

Blood vessel rupture by cavitation

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Abstract Cavitation is thought to be one mechanism for vessel rupture during shock wave lithotripsy treatment. However, just how cavitation induces vessel rupture remains unknown. In this work, a high-speed photomicrography system was set up to directly observe the dynamics of bubbles inside blood vessels in ex vivo rat mesenteries. Vascular rupture correlating to observed bubble dynamics were examined by imaging bubble extravasation and dye leakage. The high-speed images show that bubble expansion can cause vessel distention, and bubble collapse can lead to vessel invagination. Liquid jets were also observed to form. Our results suggest that all three mechanisms, vessel distention, invagination and liquid jets, can contribute to vessel rupture.

Keywords Cavitation · Vessel rupture · High-speed imaging

Introduction

Acute injury to the kidney occurs in the clinical application of shock wave lithotripsy (SWL). The primary feature of SWL-induced tissue injury is the rupture of blood vessels, with small vessels particularly susceptible to injury [1]. Cavitation is thought to play a prominent role in SWL-induced vascular rupture [2].

The study of how cavitation bubbles damage surrounding materials dates back almost a century, when the collapse of vapor bubbles near propeller blades was found to be the prime mechanism associated with damage to the blades [3]. Extensive studies have shown that cavitation bubbles cause erosion to ship propeller blades by the formation of liquid jets. It has been suggested that liquid jet impact is also one mechanism by which cavitation damages vessels [4]. In addition, vessel distention associated with cavitation bubble expansion has also been proposed as a mechanism contributing to vessel rupture [5].

To understand precisely how cavitation induces vascular injury, the most direct method is to observe the dynamics of bubbles in blood vessels in real time. In this way, bubble dynamics and their interactions with blood vessels can be investigated. Zhong et al. [5] used high-speed imaging to observe the interactions of bubbles with vessel phantoms (cellulose hollow fibers and silicon tubing). The vessel phantoms ruptured in response to large-scale cavitation; however, such in vitro tubing does not match the viscoelastic properties of real tissue. Observations of bubble dynamics in actual vessels embedded within surrounding tissue has been reported only once [6]. In that pioneering work, streak and strobe imaging were used; however, the transient dynamics of bubbles and vessels were not fully captured.

In this work, a high-speed photomicrography system was set up to directly visualize transient interactions between ultrasound-activated bubbles and blood vessels, especially microvessels whose diameters are comparable to bubble sizes. Bubble-induced vessel distention (outward push into the tissue), invagination (inward pull into the lumen) and liquid jets were all observed to be associated with vessel rupture, indicated by extravasation of bubble fragments as well as a dye injected into the vessels.

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Methods

Animal tissue preparation

The animal experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. Rat mesenteries were used as our animal model because they have good optical transparency and are easy to access. In our experience, not all strains of rats have rich mesenteric vascular networks. We examined the mesenteries of Sprague–Dawley and F344 rats (both ordered from Charles River Labs) and found that F344 rat mesenteries usually have much higher vessel densities than Sprague–Dawley rats. Therefore, F344 rats were used in our study.

To prepare the animal tissue, a rat was first anesthetized and its mesentery was exposed and kept warm and moist. Then the superior mesenteric artery (SMA) was cannulated and heparin was administered into the SMA. Another cannula was placed in the portal vein to serve as an outflow orifice. Heparinized saline was perfused through the mesentery with the intestine connected. After the mesentery was flushed clear of blood, the animal was killed and the mesentery with intestine was dissected away from the rat body. A segment of the mesentery with visually rich vascular networks was selected for targeting. One feeding artery and one draining vein of the selected region were cannulated using two polyethylene tubes, and the appropriate vessel branches leading to other regions were ligated using sutures. The selected region was then gently spread and sandwiched between two asymmetric annular plates with the two tubes passing through it as shown in Fig. 1a. In this way, perfusion was controlled into the targeted region, within the opening of the annulus.

Blood vessels (such as those illustrated in Fig. 1b) with diameters ranging from 10 to 100 μm were identified for

targeting. Ultrasound contrast microbubbles [7, 8] were perfused into the blood vessels to serve as cavitation nuclei for visualization (that is, selected nuclei were used in focusing the microscopic system described below). Green India ink at a volume concentration less than 3% was added to the perfusate to increase the contrast of the vessels compared to the surrounding tissue, and also to indicate blood vessel leakage.

