Nanoparticles Formed by Acoustic Destruction of Microbubbles and Their Utilization for Imaging and Effects on Therapy by High Intensity Focused Ultrasound

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SUPPORTING INFORMATION

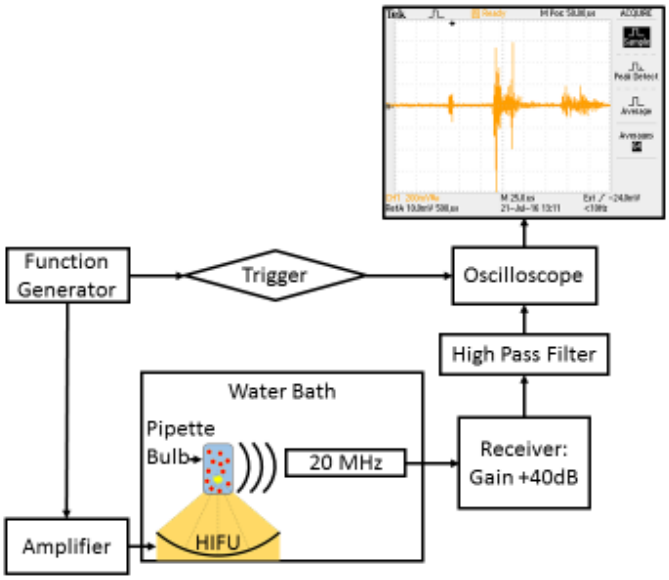

Figure S1. Schematic and sample raw data of the passive cavitation detector (PCD) setup.

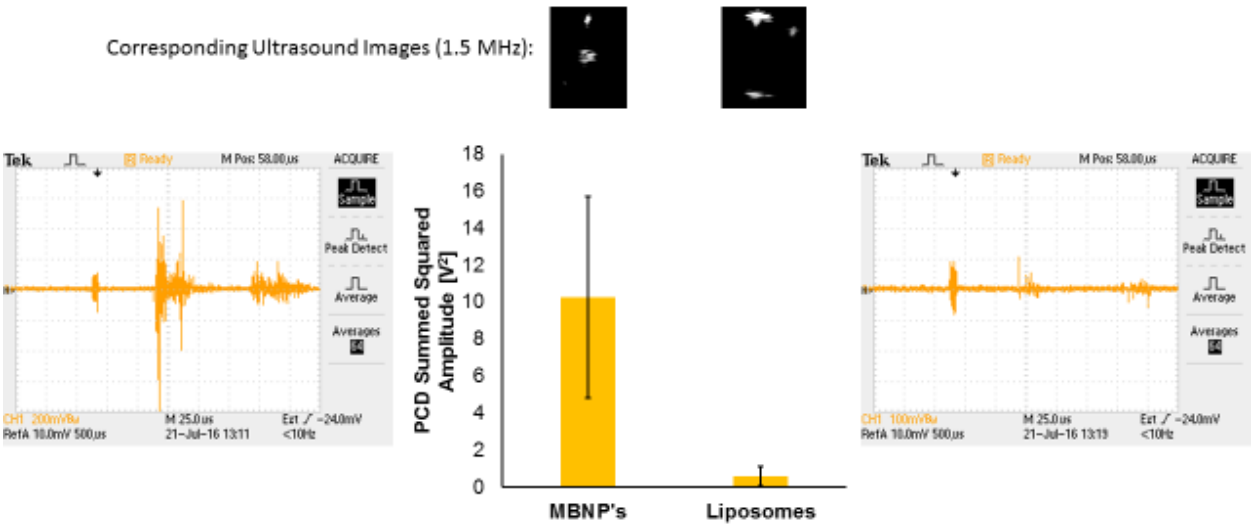

Figure S2. PCD validation of corresponding ultrasound images. This indicates that the generation of the US signal does not depend on the imaging wave being present. Above the bars are the corresponding CPS images. Representative oscilloscope screenshots for NP solutions (left) and sonicated liposome solutions (right) are included. The bar graph (center) includes the same data but quantified. Error bars denote one standard deviation taken from three independent solutions.

