakharov et al. [21] indicated that heating played a significant role in enhancing thrombolysis, as activity of tPA is known to be temperature dependant [22]. For shorter pulses and lower duty cycles and intensities, it is frequently considered that heating may not play a primary role in enhancing thrombolysis [23].

Acoustic radiation force, arising from a transfer of momentum from the wave to the propagating medium, can generate fluid streaming and tissue motion. It has been proposed that if the fluid streaming is significant relative to the existing blood flow, it can act as a ‘mild stirrer’ enhancing the supply of plasminogen and plasminogen activator from outside the clot exterior directly to the clot surface [24], [25], though there appears to be no direct evidence of this at present.

It has also been proposed that radiation forces may play a key role in sonothrombolysis by inducing clot displacements [19], [20], [26]. In particular, it has been hypothesized that a gradient in the displacements across the ultrasound beam could induce significant strain in the clot that would be associated with pore widening, which would in turn facilitate tPA

transport. Consistent with this possibility, the work of Frenkel et al. [19] for pulsed high intensity (20–80 acoustic watts) ultrasound thrombolysis observed a linear increase in the thrombolytic effects of tPA as function of transmitted power, which is proportional to the acoustic radiation force. As the clots were exposed to tPA subsequent (5 minutes after) to ultrasound treatments, thermal effects were not considered to be a significant factor in achieving these results. Jones et al. [26] found that for the case of high-intensity pulsed ultrasound of suspended *in vitro* clots peak displacements of 243 μm were observed for conditions that enhanced clot lysis (60 acoustic watts). These results provided a valuable first estimate of focused ultrasound induced displacements in thrombus and correlated it with the presence of a thrombolytic effect. These measurements were conducted using unconstrained clots and provided a single value of displacement (along the beam axis) from the end point of the transmit pulse, without examining strain.

An understanding of the potential role of ultrasound induced displacements and strain in sonothrombolysis will benefit from direct information about the kinetics and spatial distribution of displacements within focused ultrasound beams. Furthermore, clinically relevant applications such as acute ischemic stroke involve clots lodged within blood vessels, where boundary conditions will presumably influence displacements. To this end, in this study, we investigate the spatial and temporal variation of displacements and strain induced by pulsed focused ultrasound for *in vitro* clots situated within vessel mimicking phantoms.

II. Materials and methods

A. Sample preparation

Blood clots were formed in a manner similar to previously described approaches [8]. Rabbit arterial blood was withdrawn from the auricular artery of New Zealand white rabbits into citrate Vacutainer tubes (0.109 M, 3.2% sodium citrate). Clot formation was achieved through recalcification by mixing 600 μL of citrated blood with 40 μL of degassed bovine thrombin (25 NIH units/ml in saline; Sigma Aldrich, St. Louis, Missouri, USA) and 75 μL of degassed CaCl_2 (100 mmol/L in deionized water; Sigma Aldrich, St. Louis, Missouri, USA). Immediately after mixing, clots were injected into a polypropylene barbed connector (Cole-Parmer, Montreal, QC, Canada) of 6 mm inner diameter, 10 mm length. Clots were incubated at 37°C for 1 hour in a water bath.

The vessel phantom used in these experiments is illustrated in Fig. 1. A 2.5% agar phantom with an estimated attenuation coefficient α of 1 dB/cm [27] was cast within an acrylic frame (10 $\times$ 10 $\times$ 2.5 cm). A 3.5 mm diameter flow channel was created when casting the gel along the length of the phantom, 1 cm below the surface and at its centre a 5 mm long 1.5 mm diameter narrowing was created. Prior to clot introduction the channel was primed with degassed saline. After the connector housing the thrombus was attached to the phantom's acrylic casing, positive pressure from a syringe filled with degassed saline was used to dislodge the entrapped thrombus until it lodged at the narrowing.