Experimental system

The main components of the experimental system are shown schematically in Fig. 2a. The prepared tissue sample was transferred to a tank filled with phosphate buffered saline solution located on the stage of an inverted microscope (TE2000-U; Nikon Inc., Melville, NY, USA) with a 40 $\times$ water immersion objective (numerical aperture 0.8; nominal working distance 3.5 mm; nominal depth of field 0.43 μm). Both the tissue sample and the objective were immersed in water, without an air gap. Illumination for microscopic imaging was provided by a high-intensity flash lamp with duration of approximately 23 μs and nominal energy 300 J. Such high-energy light illumination is a key component in performing high-speed microphotography. The flash lamp was coupled to an optical fiber that brought the light source close to the tissue sample.

The microscope was aligned confocally with a high-intensity focused ultrasound (HIFU) transducer (H102; Sonic Concepts, Bothell, WA, USA) with a 1 MHz center frequency, a 63 mm geometrical focal length and a 0.9 f-number (see Fig. 2a). The transducer was positioned directly opposite to the microscope objective. It was driven by one cycle sinusoidal signal produced by a function generator (33120A; Hewlett Packard, Palo Alto, CA, USA) and amplified by a power amplifier (ENI A150; ENI, Rochester, NY, USA). Due to ringing of the transducer, the acoustic output pulse was about 2 cycles long as determined using a fiber-optic hydrophone (FOPH 2000; RP Acoustics, Leutenbach, Germany). An example of the pressure waveform measured at the location of the tissue sample (in the absence of tissue) is shown in Fig. 2b. In this figure, we can see both the incident wave from the transducer and the reflected wave from the microscope objective. Ultrasound pulses with peak negative pressures (PNP) of 4–7 MPa were used in this study. We note that a shock wave generator was not used as a source, but instead a HIFU transducer was used to generate signals with PNP close to those used in SWL. It should also be noted that to get rid of the reflected ultrasound wave, the transducer could be located at a 45° angle with respect to the top surface of the objective, instead of being directly above the objective as shown in Fig. 2a. In this way, the reflected wave from the objective would not impact the targeted

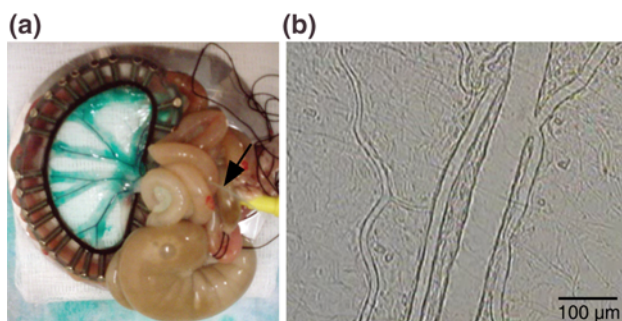


Fig. 1 **a** A selected mesenteric region is sandwiched between a magnetic and acrylic kidney-shaped annular plate. The acrylic annulus is embedded with magnetic bars, which hold the magnetic annulus to the acrylic one. Green Indian ink was perfused through a small polyethylene tube (indicated by the *arrow*) to the selected region to enhance blood vessel contrast, and indicate leakage sites. **b** A microscopic view of rat mesenteric vasculature tissue after the blood was replaced by saline

Fig. 2 **a** An illustration of the spatial locations of the main components in the experimental setup. **b** A representative pressure waveform measured at the location of the tissue sample. The time when the pulse first arrives at the tissue location is set to zero. It shows both the incident wave from the transducer and the reflected wave from the microscope objective. In this example, the peak negative pressure of the incident wave (7 MPa) exceeds that of the reflected wave (2.3 MPa)

