The effects of elastic modulus and impurities on bubble nuclei available for acoustic cavitation in polyacrylamide hydrogels

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Safety of biomedical ultrasound largely depends on controlling cavitation bubbles in vivo, yet bubble nuclei in biological tissues remain unexplored compared to water. This study evaluates the effects of elastic modulus (E) and impurities on bubble nuclei available for cavitation in tissue-mimicking polyacrylamide (PA) hydrogels. A 1.5-MHz focused ultrasound transducer with f#=0.7 was used to induce cavitation in 17.5%, 20% and 22.5% v/v PA hydrogels using 10-ms pulses with pressures up to p-=35 MPa. Cavitation was monitored at 0.075 ms through high-speed photography at 40,000 fps. At p-=29 MPa for all hydrogels, cavitation occurred at random locations within the -6 dB focal area (9.4x1.2 mm (p-)). Increasing pressure to p-=35 MPa increased bubble location consistency and caused shock scattering in the E=282 MPa hydrogels; as the elastic modulus increased to 300 MPa, bubble location consistency decreased (p=0.045). Adding calcium phosphate or cholesterol at 0.25% w/v, or bovine serum albumin at 5% or 10% w/v in separate 17.5% PA as impurities decreased the cavitation threshold from p-=13.2 MPa for unaltered PA to p-=11.6 MPa, p-=7.3 MPa, p-=9.7 MPa, and p-=7.5 MPa, respectively. These results suggest that both elastic modulus and impurities affect the bubble nuclei available for cavitation in tissue-mimicking hydrogels.

I. INTRODUCTION

In diagnostic ultrasound, acoustic cavitation is avoided as it can cause unwanted tissue damage. As such, all clinical machines must display the mechanical index (MI), a measure of the likelihood of cavitation[1]. However, acoustic cavitation is leveraged in some applications of therapeutic ultrasound to create bioeffects[2, 3] (tissue ablation, sonoporation, sonothrombolysis etc.). This prompts a need to understand bubble nuclei available for acoustic cavitation in biological tissues. In this study, we evaluate the effects of elastic modulus, as well as the size and hydrophobicity of added impurities on bubble nuclei available for acoustic cavitation in a common tissue-mimicking polyacrylamide (PA) hydrogel.

Over the decades, acoustic cavitation has been extensively studied in water, with bubble nuclei categorized as homogeneous, where vapor bubbles form in metastable liquids due to a phase transition[4], or heterogeneous, where pre-existing seed nuclei or gas pockets exist within the host liquid[5]. Many experimental researchers[6-9] have attributed heterogeneous nuclei as the predominant source of acoustic cavitation in water, as the location of bubble nuclei are random within the liquid. This results in probabilistic cavitation when an acoustic field is applied[10]. However, investigating bubble nuclei in tissues is far more complex than in water, as tissues are composed of cells and extracellular matrices with structures of differing physical and chemical properties down to sub-nanometers in size[11]. Additionally, gas levels in tissues are influenced by the tissue location, function, and disease[12]. For example, many cancerous tumors have a hypoxic core[13], which further influences the presence and distribution of bubble nuclei available for acoustic cavitation in biological tissues.

Heterogeneous bubble nuclei have been modeled according to the crevice bubble model[14], the variably permeable skin model[15], or the ion-stabilized nuclei model[16]. The earliest model of heterogeneous nuclei, the crevice model by Harvey *et al.*[14], proposed stabilization of cavitation bubbles as gaseous voids attached to hydrophobic conical crevices or motes in water. Hydrophobicity of the motes was hypothesized to arise from the physical properties of the mote itself or surface attachment of organic hydrophobic contaminants present in the medium to the motes[14]. Studies from Greenspan & Tschiegg[17] and Apfel[18] showed that decreasing the size of the solid particles or motes and reducing gas content increased the tensile strength of water as measured by acoustic cavitation. Furthermore, Greenspan and Tschiegg[17] found that gas content did not affect the cavitation threshold once motes smaller than 0.2 μm were filtered out, indicating the size of solid particles was the main contributor towards reducing the tensile strength of water measured by cavitation. Later experiments by Marschall *et al.*[19] showed that increasing the size of hydrophobic particles from 20 μm to 76 μm decreased tensile strength of water from 70 kPa to 50 kPa. These results suggest, cavitation in water is largely affected by hydrophobicity and size of motes or impurities[14, 19, 20].

