Development and Characterization of Novel Targeted Lipid-Polymer Microcapsules

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Statement of Purpose: Molecular imaging requires an imaging modality compounded with a contrast agent to aid in the detection of, and ultimately, the treatment of disease. Of the imaging modalities currently available, ultrasound provides the advantages of real-time imaging, low cost, portability and accessibility. A site-directed ultrasound contrast agent would provide insight into molecular events at specific pathologic tissues, thus providing enhancement to distinguish normal tissue from unhealthy tissue. The primary strategy of targeting contrast agents to specific sites involves modifying the surface of the contrast agent with ligands. Ligands can either be covalently attached to or physically incorporated into the shell of the contrast agent. Previously, the physical incorporation of lipid-ligand conjugates has been primarily explored in the formation of pure lipid-based contrast agents. The present study explores the feasibility of combining the physical incorporation of lipid-ligand conjugates into polymer ultrasound contrast agents and investigates its effect on echogenicity.

Methods: *Fabrication:* Microcapsules were formed with the common double-emulsion method using either ammonium carbonate or ammonium carbamate as a porogen. In order to fabricate biotinylated microcapsules, 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Biotinyl(Polyethylene Glycol)2000] (DSPE-PEG(2000)Biotin) was added to a mixture of polylactic acid (PLA) and organic solvent in a 1:100 ratio of lipid:polymer. After preparation of particles, the capsules were washed with hexane to extract the organic solvent, and lyophilized. The carbamate and carbonate sublime after lyophilization, leaving a hollow core therefore creating air-filled microcapsules.
Characterization: Biotin incorporation was confirmed by first incubating the capsules with fluorescently-labeled streptavidin, followed by visualization of fluorescence with an epifluorescent microscope and confocal microscope. The microcapsules were mounted to metal stubs with double-sided tape and coated with platinum for imaging with an Environmental Scanning Electron Microscope (ESEM) at magnifications of 3000X, 6000X, and 9000X to examine capsule morphology. *In vitro* acoustic dose and time response curves were collected using a custom-built pulse-echo set-up employing a single element broadband, 5MHz center frequency transducer.

Results / Discussion: The four different types of polymer microcapsules fabricated were PLA formed with ammonium carbonate (PLA-BON), PLA formed with ammonium carbamate (PLA-BAM), and each of those two formed with the addition of the lipid-ligand conjugate (PLA-BON-PEG and PLA-BAM-PEG). Confocal and epifluorescent microscopy confirmed the presence of fluorescently-labeled streptavidin bound to the biotinylated surfaces of PLA-BON-PEG and PLA-BAM-PEG, but streptavidin did not significantly bind to the control microcapsules. The PLA-BAM and PLA-BAM-PEG microcapsules were relatively monodisperse, hollow spheres, with smooth surfaces. The PLA-BON and PLA-BON-PEG were similarly monodisperse and hollow spheres but with more of 'wiffle-ball-like' surface morphology. The acoustic signal enhancement for the spheres after maximal dose was 18dB, 22dB, 19dB, and 24dB for PLA-BON, PLA-BON-PEG, PLA-BAM and PLA-BAM-PEG respectively. Correspondingly, the loss of signal for the respective particles over 15 min was 18, 20, 60 and 50 percent of the original signal.

Conclusions: We have demonstrated in proof-of-concept studies that lipid-ligand conjugates can be successfully incorporated into PLA microbubbles and provide enhanced echogenicity. However, the increased echogenicity leads to faster decay of the microcapsules acoustic signal. The acoustic response of these microcapsules can possibly be tuned by changing amounts of incorporated lipid. The model system used in this study can be extended to physiologically-relevant ligands by either using lipids conjugated to the physiologic ligand or linking biotinylated ligands to the current system.