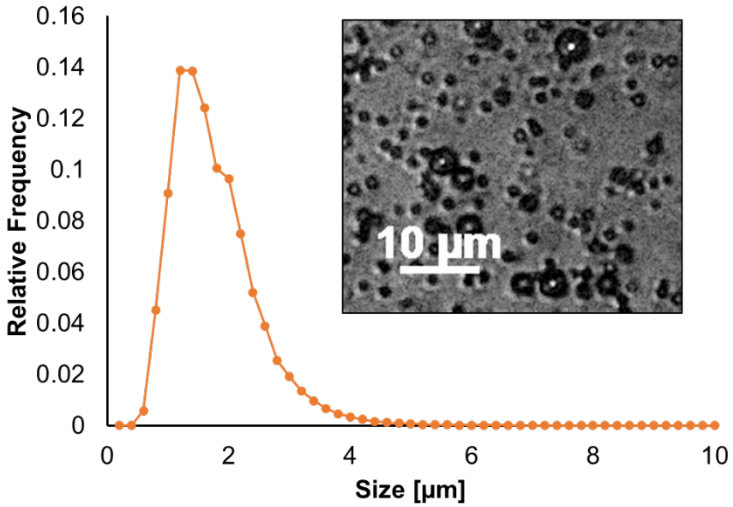

Figure S3. Histogram representing a typical size distribution of PFB microbubble suspensions as measured by optical microscopy. Inset: typical BF image of the stock microbubble solution taken at 40x. Data is averaged from three independent samples.

Table S1. Properties of microbubble stock solution, before dilution typically of about 500x, and the properties of the resulting MBNP/liposome/micelle solution generated from a diluted microbubble solution.

Property	Bubbles	NPs	Liposomes
Concentration [#/mL]	$4.36 \times 10^9 \pm 2.71 \times 10^9$	$1.64 \times 10^9 \pm 7.31 \times 10^8$	$3.17 \times 10^9 \pm 2.13 \times 10^8$
Mean Diameter [μm]	1.67 ± 0.73	$1.40 \times 10^{-1} \pm 3.70 \times 10^{-2}$	$3.63 \times 10^{-1} \pm 5.49 \times 10^{-3}$