In tissue-mimicking hydrogels, the effects of varying stiffness on acoustic cavitation have been previously studied[21 -24]. For example, Vlaisavljevich *et al.*[21] showed that no significant difference was found in the cavitation threshold (the peak negative pressure at which cavitation probability = 0.5) when the stiffness was varied from 1.13 kPa to 570 kPa in tissue-mimicking agarose gels. However, for gelatin-based tissue phantoms, Kang *et al.*[22] showed with theoretical modeling an increase in the critical pressure for cavitation nucleation with increasing concentration-dependent gel stiffness. Maximum bubble diameter has also been reported in several studies to decrease with increasing agarose gel stiffness[21, 23, 24]. Polyacrylamide (PA) hydrogels with bovine serum albumin (BSA) were developed by Lafon *et al.*[25] for thermal high intensity focused ultrasound (HIFU) studies. When exposed to thermal HIFU, opaque lesions form in the PA gel at the focus due to the denaturation of the BSA protein[25]. These PA hydrogels have also been exposed to boiling histotripsy[26], where cavitation activity caused fractionation of the hydrogel at the transducer focus. With a sound speed of 1544 m/s and acoustic impedance of 1.6 MRayls, PA hydrogels make a suitable soft tissue substitute, although the crosslinking, which stabilizes polymerization, makes a network that differs from biological tissues[27, 28]. Studies from Tse and Engler[29] and Denisin and Pruitt[30] have shown stiffness and elastic modulus can be tuned and quantified in PA hydrogels by increasing the monomer and crosslinker concentrations, making it a useful tissue-mimicking phantom for evaluating the effects of stiffness properties on the bubble nuclei available for acoustic cavitation. Furthermore, being optically transparent, PA gels also provide the advantage of allowing for monitoring of cavitation activity with high-speed photography[25].

Our objective is to evaluate the effects of PA hydrogel elastic moduli and added impurities that mimic those found in biological tissues on the bubble nuclei available for acoustic cavitation. PA hydrogels were fabricated with a range of elastic moduli and the location of acoustic cavitation when exposed to boiling histotripsy at 1.5 MHz was monitored using high-speed photography. Then, hydrophobic impurities of various size scales mimicking those found in the body including proteins (bovine serum albumin or BSA) and crystals (cholesterol and calcium phosphate) were added to the PA hydrogels to evaluate the thresholds for acoustic cavitation, defined as the peak negative pressure where the probability of cavitation exceeds 50%. We hypothesize that increasing the PA hydrogel elastic modulus will reduce the bubble nuclei available for acoustic cavitation and that adding impurities, even those as small as proteins, will reduce the cavitation threshold compared to pure or unaltered gels.

II. MATERIALS AND METHODS

A. PA phantom preparation

PA hydrogels were fabricated using the protocol in Lafon *et al.* [25] (2005). Briefly, 1 mol/L TRIS (trizma hydrochloride, Millipore Sigma, ST. Louis, MO, USA) was mixed with deionized water to dissolve 1% BSA (Millipore Sigma). 40% solution of acrylamide with acrylamide: bis-acrylamide 19:1 (Fisher Chemicals BP1406-1, Hampton, NH, USA) was added at 17.5%, 20% and 22.5% v/v concentrations to fabricate hydrogels of different elastic moduli (n=3 gel samples/PA concentration). The solution was then degassed for two hours in a desiccant chamber at 0.007 MPa. After degassing, ammonium persulfate (APS, Millipore Sigma) and N,N,N',N' tetramethylethylene/diamine (TEMED, Millipore Sigma) were added for polymerization which solidified the hydrogels. All hydrogel samples were used within 6 hours of preparation to avoid deterioration.

