Progress Report (12 months)

Focused Ultrasound Foundation Research Award for the project entitled
"The development of standards to regulate microbubble cloud formation during histotripsy pulses."

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Overview:

The stochastic nature of microbubble activity makes the development of regulatory standards difficult for cavitation-based ultrasound therapies. We propose to develop *in vitro* and *in silico* models to assess the area, location, and type of microbubble activity and ablation during histotripsy pulses. These data will elucidate a FDA regulatory framework for histotripsy devices, which will profoundly impact the advancement of clinical use of focused ultrasound.

Summary of Progress (2/2015-12/2015):

I. Develop a numerical model to predict the spatial area of a microbubble cloud nucleated from a microbubble exposed to a histotripsy pulse: The Yang/Church model was numerically integrated to predict the behavior of the cavitation nuclei exposed to measured shock scattering histotripsy pulses. The bubble motion exhibited continual growth during insonation, suggesting that the ablative action of a histotripsy pulse is related to the maximum size of the bubble. The analytic model of Holland and Apfel was extended to predict the maximum size of cavitation nuclei for both shock scattering histotripsy and microtripsy excitations. The predictions of the analytic model and the numerical model agree within 2% for fully developed shock scattering histotripsy pulses (greater than 72 MPa peak positive pressure, as shown in **Figure 1**). For shock scattering histotripsy pulses that are not fully developed (less than 72 MPa), the analytic model underestimated the maximum size by less than 5%. The analytic model was also used to predict bubble growth nucleated from microtripsy insonations, and was found to be consistent with experimental observations. Based on the extended analytic model, metrics were developed to predict the extent of the treatment zone from histotripsy pulses. A manuscript based on this study was submitted to Physics in Medicine and Biology (August 2015).

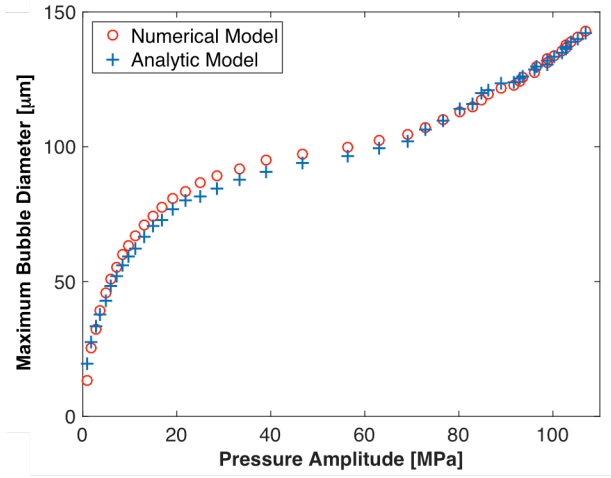

Figure 1: Predicted maximum bubble size as a function of pressure amplitude for a single cycle histotripsy pulse. The open, red circles are the predictions based on numerical integration of the Yang/Church model, and the blue crosses are the predictions of the analytic model. The predicted maximum bubble size of the two models was within 2% once the shockwave was fully developed.

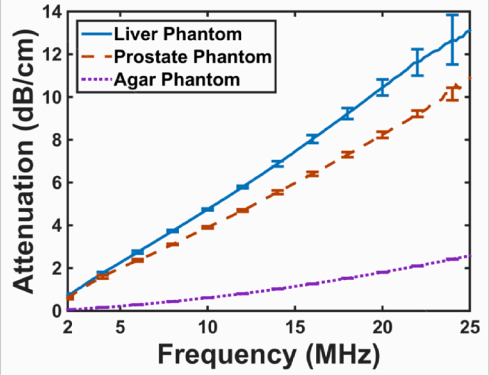

Figure 2: Attenuation spectra for each phantom between 2 and 25 MHz. The error bars are representative of the standard deviation (N = 3).

II. Correlate the parameters of histotripsy insonation with the area and location of microbubble cloud activity and lesion formation in an in vitro tissue phantom.