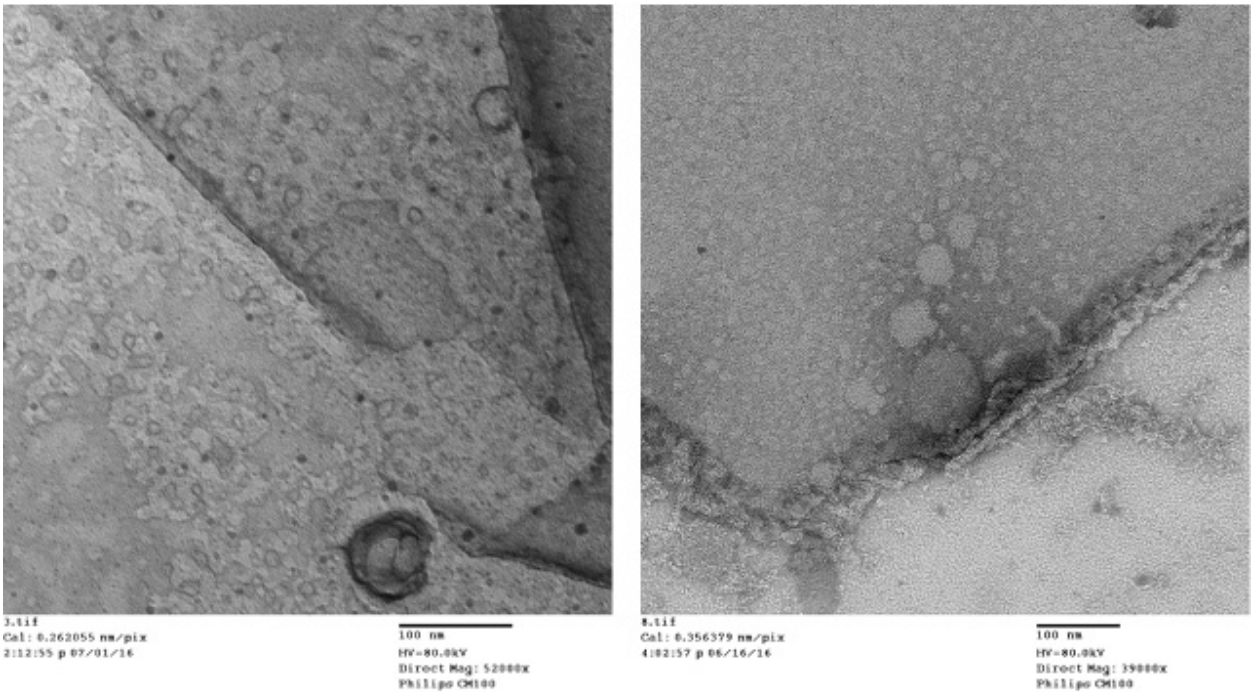

Figure S4. TEM image comparison of a uranyl acetate stained sonicated liposome solution (left) and a similarly stained destroyed microbubble solution (right).

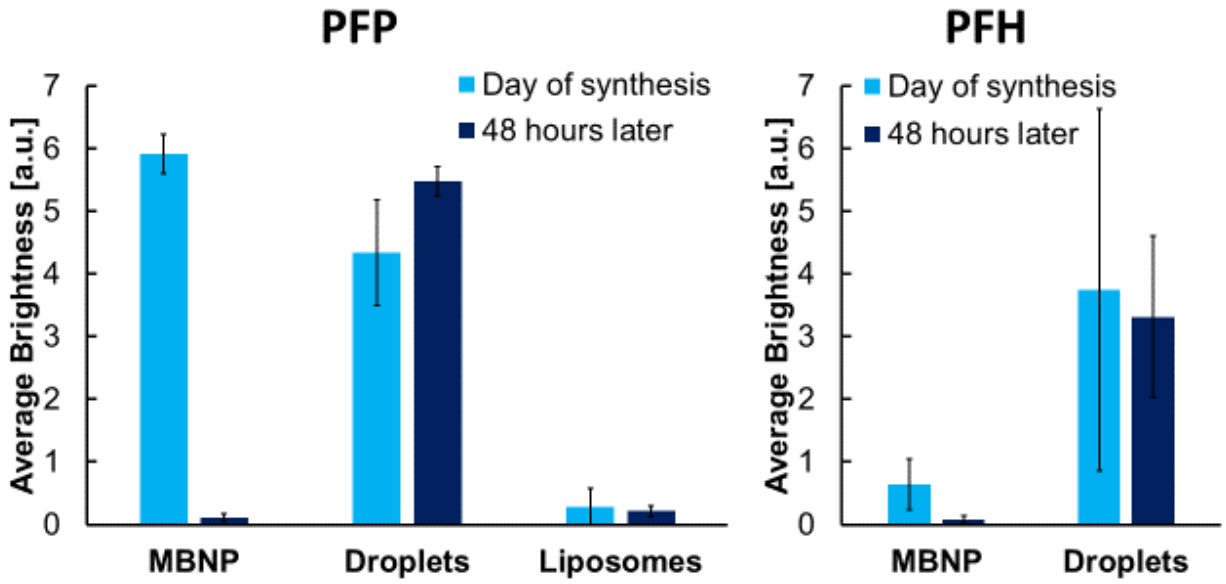

Figure S5: Acoustic contrast obtained from MBNP's derived from initial bubble concentration of ~10^7, as compared to synthesized nanodroplets also at an initial concentration of 10^7, for PFP and PFH cores. Response persisted for more than 24 hours for PFP and PFH MBNPs but was greatly reduced after the second day, while contrast from nanodroplets persisted for at least two days. MBNPs for each core were imaged with the same HIFU intensities as shown in Figure 2B.