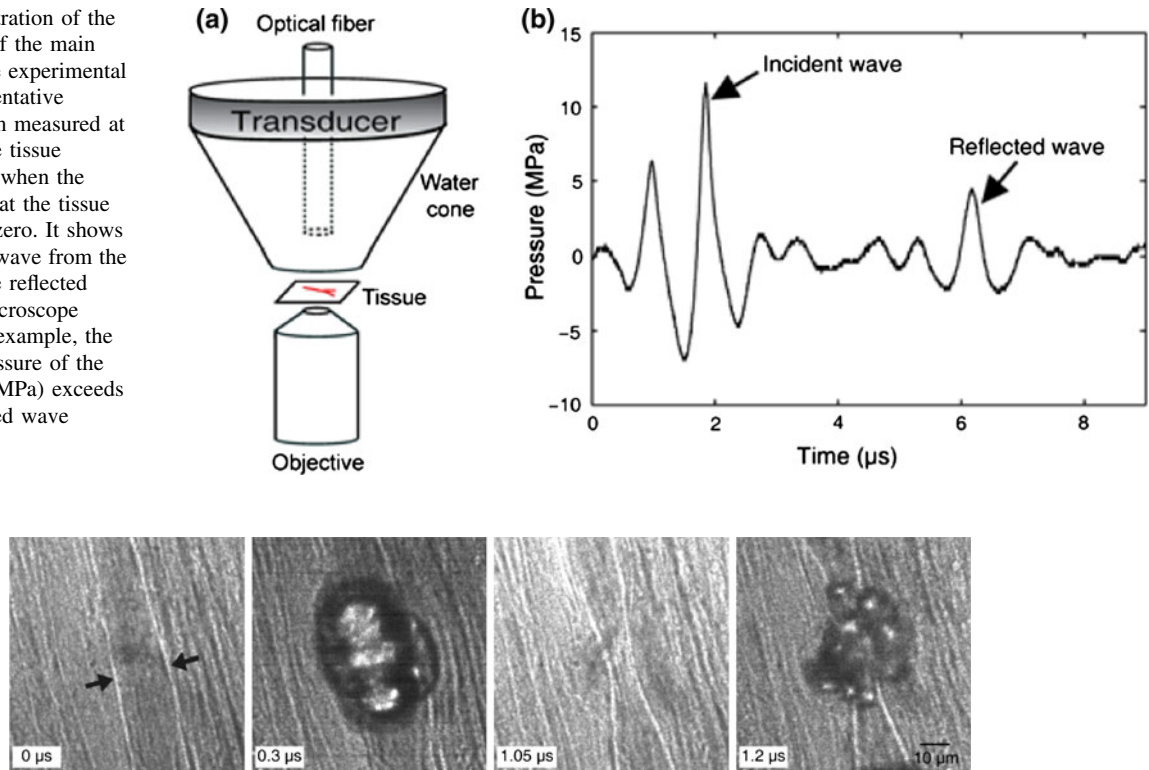


Fig. 3 A high-speed image sequence shows expansion, collapse and re-expansion of a bubble cluster, accompanied by the observation of vessel distention and invagination. The bubble fragments outside the

region on the tissue sample. We did not do so because the alignment of the optical and acoustic system is much easier in the present setup. Moreover, as shown in Fig. 2b, the amplitude of the reflected wave is only about 1/3 of the incident wave's amplitude. The impact of the reflected pulse is thus reduced compared to the incident pulse.

A high-speed camera (Imacon 200; DRS Hadland, Cupertino, CA, USA) was connected to the microscope and synchronized with the ultrasound transducer. When contrast agent microbubbles were observed flowing through the field of view of the microscope, the ultrasound pulse was generated and the camera was triggered to capture a 14-frame sequence of images at a shutter speed of 50 ns. The delay of the first frame was set to the ultrasound traveling time from the transducer to the tissue. The interframe time was set to 150, 300 or 600 ns to examine bubble dynamics over different time periods.

Results and discussion

Vessel rupture associated with vessel distention and invagination

Cavitation induced by the pressure field caused vessel distention and invagination. Vessel rupture is associated

vessel at 1.2 μs indicate that the vessel had been ruptured at some stage during this single pulse

with significant vessel distention and/or invagination, as shown by Fig. 3. Four frames were selected from one image sequence which recorded the dynamics of a bubble cluster inside a vessel of diameter 17 μm with a 7 MPa PNP driving pulse (similar to that shown in Fig. 2b). Matlab (Mathworks Inc., Sherborn, MA, USA) was used to manually highlight the vessel walls in the four frames to make it easier for the reader to identify the vessel wall. The initial position of the vessel wall is shown by arrows in the first frame. At 0.3 μs relative to the first frame, a bubble cluster of 3 or 4 bubbles expanded against the vessel. The expansion of the cluster was constrained by the vessel walls, as indicated by the elongation of the bubble cluster along the vessel wall. Bubble expansion significantly distended the vessel to approximately 2.7 times its original diameter. The bubble then collapsed at 1.05 μs leading to almost axially symmetric vessel invagination. The diameter of the vessel at maximum invagination is approximately 0.4 times its original diameter. Subsequently, several re-expanding bubble fragments are shown extravasated from the vessel, indicating that the vessel was ruptured after a single ultrasound pulse. In this case, both vessel distention and invagination may contribute to the rupture of the vessel.

