Release of Calcein from eLiposomes via Acoustic Stimulation: A Study for Enhancing Sonosensitivity of Liposomes

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With cancer incidence rates on the rise, treating patients effectively with currently available treatment methods is the biggest challenge. Currently, available cancer treatments lead to toxic side effects, and multidrug resistance, among others[1]-[3]. These limitations made biomaterials as drug delivery systems so valuable, and biomaterials composed of lipid vesicles, i.e., liposomes, are extensively being researched for the treatment of cancer effectively. Liposomes are employed as smart drug delivery systems (SDDS) for encapsulation, tumor targeting, and site-specific drug delivery while reducing systemic toxicity[4]. Liposomes have excellent biocompatibility, biodegradability, passive targeting capability, allowing their surfaces to be engineered with specific targeting ligands and control drug release. In addition, their unique structure enables them to encapsulate and confine both hydrophilic agents within their cores and hydrophobic agents within the bilayer, rendering them universal drug carriers[5]-[7]. The features above make them an attractive choice as drug delivery systems. Further manipulating the composition of liposomes can render them responsive to specific stimuli, which provides complete control over the spatial and temporal release of the drug[8], [9].

THIS study focuses on rendering transferrin-conjugated nano-liposomes sonosensitive by adding nano-emulsion droplets and studying the release of calcein upon exposure to the US. Emulsions such as perfluorocarbons have the phase-transitioning ability to change from liquid droplets to gas upon ultrasound irradiation, which would sensitize liposomes to low-intensity focused ultrasound and trigger drug release within short exposure times. This phenomenon is called acoustic droplet vaporization [10]-[16]. Liposomes are synthesized by the thin-film hydration technique and are composed of the main phospholipid dipalmitoylphosphatidylcholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- (amino (polyethylene glycol)-2000] (DSPE-PEG(2000) amine, and cholesterol. DPPC was used to construct the backbone of liposomes, whereas, the presence of DSPE-PEG(2000)amine on the surface of the vesicle, helps liposomes escape the opsonization process and prolongs the liposomal blood circulation time[7],[17]. Cholesterol, on the other hand, increases the stability of liposomes and provides fluidity to liposome membranes. Calcein is encapsulated in the liposomes during the rehydration phase. Synthesized liposomes are sonicated to achieve unilamellar configuration and extruded through a 200nm polycarbonate membrane filter. We target synthesizing liposomes in the size range of 100-200 nm, to allow extravasation into solid tumors via the enhanced permeability and retention effect (EPR)[5],[18],[19]. This enables liposomes to escape filtration by the liver and spleen.
Emulsions are prepared by mixing hydrophilic phosphate-buffered saline solution, and hydrophobic perfluoropentane (PFC5) stabilized by a DPPC surfactant film. Nanoemulsions are obtained by extruding through a 50nm filter to enable their encapsulation into the liposomes. Dynamic light scattering (DLS) confirms the size distribution of both liposomes and emulsions pre-incubation. Incubation allows the aggregation and encapsulation of nanoemulsion assembly into the liposomes. Other liposomal characterization techniques, including the Stewart assay and bicinchoninic acid (BCA) assay, are used to estimate lipid content and confirmation of

transferrin conjugation to the liposomal surface, respectively[17], [20].

Pulsed low-frequency ultrasound (LFUS) at 20 kHz was used to trigger calcein release from liposomes and eLiposomes (control and transferrin-conjugated). US power densities at will be used, namely 7.46, 9.85, and 17.31 mW/cm^2 and are pulsed for 20 seconds "ON" and 20 seconds "OFF" cycles. The release of calcein is monitored by the changes in fluorescence using a fluorescence spectrofluorometer. An example of the cumulative fractional release from liposomes and their respective eLiposomes is provided in the preliminary results shown below;

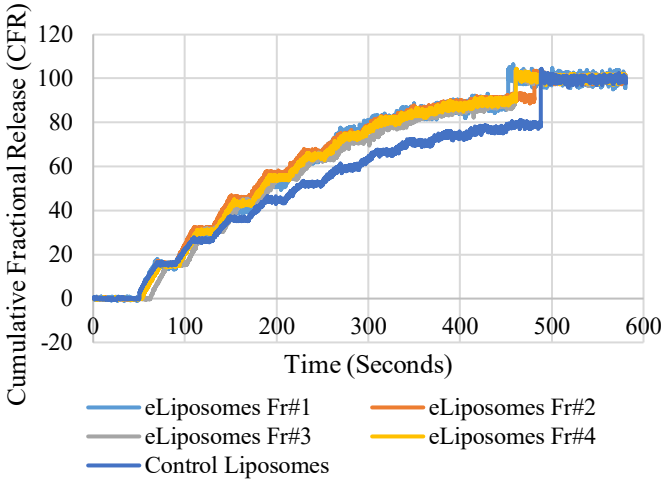

Fig. 1. Comparison between low-frequency 20kHz release behavior of Liposomes and eLiposomes using 20% power density

The figure above shows the release of calcein upon acoustic stimulation. The figure shows that eLiposomes exhibit higher release compared to liposomes. This is attributed to the presence of nanoemulsions that render liposomes more sonosensitive compared to regular liposomes. Investigation of transferrin (Tf)-conjugated eLiposomes will improve drug delivery methods. Furthermore, investigation of *in vivo* release experiments will provide a better insight into the bioeffects of acoustically stimulated Tf-eLiposomes on cells.

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