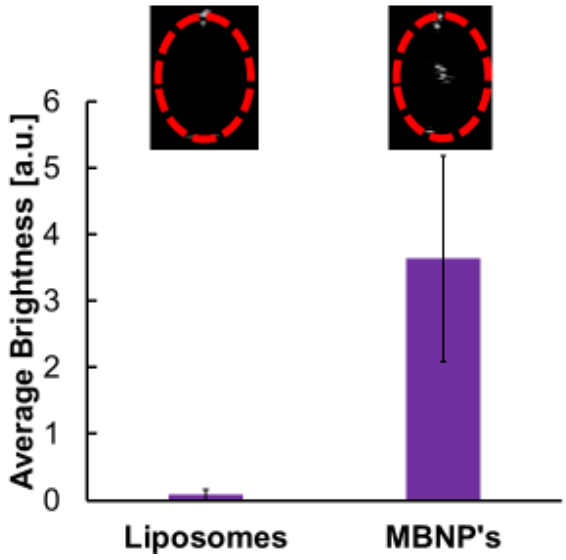

Figure S6: Ultrasound response (HIFU energy 790 W/cm^2) from PFP MBNPs isolated by centrifugation, as compared to a similar isolation procedure conducted with a liposome suspension.

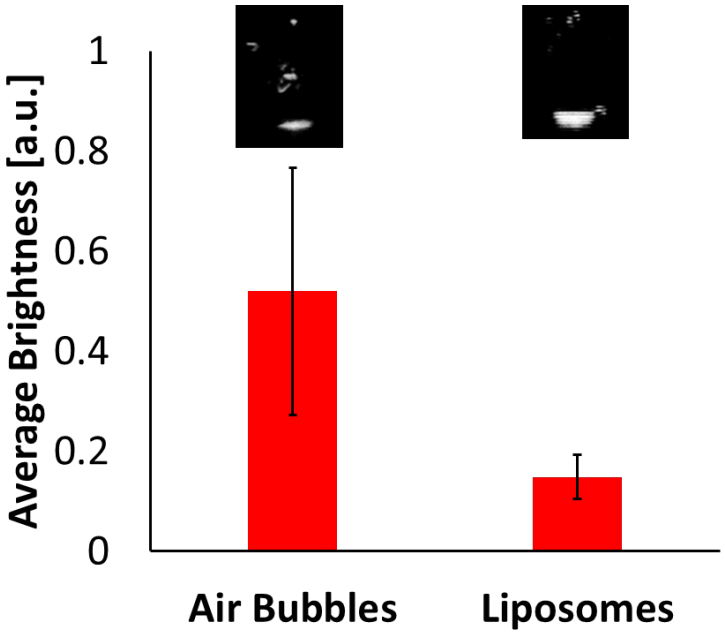

Figure S7. Ultrasound response from MBNPs generated by destroyed air microbubbles at 2x dilution as compared to a liposome solution (right) at 900 W/cm^2 HIFU intensity with 50x dilution of the original air microbubble solution imaged with CPS at 1.5MHz.