Invagination was commonly observed when bubbles collapsed near the vessel wall, which pulled the vessel

inward toward the lumen. Figure 4 illustrates that in some cases vessel invagination may play a more important role than distention in causing vessel rupture, as it may generate higher strains on the vessel wall than distention (our observations are qualitative, not quantitative). This figure consists of four selected frames from a high-speed image sequence captured when the PNP of the ultrasound pulse was 6.5 MPa. The vessel diameter was initially about 50 μm . A bubble cluster inside the vessel expanded to its maximum size in frame 2. Here, vessel distention was at its maximum, about 22 μm (calculated from the change of vessel diameter relative to its initial diameter). In this frame, the point on each side of the vessel wall that was distended the most is pointed out by a corresponding arrow. The collapse of the bubble cluster led to vessel invagination as shown in frame 3. The notch-like shape on both sides of the vessel wall suggests high strains on the vessel wall during invagination. The point on both sides of the vessel wall that invaginated the most was at about the same location as that during the maximum distention. In the last frame, no bubble was observed, yet the vessel remained invaginated, achieving a maximum invagination of 32 μm (relative to its equilibrium position), which is much larger than the maximum distention. In this particular case, no obvious leakage of the vessel was observed; however, the possible high strains associated with vessel invagination lead us to hypothesize that invagination may play an important role when there is vessel damage. The fact that the vessel remained invaginated suggests that tissue viscosity plays a significant role as well. The vessel did return to its original diameter as examined under the microscope after the high-speed images were captured.

To further study bubble–vessel interactions, we compared the high-speed images with images of vessels before and after ultrasound exposure. Figure 5 is an example that shows the rupture of a vessel caused by bubble–vessel interactions. Figure 5a shows frames selected from a high-speed image sequence captured within PNP = 5.7 MPa. A group of bubbles appeared in the upper small vessel (nominal diameter $\approx 11 \mu\text{m}$), and no bubbles in the lower bigger vessel. Under ultrasound exposure, these bubbles expanded and elongated along the vessel (frame 0.9 μs). Their collapse caused vessel invagination at multiple

locations, as seen in frame 1.2 μs with several invaginated sites pointed out by arrows. During re-expansion (1.8 μs), these bubbles expanded to much larger sizes and the vessel was significantly distended and distorted. The bubbles collapsed at 2.1 μs , and the vessel contracted irregularly and cannot be clearly identified, implying that the vessel might be damaged. The last frame (3.9 μs) shows bubbles, pointed out by an arrow, outside the original vessel.

Figure 5b and c are color images captured before and 1 min after the high-speed image sequence was taken. This delay was the time needed to take the high-speed images and switch imaging modalities from using the high-speed camera to a color CCD camera. The dye that was initially inside the blood vessel in Fig. 5b extravasated into the interstitial space (Fig. 5c), leading to a reduction of contrast and thus vessel clarity. The lower bigger vessel appears to have remained intact.

Vessel distention caused by bubble expansion pushing against the vessel wall was previously observed by Zhong et al. [5] in a vessel phantom and was considered to be one of the mechanisms leading to vessel rupture. Our study showed that vessel distention from acoustically activated bubbles can also occur in real blood vessels. And as we noted above, vessel invagination induced by collapsing bubbles may lead to high strains on the vessel wall which may also contribute to vessel rupture and may be more important in some conditions.

Small vessel rupture involving liquid jets

In the images above, no liquid jets were observed. We did, however, observe liquid jets inside blood vessels in some cases. Figure 6 shows a high-speed image sequence captured with PNP = 4 MPa. At frame 1.6 μs , a bubble in a vessel of diameter $\approx 15 \mu\text{m}$ expanded against both sides of the vessel wall. A liquid jet directed toward the right side vessel wall appears to impact on the right side vessel wall. A sketch of the jet is shown on the right corner of this frame with both sides of the vessel wall marked as dashed lines. The bubble collapses in the subsequent frame. The following frames from 2.2 to 3.1 μs display the re-expansion of the bubble with part of the cavity extravasated from the vessel. The subsequent contraction of the bubble in