B. Focused ultrasound system

For the purposes of this paper, focused ultrasound pulses used to induce clot displacements will be referred to as therapeutic ultrasound pulses. These were initiated with a custom built single element spherically focused air backed transducer (10 cm focal length, 10 cm diameter) with a center frequency of 1.51 MHz. Transmit signals were generated at a center frequency of 1.51 MHz with a function generator (model AFG 3102, Tektronix, Beaverton, Oregon, USA), amplified by 50 dB, (model A-500, ENI, Rochester, NY, USA) and passed through a custom impedance matching circuit.

The total acoustic power as a function of applied electrical power delivered to the therapy transducer was measured using a radiation force technique, as described in [28]. The maximum applied acoustic power (temporal peak) that could be measured was 45 W. Linear extrapolation was used for acoustic powers up to 85 W. Pressure measurements were conducted with a fiber-optic needle hydrophone (Precision Acoustic Ltd, Dorset, UK) in degassed water. The focal zone of the therapy transducer has a -3 dB beamwidth of 0.9 mm and a -3 dB depth-of-field of 7.1 mm. Due to limitations of the hydrophone the peak positive pressure could be only be calibrated up to 5 MPa. The spatial peak temporal peak intensity (I_{SPTP}), referred to as I , was calculated based on the peak positive pressure using [29]. The relationship between acoustic power, pressure and intensity is shown in Table 1. We note that all power levels employed were below the threshold required to produce cavitation, as confirmed in separate experiments.

C. Experimental configuration

An overview of the experimental setup is shown in Fig. 2. The therapy transducer was mounted to a micrometer controlled 3-axis positioning stage and situated below the phantom with its beam directed upwards at an angle of 15° with respect to the vertical and intersecting the phantom vessel axis at a perpendicular angle. Pulse echo measurements were used to situate the therapeutic focus in the middle of channel by placing an 18 gauge needle-mounted 1 mm diameter steel ball within the center of the channel prior to thrombus introduction. Absorbing material was placed above the phantom minimize reflected waves. All experiments were performed in a water tank filled with degassed water at room temperature.

Displacements were measured with a 12–38 MHz single element RMV710B transducer (focal length 12 mm; -6 dB beamwidth 0.16 mm; -6 dB depth-of-field 2.6 mm) operating on a VisualSonics Vevo 770 imaging system (Toronto, Canada). A nominal 1 cycle imaging pulse centered at 20 MHz was employed, which had a measured -6 dB bandwidth of 55% and corresponded to an $80\text{ }\mu\text{m}$ -6 dB pulse length. The imaging transducer was situated above the phantom to provide a longitudinal view through the center of the vessel (Fig. 3). It was oriented at an angle of 60° with respect to the vertical, thereby intersecting with the therapy beam axis at an angle of 45 degrees. It should be noted that the motivation for employing a high frequency transducer in this configuration was to obtain a high resolution (axial, lateral and elevation plane) displacement map. Configurations of single element therapy transducers with coaxially oriented imaging transducers arrays mounted within the aperture are feasible to fabricate or purchase [26]. However, such an approach is not well

suited for higher frequency transducers as there must be sufficient offset from the location of the imaging transducer relative to the therapy transducer focus in order to not interfere with the therapy beam formation.

Displacements were measured along the line of sight of the imaging transducer beam as a function of time with low amplitude single cycle imaging pulses. An overview of the pulse sequence for the imaging and therapy transducers is shown in Fig. 4. The first imaging pulse served as a reference for the initial position. The subsequent 10 imaging pulses were interleaved with ten, 500 μ s ‘pushing’ pulses which themselves were separated by 50 μ s gaps. The purpose of the 50 μ s gaps between 500 μ s pulses were to enable the acquisition of an imaging pulse without electronic and acoustic interference between the therapy transmit pulse and the receive electronics of the imaging transducer. This sequence created a 90.9% duty cycle therapy pulse of 5.45 ms duration, and afforded the opportunity to track displacements at a temporal resolution of 550 μ s during the treatment sequence. The rationale for the selection of 5 ms pulses (total on-time 5.45 ms) was based on pilot experiments that indicated displacements saturated after several milliseconds (data shown in a subsequent section). Employing longer pulses (i.e. up to 100 ms) therefore served no purpose from a displacement perspective, whereas a 5 ms pulse provided a sufficiently long time to induce displacements that were detectable at lower transmit amplitudes.