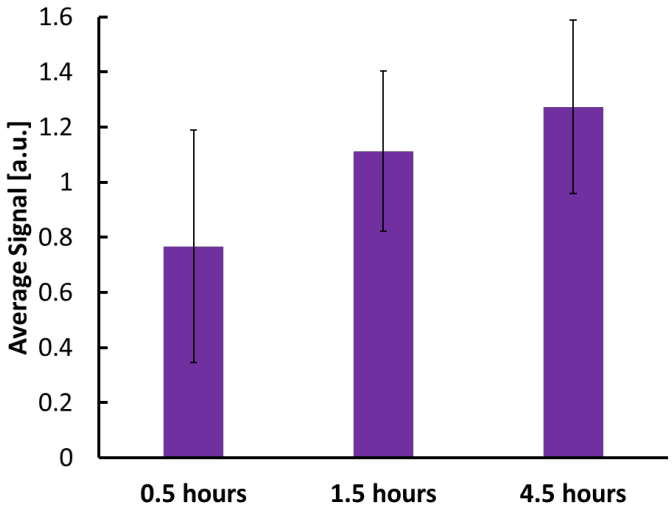

Figure S8. The brightness generated by the MBNP's from microbubble solutions aged X hours after the synthesis of the same solution. Error bars denote one standard deviation with three measurements each of independent samples. Data was taken at 415 W/cm^2 of HIFU intensity and 1.5 MHz Sequoia imaging.

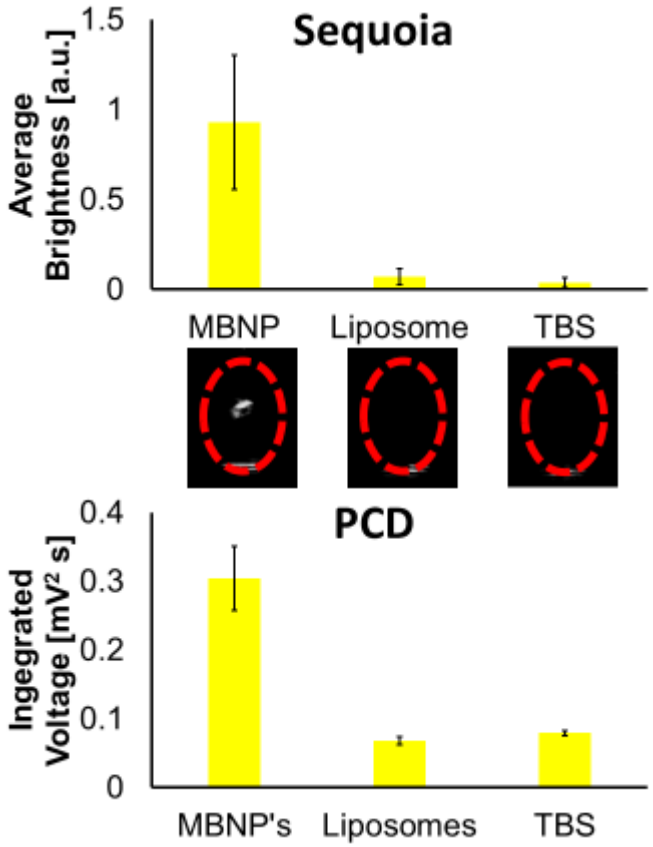

Figure S9: MBNP response quantification where the diluent was degassed TBS. Left to right: MBNPs in degassed TBS, liposomes in degassed TBS, and degassed TBS alone.

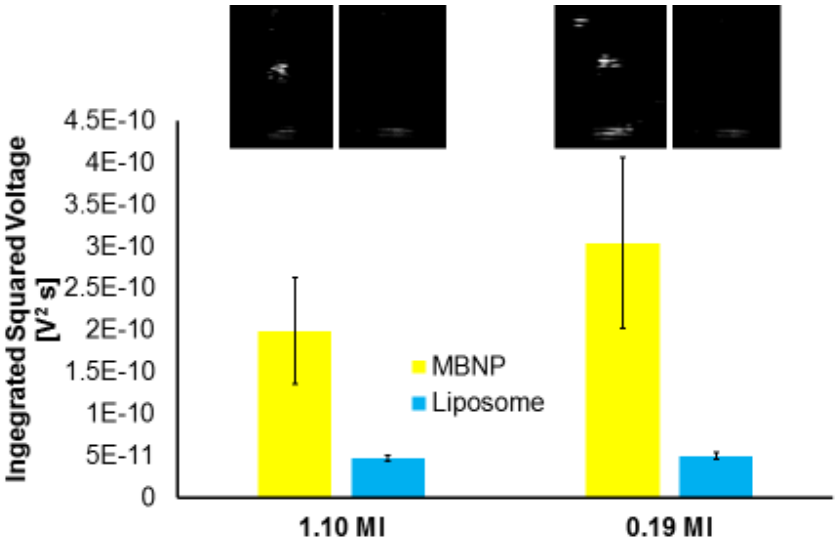

Figure S10: MBNP response comparison resulting from the destruction of microbubbles using 1.10 MI for 1 minute and 0.19 MI for 10 minutes as measured by PCD.

