Development of Multifunctional Lipid-Pluronic Nanobubble Ultrasound Contrast Agents

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Statement of Purpose: Ultrasound (US) contrast agents have shown promise in drug and gene delivery for cancer treatment, but their applicability is limited due to their 2-10 µm diameters which confine them to the vasculature[1]. Reducing the size of bubbles to below 500 nm in diameter could improve their performance in both tumor detection and drug delivery by taking advantage of the enhanced permeability and retention effect of leaky tumor vasculature. In this work we present a novel method using Pluronic, a tri-block copolymer surfactant, as the size control excipient for formulating lipid-shelled echogenic gas nanobubbles without post formulation processing.

Methods: Control microbubbles were prepared using a cocktail of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (Mw: 734.05), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (Mw: 691.97) and 1,2-dipalmitoyl-sn-glycero-3-phosphate (Mw: 670.88) in chloroform, followed by evaporation of solvent and hydration with PBS in the presence of glycerol[2]. After replacement of air with octafluoropropane (C_3F_8) gas, the shaking method was used to constitute bubbles. To make nanobubbles, five types of Pluronic (L31, L61, L81, L64 and P85; Mw 1100-4600 Da) at varying concentrations were added to the lipid mixture during hydration. Bubble size, stability and echogenicity were characterized *in vitro* using dynamic light scattering and *in vivo* with US. *In vivo* studies were carried out in a subcutaneous colorectal carcinoma tumor model in 6-8 week old BDIX rats[3]. In all studies, a bolus 50µL of bubble solution was administered into the tail vein. Tumor contrast change with time was imaged with microflow imaging (MFI) using a 6 MHz linear transducer (Toshiba). Data analysis was carried out with ImageJ.

Results: Pluronic was effective in reducing bubble size without compromising bubble echogenicity. The process was dependent on Pluronic concentration as well as Pluronic structure (Mw, hydrophilic-lipophilic balance or HLB). Among tested Pluronics, L61 and L81 showed the most favorable size modulating effect. At 0.6 mg/mL, L61 was able to reduce the bubbles size to 207.9 ± 74.7 nm compared to the 880.9 ± 127.6 nm of control bubbles; while L81 reduced bubble size to 406.8 ± 21.0 nm (**Fig 1**). *In vitro*, all Pluronic bubbles were echogenic and showed stability comparable to control microbubbles. L61 nanobubbles appeared more stable than the control bubbles (contrast decrease to 70.3 ± 9.4% of t = 0 over 30 min compared to 53.3 ± 4.7% for control). *In vivo*, tumors with nanobubble enhancement showed increased grayscale intensity compared to control. Results from contrast enhanced MFI (**Fig 2**), which combines a flash replenishment sequence and max-hold processing[4], showed that L61 bubbles lead to higher grayscale signal intensity than control bubbles in 6 out 7 tumors as measured by area under the TI curves following bubble injection, suggesting greater nanobubble accumulation in tumors.

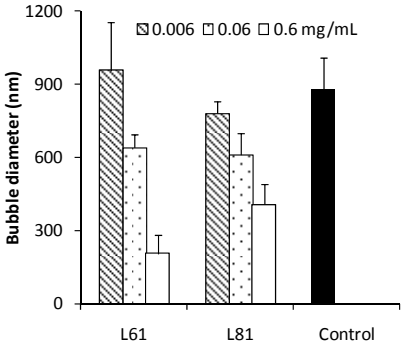

Fig 1. Bubble sizes in the presence of 0, 0.006, 0.06 and 0.6 mg/mL of Pluronic L61 or L81 (mean ± SEM; n = 3). ***** Statistically significant smaller compared to control (*P: 0.001-0.01*).

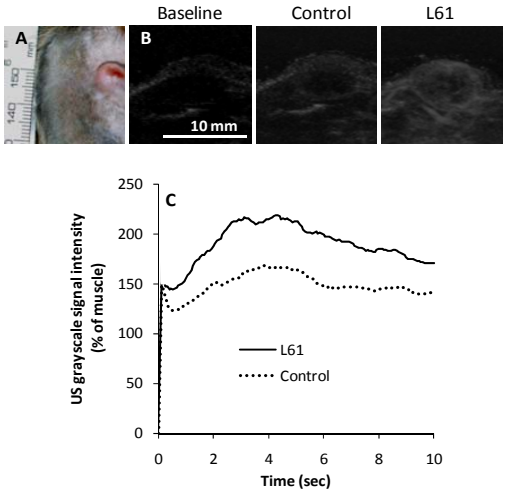

Fig 2. Tumor MFI. **A:** a tumor; **B:** representative US tumor images with contrast enhancement; **C:** quantitative analysis of the images (n = 7).

Conclusions: Pluronic is effective in reducing the size of lipid, gas-filled ultrasound contrast agents, and Pluronic Mw, HLB, and Pluronic / lipid ratio are critical factors for bubble size control. Most importantly, while the bubbles are nano-sized, their stability and echogenicity *in vitro* and *in vivo* are not compromised. Bubble pharmacokinetic studies are currently ongoing.

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References:

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