An overview of the data acquisition process is shown in Fig. 5. The received RF echoes from the imaging transducer were acquired with a 12-bit personal computer based digitizer card (Acquiris model DP310; Monroe, NY, USA) at a sampling frequency f_s of 400 MHz and stored for offline processing. To interleave imaging and therapy pulses, the external trigger of the VisualSonics machine was connected to the AWG (arbitrary waveform generator), which was used to initiate the therapy pulses and coordinate their relative timing of the imaging line acquisition as detailed previously.

D. Displacement estimation

The resulting received imaging transducer signals therefore provided a series of RF lines taken at intervals during the therapy pulse transmit sequence. These sequential imaging lines were then used to estimate displacements with a 2-D autocorrelation algorithm [30]. The 2D autocorrelator operates on the discrete analytic signal of the RF data, with the analytic signal r being defined as:

$$\hat{r} = r(m, n) - j \tilde{r}(m, n) \quad (1)$$

where $r(m, n)$ is the original RF data, $\tilde{r}(m, n)$ is the 1D Hilbert transform along the depth direction, m is the sample number along the depth direction and n is the sample number along the RF signal temporal direction. This technique can be used to estimate displacements (d_{est}) with the following equation:

$$\langle d_{est} \rangle = \frac{1}{f_{PRF}} \langle v_{est} \rangle \quad (2)$$

$$= \frac{c}{2} \frac{\langle F_d \rangle \cdot \frac{1}{f_{PRF}}}{\langle f_{RF} \rangle} \quad (3)$$

$$= \frac{c}{2} \frac{\tan^{-1} \left\{ \frac{\text{Im} \left[\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} \hat{r}(m,n) \hat{r}^*(m,n+1) \right]}{\text{Re} \left[\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} \hat{r}(m,n) \hat{r}^*(m,n+1) \right]} \right\}}{f_s \cdot \tan^{-1} \left\{ \frac{\text{Im} \left[\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} \hat{r}(m,n) \hat{r}^*(m+1,n) \right]}{\text{Re} \left[\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} \hat{r}(m,n) \hat{r}^*(m+1,n) \right]} \right\}} \quad (4)$$

where c is the speed of sound, f_s is the RF sampling rate, $\langle v_{est} \rangle$ is the estimated velocity, $\langle F_d \rangle$ is the mean normalized Doppler frequency, f_{PRF} is the pulse repetition frequency, $\langle f_{RF} \rangle$ is the mean normalized RF frequency, M is the length of the depth window, N is the length of the temporal window, and $\hat{r}^*$ is the complex conjugate of the analytic signal. Also we note that the original notation of this equation as presented in Loupas et al. [30] has been altered to be expressed in terms of displacement rather than velocity estimates. Before performing displacement estimates with this equation the RF data lines were up-sampled from 0.4 to 4 GHz. For each pulse-pair (i.e. consecutive RF lines, $N=2$) calculations were applied across a sliding depth window (75% overlap) of $M=60$ samples, corresponding to the transmitted pulse length. Higher displacements can however undergo aliasing, in particular when they exceed the Nyquist limit of 18.75 μm between imaging pulses (spaced at 550 μs). To overcome this problem an extended autocorrelation algorithm was implemented [31], which allows estimation of displacements above the Nyquist limit. With this approach, phase information is used to make initial displacement estimates through the above Eqn 2, and the envelope is then used to resolve potential ambiguities for displacement magnitudes that are above the Nyquist limit. In particular, if aliasing is considered the potential displacement candidates are:

$$d_n = \langle d_{est} \rangle - \frac{n \cdot c}{2 \cdot f_c} \quad \text{for } n=0, \pm 1, \pm 2 \dots, \quad (5)$$