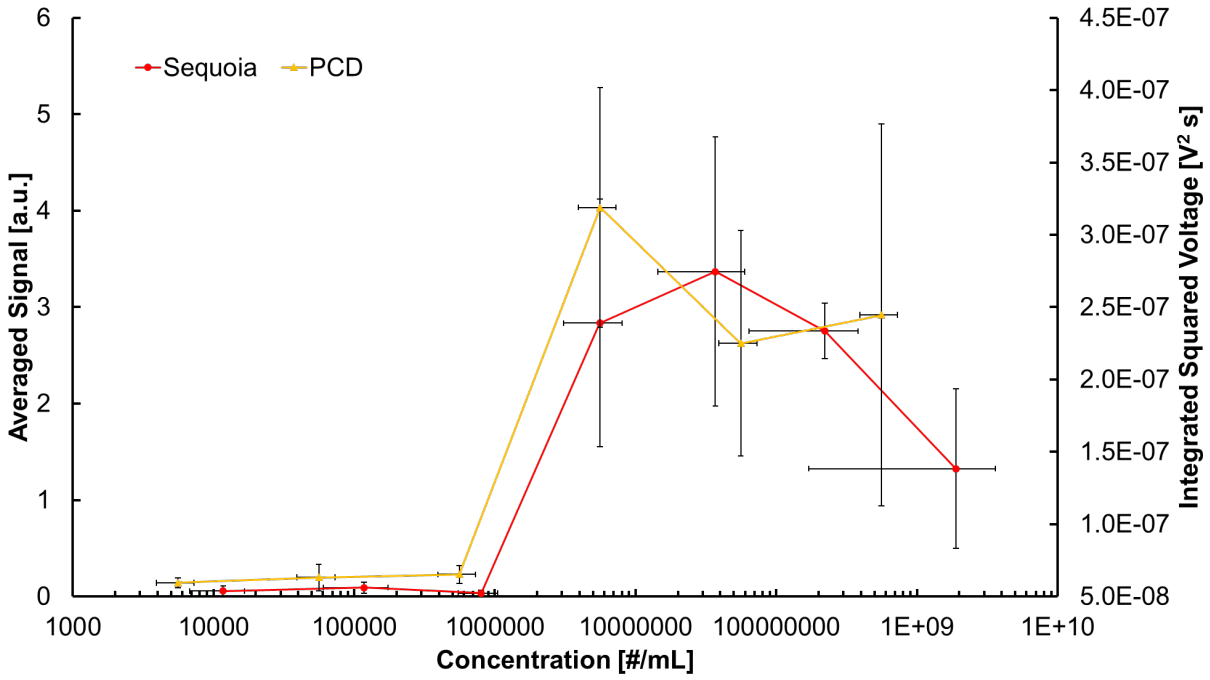

Figure S11. Corresponding PCD measurement of the concentration dependent response of the MBNP solution. For the PCD graph, the horizontal error bars represent the inherent error (approx. 30%) in the microbubble counting method. Vertical error bars represent one standard deviation in the signal response.

