Degradation of Ultrasound Contrast Agents Embedded in a Tissue Phantom

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Statement of Purpose: Ultrasound contrast agents are currently used to diagnose vascular abnormalities and image tumor perfusion. These agents (often referred to as bubbles) consist of a lipid, polymer, or protein shell that surrounds a gas core and are echogenic when exposed to moderate acoustic pressure (mechanical index > 0.05) [1]. In addition to providing image contrast, these agents also have the potential to act theranostically for localized non-invasive drug delivery to tumors. An increase in acoustic pressure causes the bubbles to burst, releasing the payload at the targeted location. When the bubbles rupture, this event can cause damage to microvessels and cellular membranes, allowing elevated extravasation of bubbles into the surrounding tissue as well as into the cell [2]. While typically the bubbles are limited to the blood pool due to their large size (>1µm), recent advances in our laboratory have led to the development of a facile means for fabricating nanoscale bubbles, which can be taken up by tumors due to the enhanced permeability and retention effect [3]. Nanobubbles are formulated by incorporating a pluronic surfactant into the lipid shell, which is also a chemo and thermal sensitizing agent for the treatment of tumors. The bubbles must be stable after leaking into the tissue in order to control and sustain the rate of drug delivery. The aim of this study is to optimize the imaging parameters for micro- and nanobubbles in a tissue phantom and study their degradation compared to bubbles in a liquid environment.

Methods: Microbubbles (~1µm) were synthesized by dissolving lipids in chloroform then evaporating the solvent to produce a lipid film which was hydrated with PBS and glycerol. The air in the vial was replaced with octafluoropropane (C_3F_8), and the solution was mechanically agitated to form the bubbles. Nanobubbles (~200nm) were produced by the same procedure, but using a solution of 0.6 mg/mL L61 pluronic in PBS during hydration. A stair shaped agarose design was used for imaging a sample of bubbles in agarose and bubbles in water simultaneously (n=3). Imaging was performed using contrast harmonic imaging with a Toshiba Aplio SSA-770A diagnostic ultrasound. Error is reported as standard deviation. A student's t-test was used to assess statistical significance.

Results: Both microbubbles and nanobubbles remained echogenic while embedded in the tissue phantom, as seen in **Fig 1**, which shows microbubbles in water (**Fig 1A**) and microbubbles embedded in agarose (**Fig 1B**). Bubbles in water and embedded in a tissue phantom were exposed to ultrasound for 30 min. Degradation of microbubbles in water (**Fig 2**) was found to be linear at a rate of -0.194 ± 0.09 dB/min while bubbles in agarose had an initial linear decay rate of -1.22 ± 0.2 dB/min. The linear decay for nanobubbles in water was -0.0840 ± 0.2 dB/min and in agarose was -1.12 ± 0.2 dB/min. The greater rate of degradation in agarose is due to the confined bubbles' repeated exposure to ultrasound, while bubbles in water are stirred so that they are replenished from the surrounding solution. Signal from microbubbles and nanobubbles in agarose was almost completely lost after 15 min. A t-test for independent samples produced a p-value of <.0001, indicating that the water and agarose data are statistically significant from each other. No statistical difference in the degradation of microbubbles and nanobubbles was observed.

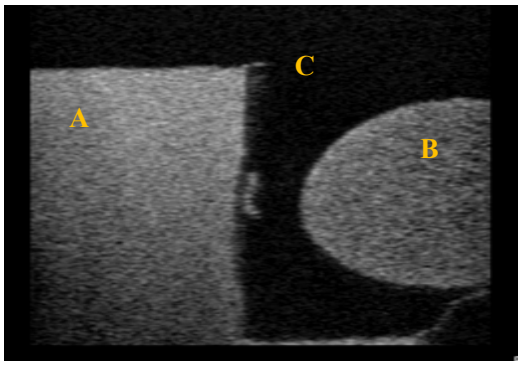

Figure 1: Ultrasound image of microbubbles dispersed in water (A), embedded in agarose (B). Agarose without added bubbles has no background signal (C)

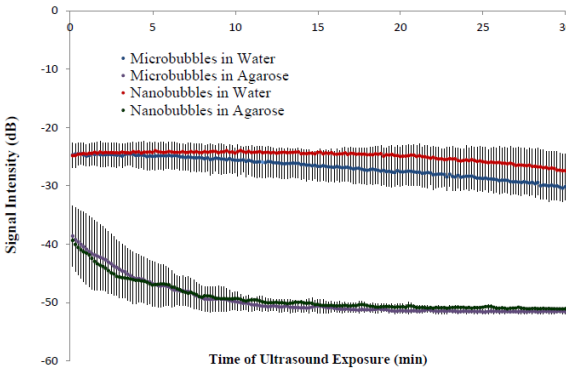

Figure 2: Average degradation curves for microbubbles and nanobubbles in agarose and in water over 30 min of ultrasound imaging.

Conclusions: Although bubbles remain echogenic in a tissue phantom, the rate of degradation proves problematic for sustained imaging of tumor treatment. The degradation rates between microbubbles and nanobubbles in both mediums were found to be statistically insignificant, indicating that the smaller size of the nanobubbles does not affect its destruction in the tissue. This was a short-term study, so the bubble degradation in water was not fully seen. The high rate of destruction in agarose could be decreased by taking less frequent images or using a lower mechanical index. This experiment was designed to mimic how bubbles behave in tissue and in the vasculature, so for an accurate comparison a new phantom will be developed with a channel so the bubble behavior can be evaluated under flow. The diffusion of the bubbles from the channel into agarose will be evaluated and compared to the Krogh tissue model of steady-state diffusion.

References:

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