When the displacements between adjacent imaging pulses are within the Nyquist limits (18.75 μm between pulses) they will be correctly estimated by equation (2) and n will equal 0. Beyond this point $\langle d_{est} \rangle$ will be aliased and n will be nonzero. According to the approach of Lai et al [31], the appropriate n value to employ (n') is found by maximizing the envelop correlation function such that $n' = \arg \max_n (R(\tau_n, 1))$ where $R(m,n)$ is the envelop autocorrelation function of the received signals and $\tau_n = 2 \cdot d_n / (c \cdot f_c)$, expressed in terms of samples. These calculations were repeated for all adjacent pulse pairs. Therefore, this process resulted in a series of displacement estimates as a function of depth along the line of sight of the imaging transducer beam and as a function of time (550 μs intervals) from the baseline point until 25

ms after the therapy pulse is terminated. Similar to the situation for blood flow applications, these displacement estimates were then corrected for the relative angle between the axes of the imaging and therapy transducer beams (45 degrees) to obtain the estimates of the *axial* component of displacements along the therapy beam axis. Implicit in doing this is the assumption that radiation force induced displacements will be along the therapy transducer beam axis. In the case of radiation force induced displacements with a highly focused ultrasound beam it is generally considered that the axial displacements at the focal zone are the dominant form of displacement and that lateral displacements are much smaller [32], [33].

Prior to estimating displacements in the clot, displacement corrections were first applied to compensate for motion of the agar phantom due to ambient vibrations, likely associated with background building motion. This was accomplished by selecting a depth range within the phantom, (1–2 mm preceding the vessel boundary) estimating displacements in this region, and using these displacement estimates to correct for agar motion as a function of time along each line of sight, based on the assumption of rigid body translation of the phantom. The compensations were typically on the order of several microns (8 μm maximum) over the time range of the experiments, and were found to effectively compensate for these effects. This correction assumes that the therapy pulse does not induce substantial motion in the agar phantom and that the vibrations are uniform throughout the phantom. To verify this, displacements were assessed in the phantom after this compensation was applied and were found to be undetectable (15 W transmit pulse).

E. Experiments performed

The first set of experiments examined displacements along the imaging transducer beam axis when it was in a location that passed through the center of the therapy beam axis. The second set of experiments were conducted when the imaging transducer beam axis was located at a series of locations with different offsets along the vessel axis with respect to the intersection point with the therapy beam axis. These are referred to as lateral offset experiments.

1. Measurements of thrombus displacement and strain across the therapy beam axis—Each focused ultrasound pulse creates a clot displacement profile that varies spatially and temporally and is dependent on the transmit parameters. The peak clot displacement value coincided with the maximum displacement in both the spatial and temporal domain. In these experiments, the mean and standard deviation peak clot displacement vs. transmit power was assessed from 1–85 acoustic watts. Clot displacements below 3 μm could not be resolved due to poor signal to noise ratio (SNR).

The temporal kinetics of clot displacements were also assessed in this study. The time to peak (TTP) was defined as the time required for the clot to reach 90% of its peak displacement. The recovery time (RT), which gives an indication of the amount of damping present in the tissue, was defined as the time interval between the displacement value immediately after pulse termination and 10% of that value. To increase temporal resolution, low-pass interpolation was used to up-sample the results from 550 to 55 μs [34].

An indication of shear strain induced by the therapy pulse was assessed by computing differential displacements at each point along the depth direction for each tracking pulse pair. Due to the noise involved for such estimations a fifth order lowpass Butterworth filter was applied to the displacement data prior to differentiation. The absolute strain value S_i at the midpoint between two adjacent displacement values was calculated by taking the spatial gradient in displacements along the imaging beam axis when it was in a location intersecting the therapy beam axis:

$$S_i = \frac{d_i - d_{i-1}}{\Delta D \cdot \cos(45)} \quad (6)$$

where d_i and d_{i-1} are magnitudes of displacement at the depth points (along the imaging beam axis) on either side of the mid-point, D is their separation in the depth direction and the cosine term accounts for the intersection angle.