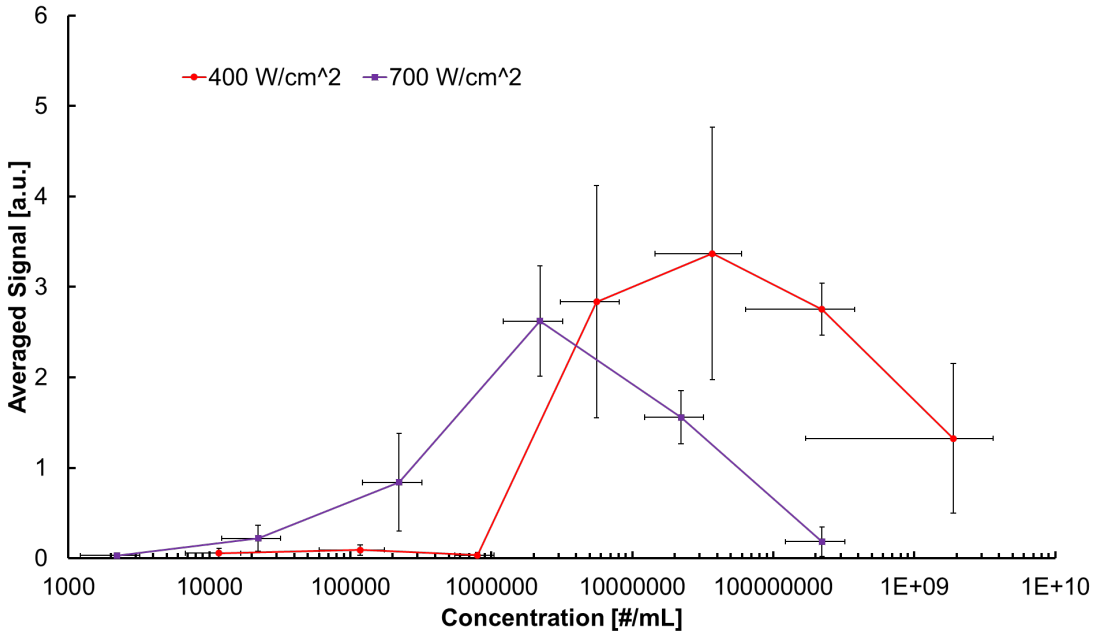

Figure S12. Concentration series at higher HIFU intensity overlaid with series from Figure 2A. Note that at higher HIFU intensities, lower concentrations can be re-imaged. Error bars denote one standard deviation.