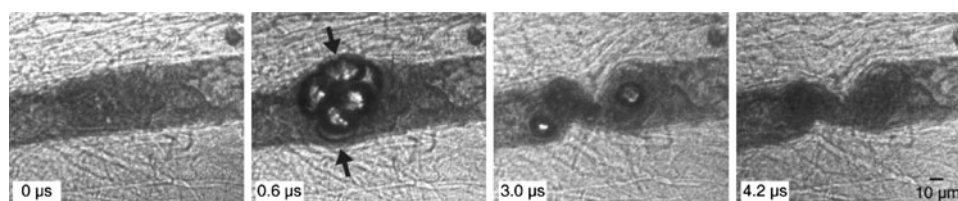


Fig. 4 A representative high-speed image sequence that shows vessel invagination may play a more important role in causing vessel rupture than distention, as invagination may generate higher strains on the vessel wall compared with distention

Fig. 5 A vessel was ruptured by cavitation bubbles inducing vessel distention and invagination. **a** Selected frames from a high-speed image sequence recorded at PNP 5.7 MPa. Vessel invagination was observed at 1.2 μs with several invaginated sites pointed out by *arrows*. Vessel distention was clearly observed at 1.8 μs . At 3.9 μs , bubbles, pointed by the *arrow*, are outside the vessel. **b** An image of the vessel just before the high-speed images were captured. **c** An image of the vessel 1 min after the high-speed images were taken shows the upper vessel was ruptured

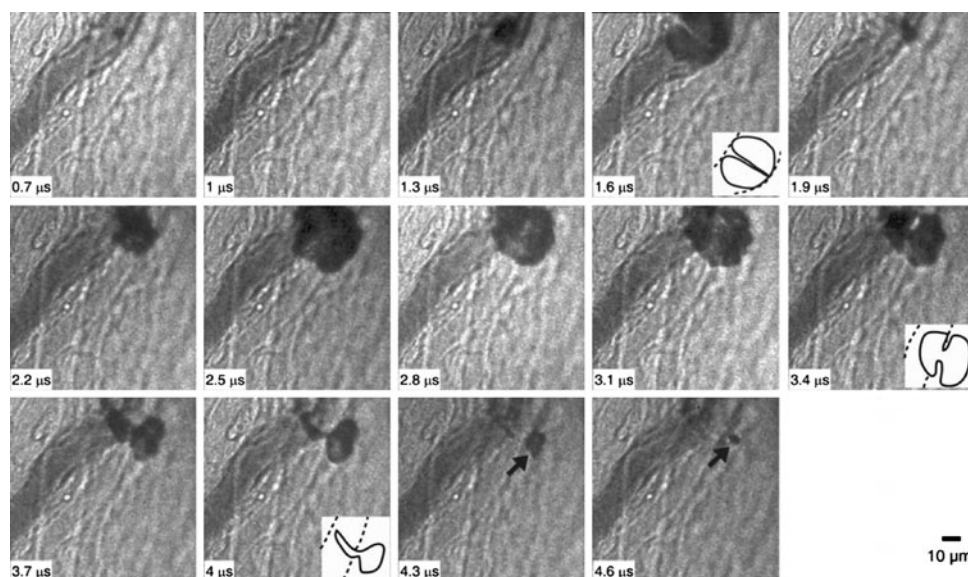
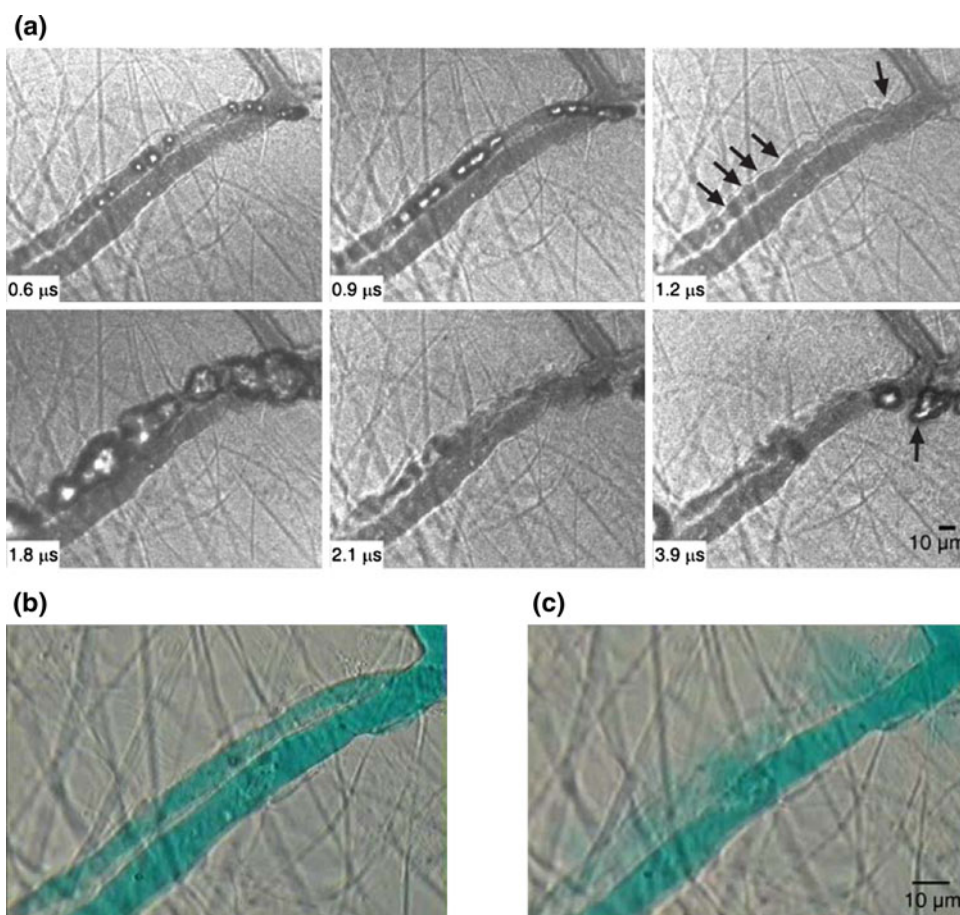