B. Elastic modulus measurement

A pair of 1 MHz contact transducers was placed directly on the clear polycarbonate[31] casing (1.72 mm thick, c_{casing}=2270 m/s) containing the gel samples to transmit ultrasonic pulses through the 17.5%, 20% and 22.5% v/v PA hydrogels (n=3 points/PA gel and n=3 PA hydrogels /elastic modulus). Speed of sound was calculated from the time difference between the transmitted and reflected maxima and the distance of propagation[32] of 54±0.24 mm. Then, the P-wave modulus[33] (M) was calculated from $M = \rho c^2$, where ρ is the density of the hydrogel and of c is the speed of sound measured using the ultrasonic data. Using the Poisson's ratio $\nu = 0.48$ measured by Boudou *et al.*[34] for PA hydrogels of varied elastic moduli, Elastic moduli (E) were estimated from the relation[33] $E = \frac{M(1+\nu)(1-2\nu)}{(1-\nu)}$. After obtaining the elastic modulus values (n=27 with n=3 points/PA gel and n=3 PA hydrogels /elastic modulus), Pearson's correlation coefficient, r, was measured from linear regression model using RStudio (R, Boston, MA, USA) to evaluate for a linear relationship between PA concentration and elastic modulus.

C. Acoustic cavitation

Fig. 1(a) shows the experimental arrangement for the study. A single-element 1.5 MHz focused ultrasound transducer with f# = 0 .7 (modified H-234 with a 41.2 mm center opening, Sonic Concepts, Bothell, WA, USA) was operated inside a water tank containing deionized and degassed water (<20% dissolved oxygen). An arbitrary waveform generator (Keysight, 33600A series, Colorado Springs, CO, USA) and RF amplifier (ENIA500, Rochester, NY, USA) were used to generate 10-ms pulses with peak negative pressures ranging up to p-=35 MPa. PA hydrogels were aligned with the transducer focus using a 3D positioning system (Bislide, Velmex Inc., Bloomfield, NY, USA) and acoustic cavitation at the treatment depth of 1.5 cm was recorded through high-speed photography (Photron Nova S9, Tokyo, Japan) at 40,000 fps with backlight illumination (Zaila Daylight LED Fixture, Nila, Altadena, CA, USA).

The acoustic pressure field of the transducer was measured in deionized and degassed water using a fiber optic probe hydrophone (FOPH, HFO – 690, ONDA Corporation, Sunnyvale, CA, USA). As the FOPH probe tip broke for peak negative pressures exceeding p-=20 MPa, waveforms for higher pressure amplitudes were modeled using the HIFU-beam simulator[35]

(Laboratory for Industrial and Medical Ultrasound, Moscow State University). The -6 dB focal dimensions of the 1.5 MHz transducer was measured at p-=18 MPa to be 9.4x1.2 mm.

D. Evaluation of spatial locations of bubble nuclei for acoustic cavitation

The location of acoustic cavitation was evaluated in the 17.5%, 20%, and 22.5% v/v PA hydrogels at 0.075 ms, a time point chosen as it represented the first evidence of cavitation in the majority of samples. A total of 27 points were assessed (n=3 points/PA hydrogel and 3 gel samples/PA concentration) for peak negative pressures from 29 MPa to 35 MPa. The captured grayscale images were processed using ImageJ (NIH, Bethesda, MD, USA) and MATLAB (Mathworks, Natick, MA, USA) for image binarization. Binarized images of three different locations within the same hydrogel exposed to the same peak negative pressure were overlaid to observe and measure overlap amongst the cavitated bubbles. When bubble overlap was observed, one-way ANOVA with Tukey pairwise testing was performed ($p<0.05$ considered significant) for 17.5%, 20% and 22.5% v/v PA hydrogels (n=3 gel samples/PA concentration). To measure the strength and direction of any linear relationship between elastic modulus and bubble overlap areas, Pearson's correlation coefficient, r, was estimated using linear regression model in RStudio.