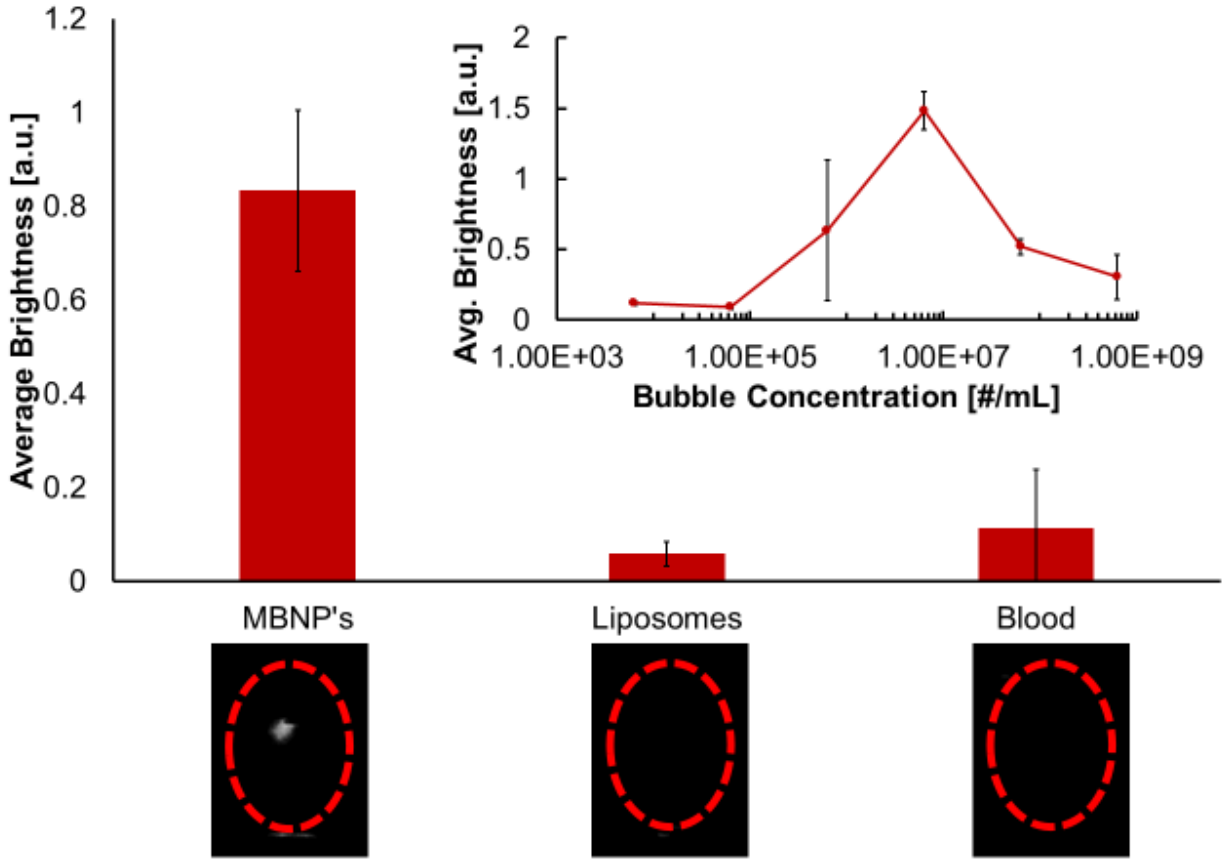

Figure S13: MBNP response quantification where bovine whole blood was used as the diluent. Left to right: MBNPs in bovine whole blood, liposomes in blood, and blood alone.

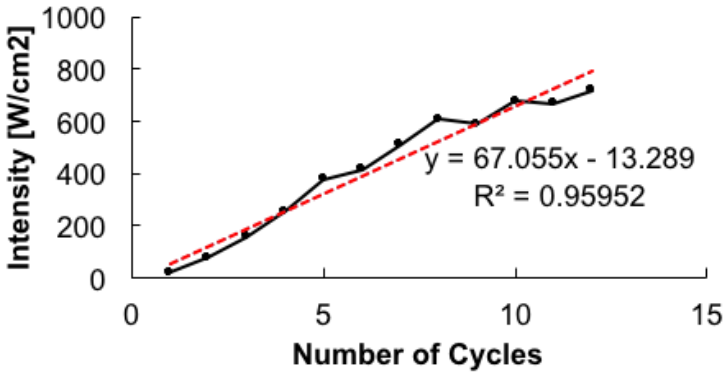

Figure S14. HIFU calibration curve corresponding number of cycles with HIFU intensity taken with needle hydrophone. Values outside of this range were extrapolated using the above equation.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	13.307109	4.43570	19.3911
Error	14	3.202500	0.22875	Prob > F
C. Total	17	16.509609		<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Diluent	2	2	1.947811	4.2575	0.0359*
Sample	1	1	11.359298	49.6581	<.0001*

Levels not connected by same letter are significantly different.

Level		
Buffer	A	
Serum	A	B
Blood		B

Level		
MBNP	A	
Liposome		B

Figure S15: Statistical analyses conducted in JMP 12 (alpha = 0.05 for all tests). Using Diluent (Blood, Serum, and Buffer) and Sample (MBNP, Liposome) as explanatory variables, the ANOVA test indicated that these factors did have a significant effect on the result (avg. brightness). Further, testing the significance of each effect and found that both Sample and Diluent both contributed significantly to the model. Lastly, a student HSD test was conducted for both effects to determine which levels within each effect were significantly different from each other. It was found that for Sample: MBNP and Liposomes were statistically significantly different for all cases. It was found for Diluent that Buffer and Blood were significantly different in their response, but neither was significantly different from Serum.