Fig. 6 Vascular rupture involving a liquid jet. PNP = 4 MPa. Vessel diameter = 15 μm . At frame 1.6 μs , a liquid jet appears to be formed inside the bubble and directed toward the right vessel wall. The jet appears to impact the vessel wall. At frame 3.4 μs , the re-expanded bubble contracted showing two connected parts: one outside the vessel and one inside. This leads to a mushroom-shaped form with its

stem stretching through the vessel wall (frame 4 μs). Sketches of the bubble in the three characteristic frames are marked with the bubble in *solid lines* and vessel in *dashed lines*. In the last frame, the collapsed bubble (*arrow*) was observed in the interstitial space outside the vessel wall

frame 3.4 μs shows two connected parts of the cavity: one inside the vessel lumen and the other outside the vessel as illustrated by the sketch on image frame. The bubble continued to contract in frames 3.7 and 4 μs forming a mushroom shape, with the cap outside the vessel and stem extending through the vessel wall as illustrated by the drawing in frame 4 μs . In frames 4.3 and 4.6 μs , the cavity outside the vessel (arrow) collapsed. The cavity inside the vessel is not clearly identifiable. In the last frame, the distance from the bubble fragment outside the vessel to the right side vessel wall is about 16 μm .

Unlike the examples shown above, in this particular example we hypothesize liquid jetting as a mechanism for vascular rupture. The formation of liquid jets is controversial, as bubble expansion is somewhat constrained by the vessel walls, which is thought to limit the strength of collapse that leads to jet formation. However, our observations clearly show that liquid jets can form in constrained environments. Even though bubble expansion is constrained by the vessel wall and surrounding tissue, the rebounding of the vessel wall during bubble collapse can ‘inject’ momentum back into the liquid [9] that may contribute to the formation of liquid jets. We observed that in larger vessels, at least twice the size of the maximum bubble diameter, liquid jets are always directed away from the nearest vessel wall, not towards it. In those cases, damage due to liquid jets impinging on the vessel wall is probably not occurring. But for small vessels like that shown here, a liquid jet can impact the opposite vessel wall leading to damage.

Conclusion

High-speed imaging was used to record cavitation within blood vessels in an ex vivo rat mesentery preparation exposed to an ultrasound source generating negative pressures in the range produced by shock wave lithotripters.

The data provide visual evidence that cavitation can cause obvious rupture of microvessels smaller than 20 μm in diameter as indicated by bubble fragments growing outside the vessel and dye extravasation. The precise mechanisms that were observed included vessel distention, invagination and liquid jetting. All experiments involved a single ultrasound pulse lasting no more than a few microseconds. Future work will involve the use of shock waves that mimic actual SWL devices.

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