A. Acoustic spectroscopy of tissue mimicking phantoms: Tissue-mimicking phantoms are employed for the assessment of shocked histotripsy pulses *in vitro*. These broadband shockwaves are critical for tissue ablation, and are influenced by the frequency-dependent attenuation of the medium. The density, sound speed, and attenuation spectra (2–25 MHz) were measured for phantoms that mimic prostate and liver tissue, key histotripsy targets (**Figure 2**). In addition, the properties of an agar phantom previously employed by other research groups for *in vitro* studies of histotripsy were also measured. Histotripsy waveforms measured with a fiber optic hydrophone were

digitally attenuated according to the measured attenuation spectra for each phantom. Although the measured bandwidth of the attenuation spectral data was 23 MHz, only 10 MHz of bandwidth was required to ascertain the influence of attenuation on shockwave formation. The degree of shockwave formation appears to be most strongly influenced by attenuation near the fundamental frequency, and the higher harmonics play a secondary role. Thus, the attenuation only needs to be measured at the fundamental to determine the shockwave amplitude and rise time during histotripsy shock formation. The rarefactional pressure amplitude, and the shockwave amplitude and rise time are necessary metrics for the prediction of microbubble cloud formation, which is the dominant mechanism of histotripsy therapy. A manuscript based on this study was submitted to Ultrasound in Medicine and Biology (September 2015).

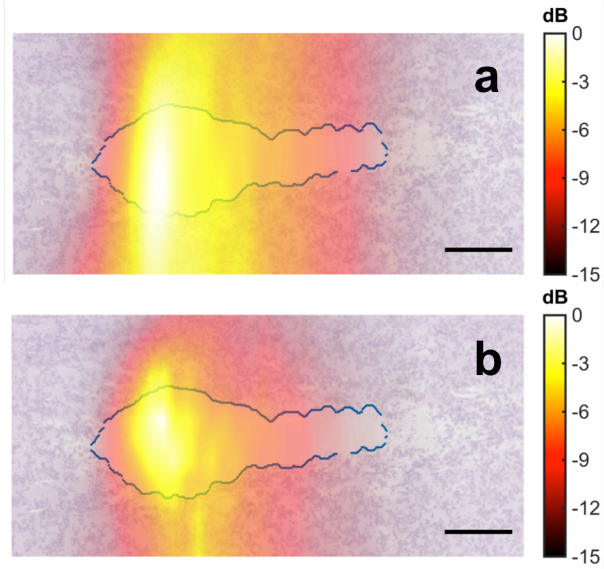

Figure 3: Histotripsy pulses (10 μs pulse duration, 18 MPa peak negative pressure, 20 Hz pulse repetition frequency) were employed to generate microbubble clouds in a soft tissue phantom. A periodic acid Schiff stain was used to delineate the ablation zone resulting from the mechanical action of the microbubble clouds (blue outline, all panels). Cavitation activity was monitored and passive cavitation images beamformed using the delay, sum, and integrate method (hot color map overlay in panel **a**) or robust Capon beamforming (hot color map in panel **b**). Note the improved range resolution (up and down in the image) with the robust Capon beamforming over the delay, sum, and integrate method. The black bar in the lower right corner of each panel is 5 mm.

B. Passive cavitation imaging for monitoring mechanical ablation of histotripsy pulses: Microbubble clouds were generated with histotripsy pulses (10 μs pulse duration, 18 MPa peak negative pressure, 20 Hz pulse repetition frequency) in a prostate tissue phantom. Cavitation emissions were passively recorded during the histotripsy insonation with a diagnostic imaging array, and processed using the delay, sum, and integrate method. In addition, we have recently implemented the robust Capon beamforming method that provides substantially improved axial resolution of the passive cavitation image. After insonation, the phantom was sectioned and processed with a periodic acid-Schiff stain. Fiducial markers composed of hyperechoic nylon wire imbedded in the phantom were used to align the beamformed passive cavitation images with the phantom histology images (**Figure 3**). A receiver operator characteristic (ROC) was computed by comparing the lesion width on the histology with the time-averaged passive cavitation image. The area under the ROC curve was in excess of 0.99 for both methods to process the passive cavitation images. These studies indicate that passive cavitation imaging can be used as a predictive measure of the ablative damage from histotripsy pulses. A manuscript based on this data is currently in preparation, and will be submitted to Radiology.