E. Addition of impurities

To observe the effects of impurities in PA hydrogels on bubble nuclei available for cavitation, separate 17.5% v/v PA hydrogels (n=5 gel samples/added impurity) were fabricated as described previously with the addition of 0.25% w/v cholesterol crystals or 0.25% w/v calcium phosphate crystals (composed of submillimeter powder up to crystals of 0.6 mm and 0.4 mm maximum dimension, respectively), or 5% w/v BSA or 10% w/v BSA (average dimension[36] 140e-07 mm). These impurities were chosen to represent a range of size and hydrophobic properties similar to those found in tissues. Hydrogels with impurities were targeted using the same 1.5 MHz focused ultrasound transducer with 10-ms boiling histotripsy pulses as described previously. At each location in the hydrogel, acoustic pressure was increased until cavitation was observed at 0.075 ms using high speed photography. For each tested peak negative pressure, three locations on each gel were exposed. Cavitation probability was calculated as the number of times acoustic cavitation was observed at a particular pressure over total number of times gels were exposed to that pressure. Mean and standard deviation of the cavitation probabilities were then calculated from the set of samples for each PA gel with impurities (n=5 gel samples/added impurity). As the mean probabilities of cavitation were measured at discrete peak negative pressures, sigmoid curves were fitted using the MATLAB curve fitting toolbox. Cavitation thresholds were reported as the peak negative pressure at which acoustic cavitation probability was 50%.

III. RESULTS

A. Effect of elastic modulus on bubble nuclei available for cavitation

The sound speeds of the 17.5%, 20% and 22.5% v/v PA hydrogels were measured with 1 MHz contact transducers as 1544±20 m/s, 1566±20 m/s and 1600±23 m/s respectively, resulting in respective P-wave moduli of 2.48±0.06 GPa, 2.55±0.08 GPa and 2.66±0.08 GPa. The elastic moduli of the 17.5%, 20% and 22.5% v/v PA hydrogels were estimated using the previously reported Poisson's ratio[34] and P-wave modulus[33] as 282±7 MPa, 291±8 MPa and 300±12 MPa, respectively. As expected, increasing the concentration of PA increased the elastic modulus of the PA hydrogel (r=0.62) as shown in Fig. 2.

Upon exposure of the E=282 MPa hydrogel to 1.5 MHz focused ultrasound and peak negative pressures of 29 MPa, cavitation activity was first observed at 0.075 ms (Fig. 3a). As the focused ultrasound exposure progressed, several more bubbles were observed 0.125 ms. However, when hydrogels with the same elastic modulus of E=282 MPa were exposed to a higher peak negative pressure of 35 MPa, we observed a shock-scattering-induced bubble cloud at 0.075 ms (Fig. 3b). This bubble cloud continued to oscillate and collapse throughout the 10-ms boiling histotripsy pulse.

When the E=282 MPa hydrogels were exposed to a peak negative pressure of 29 MPa, acoustic cavitation occurred at random locations within the -6 dB focal area of the transducer, with no bubble overlap among the repeat exposures in the same hydrogel (Fig. 4). Similar lack of overlap was observed for E=291 MPa and E=300 MPa hydrogels when exposed to a peak negative pressure of 29 MPa. As the peak negative pressure increased from 29 MPa to 33 MPa, a combination of a few solitary cavitation bubbles and shock scattering was observed in separate exposures for the same hydrogel elastic modulus. Increasing the peak negative pressure to 35 MPa for the E=282 MPa PA gels further increased shock scattering occurrences at 0.075 ms, increasing the bubble overlap among repeat exposures in the same hydrogel (Fig. 5 a and b). However, when the elastic modulus was increased from E=282 MPa to E=300 MPa, acoustic cavitation and thus shock scattering became less consistent, reducing the overlap of bubbles in the overlaid binary image (Fig. 5 c and d).