2. Measurements as a function of lateral offset with respect to therapy beam axis—For these experiments a single pushing location was used with a 2-D lateral tracking region. The therapy transducer was held stationary and lateral clot displacements were assessed by translating the imaging transducer at discrete offsets (0.25 mm) from the transmit focus and acquiring displacement profiles up to ± 1.5 mm of the fixed pushing location. A representative B-mode cross-sectional slice through the blood clot is shown in Fig. 6, with illustrative lines of sight used to track lateral displacements. All exposures were performed at 15 W, and at each distance from the focus the mean and standard deviation peak displacement was recorded. Lateral strain was also computed from the derived displacements using equation (1).

III. RESULTS

A. Measurements of thrombus displacement and strain across the therapy beam axis

Representative maps of displacement along one line of sight as a function of time for a range of acoustic powers (1–20 W) for a sample plot are shown in Fig. 7.

The mean and standard deviation peak clot displacement versus acoustic power is shown in Fig. 8 ($n = 9$ clots). At 1 W the mean peak displacement was 3 μm . From 1–60 W a linear trend is observed between peak clot displacement and acoustic power, with a measured slope 2 $\mu\text{m}/\text{W}$ using linear regression ($p < 0.05$). As the acoustic power reaches 70 and 85 W, peak clot displacement begins to saturate close to 128 μm .

If the clot depth is fixed the kinetics of displacement are more evident. An example of clot displacements for a 20 W acoustic pulse at four specific depths: at the peak displacement depth (indicated to be 0 mm for reference purposes), and distances of 0.5, 1 and 1.5 mm from this depth, are shown in Fig. 9. At the peak displacement depth it is observed that the clot reaches its peak before the termination of the transmit pulse with a TTP of ~ 2.2 ms (arrow). It is evident at higher transmit powers that an overshoot occurs. After the transmit pulse is turned off the clot decays rapidly ($RT = \sim 2.2$ ms). During the recovery process an overshoot is also observed

before the clot returns to its original position. Away from the center of the beam the displacements become lower in magnitude and finally, at a 1.5 mm radial position, the displacement cannot be distinguished from background noise.

The TTP values ($n = 9$ clots) are summarized in Fig. 10a. At 1 W the TTP has an average value of 3.5 ms, and drops by almost a full millisecond at 5 W with a value of 2.7 ms. For acoustic powers > 5 W the TTP is relatively constant with a mean value near ~ 2.2 ms. The RT for clots was largely independent of transmit power with a mean value of ~ 2.2 ms (Fig. 10b).

Axial strain measurements are summarized in Fig. 11 and 12. Mean axial spatial and temporal maps of clot strain as a function of position from the depth of peak displacement for transmit pulses of 20 W is shown in Fig. 11. Axial strain is pronounced, reaching a value of 6%. Peak strain was observed at ~ 0.7 mm from the peak displacement depth and this observation was consistent for all transmit powers investigated. Fig. 12 shows the relationship between peak strain and transmit power, where strain was observed to increase linearly with power, reaching a maximum value of 11% at 35 W.

B. Measurements of thrombus displacement and strain at laterally offset positions

A comparison of the experimental pressure squared profile of the therapy transducer in water vs. the laterally offset peak displacement profile in thrombus ($n = 6$ clots) at 15 W is shown in Fig. 13. In Fig. 13a), the lateral pressure profile reaches its -3 dB value at 0.5 mm from the focus. In contrast, at the 15 W transmit pulse the decay of the laterally offset peak clot displacements (Fig. 13b) is more gradual reaching -3 dB at ~ 0.85 mm from the focus. Fig. 14 demonstrates the dependence of strain as a function of lateral position. For transmit pulses of 15 W ($I = 1620$ W/cm²) peak strains of 2.5% were observed at 0.75 mm away from the focal zone. The peak lateral strain magnitude is twice that of the axial measurements (5 vs. 2.5%, respectively).