III. Efficacy of histotripsy combined with rt-PA *in vitro*: Histotripsy is under development to treat chronic deep vein thrombosis (DVT). We hypothesize that combining thrombolytic agents with histotripsy will enhance clot lysis. Recombinant tissue plasminogen activator (rt-PA) and rt-PA-loaded echogenic liposomes (t-ELIP) that entrain octafluoropropane microbubbles (OFP t-ELIP) were used in combination with highly shocked histotripsy pulses. Fully retracted porcine venous clots, with similar features of DVT occlusions, were exposed to histotripsy pulses alone (peak negative pressures of 7-20 MPa), histotripsy and OFP t-ELIP, or histotripsy and rt-PA. Microbubble cloud activity was monitored with passive cavitation imaging during histotripsy exposure. The energy levels of cavitation emissions from within the clot were not statistically different between treatment types, likely due to the near instantaneous rupture and deletion of OFP t-ELIP. The thrombolytic efficacy was significantly improved in the presence of rt-PA. These results suggest the combination of histotripsy and rt-PA could serve as a potent therapeutic strategy for the treatment of DVT. These data were presented at the 170th meeting of the Acoustical Society of America (Jacksonville, FL), and a manuscript was submitted to Physics in Medicine and Biology (November 2015). In addition, an R01 proposal has been submitted to the National Heart, Lung, and Blood Institute at the National Institutes of Health (submission date: October 2015) for further the study of this topic.

Conference Abstracts Funded in Part by FUS 319R1

1. Kenneth B. Bader, Kevin J. Haworth, Tao Peng, David D. McPherson, Adam D. Maxwell, Christy K. Holland, "Fibrin-targeted echogenic liposomes for localized ablation of thrombi with histotripsy pulses," 170th Meeting of the Acoustical Society of America, Jacksonville, FL, November 2015. *Journal of the Acoustical Society of America* **138** (3): 1819.

2. Kenneth B. Bader, Christy K. Holland, "The development of a hybrid finite difference solution of the Westervelt equation using the Fast Nearfield Method as a boundary condition for focused sources," 20th International Symposium on Nonlinear Acoustics, Lyon, France, June 2015. *AIP Publishing* **1685**: 070005.

3. Michael J. Crowe, Jason L. Raymond, Christy K. Holland, Kenneth B. Bader, "Broadband attenuation measurements of tissue-mimicking phantoms employed for histotripsy," 169th Meeting of the Acoustical Society of America, Pittsburgh, PA, May 2015. *Journal of the Acoustical Society of America* **137** (4): 2399.

Submitted Manuscripts Funded in Part by FUS 319R1

1. Kenneth B. Bader, Kevin J. Haworth, Himanshu Shekar, Adam D. Maxwell, Tao Peng, David D. McPherson, Christy K. Holland, "Efficacy of histotripsy combined with rt-PA *in vitro*," *Physics in Medicine and Biology* (submitted 11/2015)

2. Kenneth B. Bader, Michael J. Crowe, Jason L. Raymond, Christy K. Holland, "The effect of frequency-dependent attenuation on predicted histotripsy waveforms in tissue mimicking phantoms," *Ultrasound in Medicine and Biology,* (submitted 9/2015)

3. Kenneth B. Bader, Christy K. Holland, "Predicting the growth of nanoscale nuclei by histotripsy pulses," *Physics in Medicine and Biology,* (submitted 8/2015)

Submitted Grant Applications Based on Preliminary Data Funded in Part by FUS 319R1

"Localized thrombus ablation with histotripsy and echogenic liposomes," submitted to National Institutes of Health (October 2015)

Manuscripts in Preparation Funded in Part by FUS 319R1

Kenneth B. Bader, Kevin J. Haworth, Adam D. Maxwell, Christy K. Holland, "Passive cavitation images as a predictive metric of mechanical ablation with histotripsy pulses," *Radiology*