Comparing the bubble overlap area (n=3 targeted locations/PA hydrogel and n=3 PA hydrogels/elastic modulus) across all three hydrogel elastic moduli at p-=35 MPa, we found that increasing the elastic modulus of the gel decreases bubble overlap area, or the consistency of available bubble nuclei for acoustic cavitation and shock scattering. PA hydrogels (n=3 PA hydrogels/elastic modulus) with the lowest elastic modulus (E=282 MPa) were shown to have the largest bubble overlap area. Increasing the PA gel elastic modulus to E=300 MPa significantly decreased the bubble overlap area compared to the E=282 MPa hydrogels (ANOVA p=0.045, n=3 PA hydrogels /elastic modulus, Fig. 6).

B. Effect of impurities on acoustic cavitation threshold

Unaltered 17.5% v/v PA hydrogels were found to have a cavitation threshold of p-= 13.2 MPa. When 0.25% w/v calcium phosphate crystals were added in separate 17.5% v/v gels, the cavitation threshold drops to p-=11.6 MPa (Fig. 7a). The addition of 0.25% w/v cholesterol crystals in separate PA hydrogels drops the cavitation threshold even further to p-=7.3 MPa (Fig. 7b).

When BSA protein was added at different concentrations to the 17.5% v/v PA hydrogels, we see a similar decrease in the cavitation threshold. For 5% w/v BSA in the 17.5% v/v PA, cavitation threshold was reduced from p-=13.2 MPa in the unaltered hydrogel to p-=9.7 MPa (Fig. 8a). When the BSA concentration was increased to 10% w/v in separate PA hydrogels, the cavitation threshold further decreased to p-=7.5 MPa (Fig. 8b). Interestingly, for both 5% and 10% w/v BSA, cavitation did not occur 100% of the time until the peak negative pressure reached p-=12.8 MPa.

IV. DISCUSSION

This study evaluated the effect of varying elastic moduli and the addition of impurities on the bubble nuclei available for acoustic cavitation in tissue-mimicking PA hydrogels. When PA hydrogels were exposed to a peak negative pressure of 29 MPa, bubble nuclei were found at random locations within the -6 dB focal area of the transducer for all PA hydrogel elastic moduli. Increasing the peak negative pressure to 35 MPa caused shock scattering in all concentrations of PA hydrogels at 0.075-ms, with the consistency of shock scattering decreasing with increasing elastic modulus. When cholesterol, calcium phosphate, or BSA impurities were added to the PA hydrogels, a decrease in the cavitation threshold was observed compared to unaltered 17.5% PA hydrogels. The addition of hydrophobic cholesterol crystals or 10% BSA showed the largest decrease in acoustic cavitation threshold to p-=7.3 MPa and p-=7.5 MPa, respectively, whereas the addition of the calcium phosphate crystals showed the smallest change in acoustic cavitation threshold of 1.6 MPa from unaltered PA hydrogels (p-=11.6 MPa and p-=13.2 MPa, respectively). These results suggest the importance of elastic modulus and the size and hydrophobicity of added impurities influences the acoustic cavitation threshold in tissue-mimicking PA hydrogels.

The shock scattering observed in this study was more predominant for higher peak negative pressures and lower elastic moduli hydrogels. Shock scattering has been identified by Maxwell *et al.*[10] as a fundamental mechanism for bubble cloud formation during a single multicycle histotripsy pulse, where the impinging positive shock is backscattered and phase inverted by the initial bubble, creating a larger rarefaction-driven bubble cloud formation. Previously, studies of Vlaisavljevich *et al.*[21, 37], and Wilson *et al.*[24] reported that increasing the medium stiffness negatively impacted bubble expansion and maximum bubble diameter. Associated to the stiffness and size of bubble, the analytical computations of Bader *et al.*[38] showed that for stiffer media, expansion of initial bubble nuclei became inhibited enough to fail initiating shock scattering during histotripsy pulses, which is further supported by our observation for increasing PA elastic modulus.

