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Stability of e-Liposomes at Higher Temperatures

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Extended Abstract

The newest generation of drug delivery vehicles in cancer treatment are e-Liposomes (which are ultrasonically sensitive liposomes). These carriers encapsulate a small perfluorocarbon nanoemulsion inside a liposome. Ultrasound is then used to produce pressure fluctuations inside the nanoemulsion, causing it to vaporize, burse the liposomes open and release the antineoplastic agent only at the site where the ultrasound is focused (i.e. the tumor tissue).

Nanoemlusions of PFCs are made by placing 0.1 mL of ice cold 1,1,1,2,2,3,3,4,4,5,5,5-perfluoropentane (PFC_5) (with a boiling point of 28 °C) in a cold flask previously coated with a layer of phospholipids and sonicating on ice with a 20-kHz ultrasound probe for 1 min. The resulting droplets have a size range between 100 and 200 nm. They are extruded through a 100-nm filter to decrease their size distribution.

The liposomes were made as follows. Phospholipids were dried onto a glass flask and then hydrated with buffer, forming liposomes of various sizes. The mixture was extruded through a 200-nm filter to produce a uniform size distribution of unilamellar liposomes.

The e-liposomes were made by mixing 100-nm PFC_5 emulsions with 200-nm liposomes and sonicating the mixture on ice for 10 minutes. The sonication transiently breaks open a liposome and the emulsion droplet enters inside the drug delivery vehicle before the liposome membrane re-seals. External emulsions (not captured inside the eLiposome) are removed by centrifugation on a sucrose/glucose/NaCl density column with a resultant eLiposome having an average diameter of 200-nm (The size distribution of the liposomes is measured by dynamic light scattering (DLS) on a *Brookhaven 90Plus* Particle Sizer (Brookhaven Instruments, NY)). Inside the resultant eLiposomes a 100-nm PFC_5 droplet was also imaged using cryoTEM. To load calcein, the model drug was added before making the transient pores in the liposome using ultrasound. The calcein concentration used was 20 mM which is in the self-quenched region and thus has minimal fluorescence inside the carrier. The fluorescence then increases upon the rupture of this drug delivery vehicle during the application of ultrasound when the emulsion vaporizes causing the liposome bilayer to break open and the drug concentration to decrease thus increasing its emitted fluorescence.

The purpose of this abstract is to show that PFC_5 is stable above its boiling point (including at 37 °C) and thus will not rupture when introduced in a patient. Thus, we performed fluorescence experiments on our newly synthesized e-Liposomes to measure any premature release of calcein at various temperatures.

To measure the amount of calcein released from these e-liposomes, 30 µL of the calcein loaded nanocarriers were mixed in 2 mL of PBS (pH = 7.4). Excitation and emission wavelengths were set at 488 and 520 nm, respectively and the fluorescence level continuously recorded. Figure 1 shows the release of calcein over a period of one hour. Rapid release was observed at 59°C and 49°C. At 37° and 25° there was very little release and release was not statistically different above and below 28°C (PFC_5 boiling point) suggesting Laplace pressure prevented gas formation. This proves these carriers to be stable upon

injection into the patient and then released once the local temperature of the tissue is increase above 45-50 °C via acoustic power.

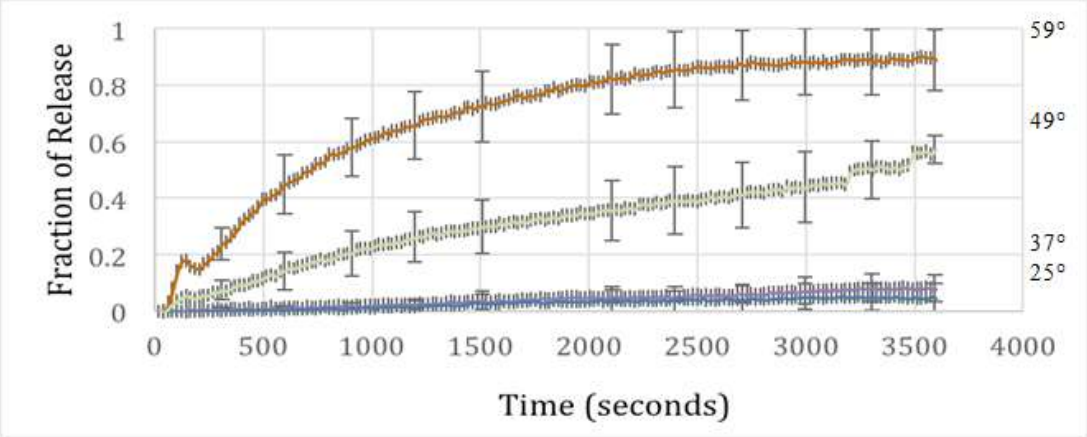

Fig. 1. Fraction of calcein release from eLiposomes incubated, NOT insonated, at various temperatures.