IV. DISCUSSION

This study has investigated displacements and strain in constrained in vitro clots exposed to focused ultrasound pulses both at the center of and at laterally offset positions of the therapy beam.

A linear relationship between peak axial clot displacement and transmit power was observed up to 60 W. This behavior has been previously observed in other tissue types [35]–[37] and simulated for the case of ‘free’ clots, where Frenkel et al. [19] indicated a linear relationship between transmit power and peak displacement for clots insonified with 1 MHz 100 ms pulses for acoustic powers between 20–80 W. At higher transmit powers (> 60 W) the displacements observed in the present study departed from a linear relationship with power and appeared to approach a saturation level (128 ± 24 μ m) by 85 W. As a point of comparison, in [26] a 243 μ m displacement was observed at 60 W, where the I_{SATP} was estimated to be 4022 W/cm² by dividing the acoustic power by the -3 dB beam cross-sectional area (4 cm focal length, 5 cm diameter). Using this metric, 45 W for our transducer corresponded to an I_{SATP} of 4640 W/cm², at which point a peak displacement of 95 ± 26

μm was observed, substantially lower than that reported in [26]. It is possible that variations in the clot types employed in these two studies may result in differences in absorption and mechanical properties that impacted displacement values. In the present study thrombin was used to initiate clots and in [26] a whole blood incubation approach was employed. However, the presence of frequency dependant absorption [38] would be expected be a factor that would increase displacements in our study (1.5 MHz) relative to [19], which employed 1.0 MHz. Due to the degree of reduction in displacements in the present study relative to [26] it is hypothesized that the primary factor causing this is that the presence of boundary conditions for the constrained clots relative to the 'free' clots, which has implications for displacement levels of clots confined within blood vessels.

Dynamic axial displacement measurements of blood clots were measured in this study. It was demonstrated that the TTP was longer for lower transmit powers, ranging from 3.5 to 2.5 ms at 1 and 5 W, respectively. At powers above 5 W the TTP reached a steady state with an average value of ~ 2.2 ms. These observations give an indication of the balance between tissue acceleration generated by the acoustic radiation force which is opposed by the elastic restoring force for constrained blood clots [39]. Interestingly, the RT for clots exposed to focused ultrasound pulses was almost identical for powers from 1–85 W at a value of ~ 2.5 ms, slightly higher than the average TTP value. This effect has been observed in simulations before with other tissues [37] where tissue exposed at 1 MHz with 100 ms pulse lengths measured an identical recovery time independent of transmit power. The time taken for tissue to return back to its original state will be related to its viscoelastic properties. These results have implications relevant to the design of ultrasound pulses that attempt to maximize clot displacements and strain. In particular, pulse lengths above the mean TTP will not increase the peak displacement, while interpulse spacing less than the sum of the RT and pulse length will be sufficient to allow the clot to return to its initial state. Given the apparent influence of confinement on the results, it seems likely that the vessel size, beam size, frequency and orientation will also influence the resulting displacements and strains.

It has been postulated that the mechanism for improved clot lysis in the context of using pulsed sub-cavitation threshold HIFU to potentiate tPA is the generation of steep displacement gradients occurring near the focal zone. These gradients may act to increase pore sizes within clots and thereby facilitate tPA transport into the clot (i.e. increase bioavailability) [20], [26]. The impact of clot strain on pore size appears not to have been directly investigated to date, but conceivably could be associated with either transient widening of gaps within the fibrin matrix during sonication or permanent structural alterations sustained after each ultrasound pulse. Indeed, Jones et al [26] showed that the diffusion coefficient was increased by 6.3 times for fluorescently labeled dextrans in HIFU treated clots. While considerable peak displacements ($243 \mu\text{m}$) were observed in the 'free' clots employed in that study, the strain levels were not measured. In the present study it was directly shown that substantial strains could be induced in clots with focused ultrasound, which is at least consistent with the hypothesis that shear forces may be playing a role in enhancing tPA diffusion. We observed that the maximum axial strain, independent of transmit power, occurred at ~ 0.7 mm from the focal zone of the therapy transducer. When the therapy beam was fixed and the imaging transducer moved laterally, at 15 W the mean peak strain occurred at 0.75 mm, with an average magnitude of 2.5%. The discrepancy in