The addition of cholesterol crystal impurities decreased the cavitation threshold more than the addition of calcium phosphate crystal impurities, which may be explained through hydrophobicity. Cholesterol is more hydrophobic than calcium phosphate, with calcium phosphate[39] having a polar surface area of 173 Å² compared to 20.2 Å² for cholesterol[40]. Previous research[14] in water has shown that that more bubbles are stabilized on hydrophobic motes, which is supported in our study by the lower cavitation threshold of cholesterol compared to calcium phosphate. In addition to hydrophobicity, particle size has been shown to affect the peak negative pressures required for cavitation[17, 19]. In this study, cholesterol crystals were slightly larger than calcium phosphate crystals (0.6 mm and 0.4 mm respectively) and higher peak negative pressures were required for cavitation in hydrogels with the added calcium phosphate crystals. Similarly, BSA, which has a reported average dimension[36] 140e-07 mm, also reduced the cavitation threshold, even though BSA is much smaller than the previously proposed minimum of 0.2 μm for solid motes to affect cavitation[14]. While BSA is generally hydrophilic with pH dependent charge[41] (effective charge of -8.4 ± 0.3 at pH=6.8), hydrophobic lipid-binding pockets[42] also exist which may allow bubbles to be stabilized that can influence cavitation. Taken together, the impact of these added impurities on cavitation threshold suggest that hydrophobicity may have more impact than size in reducing the cavitation threshold in tissue-mimicking PA hydrogels.

One major limitation of this study was the 40,000-fps used for high-speed photography. While this frame rate was sufficient to capture the spatial distribution of early acoustic cavitation at 29 MPa, shock scattering was present when the peak negative pressure was increased. Another limitation is that we only evaluated cavitation for a single frequency of 1.5 MHz. Although this frequency is well within the frequency range of therapeutic ultrasound, future studies will include evaluating the effect of frequency on the distribution of bubble nuclei available for acoustic cavitation.

Additionally, all reported pressures are unattenuated. Lafon *et al.*[25] reported a linear increase in the attenuation of polyacrylamide with BSA concentration ranging from 3% to 9% for frequencies of 1 to 5 MHz. The attenuation coefficient[25] was reported as 0.009 Np/cm/MHz at 3% BSA and 1 MHz. Extrapolating from their results, 1%, 5% and 10% BSA would cause attenuation coefficients of 0.005, 0.013 and 0.023 Np/cm/MHz, respectively at 1.5 MHz. Hence, the derated pressures were found to be close to the unattenuated pressures at the treatment depth of 1.5 cm. Finally, P-wave moduli of PA hydrogels were calculated through speed of sound measurements at 1 MHz, resulting in elastic moduli on the order of 100s of MPa instead of kPa as has been previously reported using laser-ultrasonic pulses and phase velocity or microscale mechanical characterization[43, 31, 44]. However, our speed of sound and density measurements were similar to the values reported by Zell *et al.*[45] which were measured from ultrasonic pulses, showing the influence of measurement technique and shear rate on the reported values of elastic modulus in PA hydrogels. Future investigations may include mechanical characterization of PA hydrogels for easier comparison to biological tissues.

V. CONCLUSIONS

The increasing number of applications of diagnostic and therapeutic ultrasound necessitate understanding bubble nuclei available for acoustic cavitation in biological tissues for clinical safety. Results from this study show that acoustic cavitation is influenced by both elastic moduli and added impurities in tissue-mimicking PA hydrogels. As the PA elastic modulus increased, the number of bubble nuclei available for acoustic cavitation decreased, as evidenced by the reduced bubble overlap for higher elastic modulus hydrogels. Introducing impurities in PA hydrogels with hydrophobic properties, even those as small as BSA proteins, decreased the acoustic cavitation threshold. These results suggest that tissue elastic moduli and impurities as small as proteins affect the number and distribution of bubble nuclei available for acoustic cavitation.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Andrea Arguelles from the Pennsylvania State University for providing equipment and advice in measuring sound speeds in the hydrogels. Funding for this research were provided by NSF CAREER 1943937 and the Pennsylvania State University Center for Biodevices Leighton Riess Graduate Fellowship (Rawnaque).

