Stable Thermally Modulated Nanodroplet Ultrasound Contrast Agents

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Statement of Purpose: Droplet vaporization (acoustically or thermally triggered) has been a long-established method for inducing echogenicity of otherwise stable but poorly echogenic liquid perfluorocarbon-based ultrasound contrast agents. This process drives a phase transition of the liquid perfluorocarbon core into the gas phase, resulting in enhanced echogenicity.However, in this case, the increase in echogenicity comes at a cost of increased size and reduced stability of the particles, which limits the range of their diagnostic and therapeutic applications. We have discovered that liquid tetradecafluorohexane (TDFH) nanodroplets stabilized with a bovine serum albumin (BSA) shell exhibit an unexpected, thermally responsive behavior that is independent of vaporization. Hence, we have developed a method for increasing echogenicity of these particles by subjecting them to a heating-cooling cycle, the process which we will refer to as thermal modulation. In this study, we evaluated the signal intensity of BSA-TDFH nanodroplets before and after the thermal cycle, and showed that thermal modulation induces echogenicity of the particles while preserving their nanoscale size and stability.

Methods: Nanodroplets were prepared by combining 850 µl of 1.4% solution of BSA in 1X phosphate-buffered saline (PBS) with 250 µl of liquid TDFH followed by air removal and mechanical agitation. Prior to any experiment, nanosized particles were isolated from a polydisperse nanodroplet population by using centrifugation. During the thermal modulation process, the nanodroplet solution was first heated from room temperature to 44 °C, and then cooled down to 37 °C. Thermally modulated BSA-TDFH nanodroplets were characterized in terms of structure, size, echogenicity, stability, and thermal responsiveness over the range of temperatures from 25 °C to 44 °C. The ultrasound images of nanodroplets were obtained in a glass beaker that contained PBS using an MX250 linear array transducer equipped to a Vevo 3100 preclinical ultrasound imaging system (Fujifilm VisualSonics). The images were acquired using the nonlinear contrast (NLC) mode at an 18 MHz frequency and 75% power.

Results: The data showed that thermal modulation significantly increased the echogenicity of the nanodroplets ($p<0.001$). The mean fold-increase in nanodroplet echogenicity after the thermal cycle was 10.4±3.3 on the B-mode (**Fig.1)** and 12.5±4.6 on the NLC mode. The average diameters of nanodroplets before and after thermal modulation were 199.1±45.0 nm and 169.4 ± 43.3 nm, respectively. Once nanodroplet echogenicity was induced via the thermal cycle, particles retained enhanced signal for 13 hours. When nanodroplets were subjected to a heating and cooling ramp, the particle signal intensity generally increased with rising temperature and declined with decreasing temperature. However, nanodroplets had higher signal intensity at 37 °C during the cooling portion of the thermal cycle. Therefore, higher echogenicity of BSA-TDFH nanodroplets was induced with thermal modulation compared to direct heating to 37 °C. Since the temperature range selected for thermal modulation experiments lies far enough from the vaporization threshold of pure liquid TDFH (56 °C) and no increase in the particle size was observed, it is unlikely that the increase in nanodroplet signal intensity is due to the transient phase shift of the liquid perfluorocarbon core. Further investigations are required to elucidate the mechanism behind thermally induced echogenicity of stable BSA-TSDH nanodroplets.

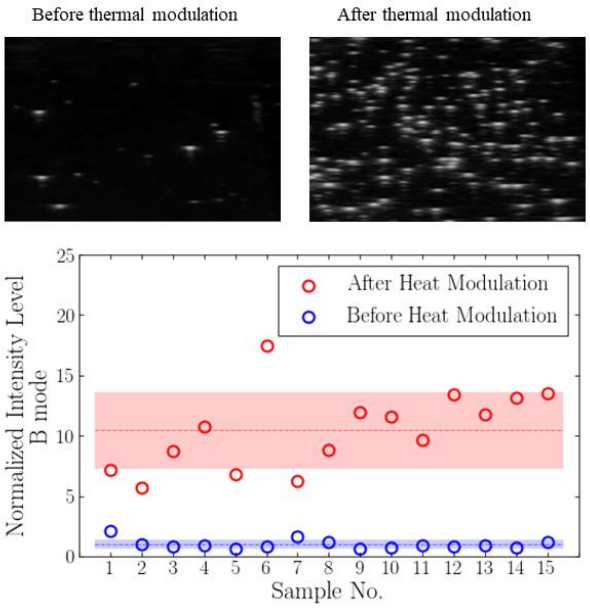

Figure 1. Analysis of nanodroplet echogenicity before thermal modulation at room temperature and after thermal modulation at 37 °C on the B-mode (n=15). The data was normalized by the grand mean of signal intensity before thermal cycle.

Conclusions: Our study shows that thermal modulation can be used as a simple, easily controlled, and effective method to significantly increase the echogenicity of high boiling point perfluorocarbon nanodroplets while preserving their nanoscale size. BSA-TDFH nanodroplets subjected to a heating-cooling thermal cycle averaged more than a tenfold increase in ultrasound signal intensity. Therefore, they can potentially serve as theranostic agents for various applications, such as extravascular tumor imaging.