these results can in part be attributed to the low spatial sampling in the lateral direction (0.25 mm). The axial strain generated by the acoustic radiation force is dependent upon the material properties of the tissue, the transmit frequency and the shape of the beam intensity. It was also observed that a linear relationship between peak axial strain and transmit power occurred (up to 35 W), where values reached nearly 11%. While no previous results have been obtained measuring strains in clots, it is useful to consider the results of Hancock et al [37]. In this study, experimental measurements of displacements were made in muscle tissue with pulsed HIFU exposures, and theoretical strains were calculated. For conditions that resulted in enhanced extravasation of 100–200 nm particles, the estimated peak strains were similar to the experimental values measured in the present study.

It is useful to consider the implications of these results to the case of the Alexandrov transcranial Doppler clinical trial [12] where clot displacements and/or strain may play a role in facilitating thrombolysis. Under these conditions, the derated pressure has been estimated at 70 kPa in the brain [14] for a 2 MHz, 15 μ s pulse. If one assumes the following: at short pulse lengths the clot does not reach a steady state, and that clot displacement is linear with transmit power, a rough extrapolation of our results for their case would amount to displacements on the order of several nanometers. Moreover, the associated strain would be substantially lower due to the width of their beam which suggests clot strain for each individual ultrasound pulse would be far less pronounced in this case, though it is also notable that the high PRF (10 kHz) and duration of treatments (0.5–1 hr) may also be a consideration.

This study has a number of limitations. First, it can be expected that clots with different compositions and ages will produce results that will be different from the results of this study. Second, while this study provided experimental evidence of the spatial and temporal dependence of displacements and strain in response to focused ultrasound stimulation, it did not do this in a full 3D manner but rather sampled particular spatial regions with a time resolution of 0.55 ms. Further, while the examination of clot motion within an agar channel is arguably a far more clinically relevant situation than the ‘free’ clots employed in previous studies [19], [20], [26], the behavior of a clot within a vessel *in vivo* may differ from this. Two aspects to consider in this regard are the mechanical properties of the vessel and surrounding tissue, and, if the particular situation of interest involves the ultrasound beam exposing both tissue and clot, then the absorption properties of these tissues will also be relevant. We note that the agar employed has previously been measured to have a shear modulus of 80 kPa [40], which is within the range recently measured in for example carotid arteries [41], suggesting that this is a reasonable value to capture the primary influence of the mechanical effects of a constraining artery. However, the results obtained in the present study suggest that it would be of interest to investigate displacements in more refined models that more closely mimic *in vivo* conditions such as perhaps incorporating different wall and surrounding medium as well as simulation studies [42]. Ultimately, it will be of interest to conduct such experiments in an *in vivo* context, though these will be complicated by issues of motion due to tissue and blood flow.

V. CONCLUSIONS

In this study we measured the magnitude, spatial and temporal variation of displacements and strain initiated by focused ultrasound for constrained *in vitro* blood clots. Complex spatial and temporal displacement and strain patterns were observed, which suggest that clot displacement will be strongly influenced by confinement. An understanding of the potential role of these displacements in ultrasound potentiated tPA clot lysis will require an examination of these effects in the context of tPA lysis experiments. If it is established that displacements are a mechanism of clot lysis, the optimization of pulsing strategies must take into consideration the coupled power and time dependant aspects of clot displacements, which will impact parameters such as transmit amplitude and PRF.