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Collected Figure Captions

Fig. 1. (Color online). (a) Experimental arrangement for a 1.5 MHz focused ultrasound transducer to induce acoustic cavitation in PA hydrogels. A high-speed camera was used to monitor cavitation at 40,000 fps. (b) Representative pressure waveform measured with a fiber optic probe hydrophone at 400 mV. Peak negative pressures above 20 MPa were estimated using HIFU-beam simulator software[35].

Fig. 2. (Color online). Plot of elastic modulus versus PA concentration (n=3 points/PA gel and n=3 PA hydrogels /elastic modulus). Increasing acrylamide content increases elastic modulus of the hydrogels (r=0.62).

Fig. 3. High speed photographs of an E=282 MPa PA hydrogel during exposure to a 10 ms boiling histotripsy pulse at 1.5 MHz with peak negative pressures of (a) 29 MPa and (b) 35 MPa. For both applied peak negative pressures, no bubbles were observed in the first frame at 0.025 ms (left column). As time progressed to 0.075 ms (middle column), the first acoustic cavitation bubble appears in (a) the PA gel exposed to 29 MPa peak negative pressure while (b) shows shock-scattering-induced bubble cloud formation for the PA gel exposed to 35 MPa peak negative pressure. As the ultrasound pulse continues to 0.125 ms (right column) additional bubbles are observed for (a) the 29 MPa exposure and (b) collapse followed by growth of the bubble cloud is observed for the 35 MPa exposure.

Fig. 4. (Color online). (a,b,c) Representative high-speed images of acoustic cavitation for 282 MPa hydrogels (n=3 targeted locations/PA hydrogel) exposed to p-=29 MPa at 0.075 ms. The oval in (a) shows the -6 dB negative pressure focal area of the transducer. d) Binarized and overlaid high-speed photographs from a, b, c show no bubble overlap between the three exposures (darker spots indicate increasing overlap).

Fig. 5. (a, c) Representative high-speed images (n=3 targeted locations/PA hydrogel) of acoustic cavitation occurring at 0.075 ms in a) E=282 MPa PA hydrogels and c) E=300 MPa PA hydrogels at p-=35 MPa. (b, d) Binarized and overlaid high-speed photographs at 0.075 ms for three separate locations exposed to the same peak negative pressure in the same hydrogel. (a) At 0.075 ms, shock scattering was observed in most of the E=282 MPa samples, which (b) increased bubble overlap in the binarized and overlaid images. (c) When the gel elastic modulus increased to E=300 MPa, acoustic cavitation and thus shock scattering was reduced at 0.075 ms, leading to (d) less bubble overlap in the binarized images.

Fig. 6. Plot of average bubble overlap area at p-=35 MPa vs. PA hydrogel elastic modulus (n=3 PA hydrogels /elastic modulus). Increasing the PA elastic modulus decreased the average bubble overlap area caused predominantly by shock scattering (r=–0.79). A significant difference was found between the average bubble overlap area of the 282 MPa and 300 MPa elastic moduli gels using post-hoc one-way ANOVA with Tukey pairwise comparison (ANOVA p=0.045, n=3 PA hydrogels /elastic modulus).

Fig. 7. (Color online). Acoustic cavitation probability vs. peak negative pressure for (a) calcium phosphate crystals and (b) cholesterol crystals added to 17.5% v/v PA hydrogels. The asterisk indicates the cavitation threshold measured for unaltered 17.5% v/v PA hydrogels (n=5 gel samples/added impurity).

Fig. 8. (Color online). Acoustic cavitation probability vs. peak negative pressure for (a) 5% w/v BSA and (b) 10% w/v BSA in 17.5% v/v PA hydrogels. The asterisk indicates the cavitation threshold for unaltered 17.5% v/v PA hydrogels (n=5 gel samples/added impurity).