Acknowledgments

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Fig. 1.

Lateral cross-section of vessel phantom. A thrombus 3 cm in length is constrained within a 3.5 mm diameter saline channel and surrounded by agar-silica to simulate a constrained clot embedded in tissue.

Fig. 2.

a) Schematic illustration and b) photographic overview of experimental setup. Pushing pulses are administered below the clot from a focused transducer and displacements are tracked from above with a high frequency imaging transducer. c) Expanded illustration of the relative angles and locations of the therapy and imaging beams with respect to the clot cross-section.

Fig. 3.
25 MHz cross-sectional B-mode image acquired from the imaging transducer. The constrained clot is delineated by its hyperechogenicity.

Fig. 4. Timing diagram of pulse sequence. Therapeutic and imaging pulses are interleaved for initiation and detection of clot displacements, respectively. After ten pushing pulses, imaging pulses are transmitted in isolation to monitor dynamics of tissue recovery.

Fig. 5.

Diagram of data acquisition. The VisualSonics Vevo 770 is synchronized with the AWG (arbitrary waveform generator) to simultaneously output a therapy pulse and digitize RF data on a personal computer.

Fig. 6.

25 MHz cross-sectional B-mode image acquired from the imaging transducer. The centered bold arrow designates the line of sight used to track clot displacements and is coincident with the therapy beam. The smaller arrows depict the laterally offset displacement estimations in 0.25 increments (not to scale). Displacements were measured up to 1.5 mm of either side of the center of the therapy beam axis.

Fig. 7.

Spatial maps of clot displacements as a function of depth along one line of sight as a function of time (imaging beam intersecting the therapy beam axis). The pushing pulse is on from 0 to 5.45 ms. Results are shown for a) 1W, b) 5W and c) 20W which indicate progressively higher displacements as a function of transmit power.

Fig. 8.

Mean and standard deviation peak clot displacement as a function of transmit power. A linear trend is observed between peak displacement and acoustic power up to 60 W before an apparent saturation point approached towards 85 W.

Fig. 9.

Time dependant displacement at peak displacement depth. The tissue is observed to reach its peak before the termination of the pulse (arrow) and then recovers rapidly once the pushing pulse is turned off (5.45 ms). An overshoot past the baseline value is observed as the tissue recovers to its original value.

Fig. 10.

Mean and standard deviation TTP and RT as a function of acoustic power. a) At lower powers (1–5 W) an inverse relationship between transmit power and TTP is observed, ranging from 3.5 to 2.5 ms at 1 and 5 W respectively. At powers > 5 W the TTP is relatively constant at a value near 2 ms. b) the RT for all clots is relatively independent of transmit power with a mean value near 2 ms.

Fig. 11.

Spatial map of average on-axis clot strain along one line of sight as a function of time. At 20 W evidence of large strain is observed at radially offset positions from the depth of peak clot displacement, reaching magnitudes of 6%.

Fig. 12.

Mean and standard deviation on-axis peak strain as a function of transmit power where a linear relationship is observed, with peak strain attaining 11% at 35 W. Only the positive error bars are displayed.

Fig. 13.

Lateral map of mean and standard deviation peak clot displacements. a) Normalized pressure squared (proportional to intensity) profile in water. b) Peak displacement profile in thrombus at 15 W. It is observed that the peak displacements in clot decay slower than those in water. By 1.5 mm clot displacements are approaching the noise floor.

Fig. 14.

Measurement of clot lateral strain induced by the focused ultrasound pulse for a sonication power of 15 W. The largest strain value is close to 2.5%, which occurs 0.75 mm from the peak displacement value. Only the positive error bars are displayed.

TABLE I

Therapy transducer calibration data

Power (W)	P _P (MPa)	I _{SPTP} (W/cm ²)
1	1.0	65
2.5	1.7	187
5	2.6	439
10	3.8	937
15	5.0	1620

P_N = peak negative pressure, I_{SPTP} = spatial peak temporal peak intensity