Effect of Temperature and Size on Release of Calcein from eLiposomes

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Statement of Purpose: A nano-sized drug carrier was made that can be burst by applying ultrasound (US). Whereas most ultrasonic drug delivery requires gas bubbles, this technique does not. The carrier, called an eLiposome, is a liposome containing therapeutics and a liquid nanodroplet of perfluorocarbon (PFC) that can vaporize to gas upon application of US.[1, 2] Previous studies suggests that during insonation, the local pressure drops below the PFC vapor pressure which induces the formation and expansion of a vapor phase, which expansion ruptures the liposome and releases the drug or genes locally. However no studies have examined the effect of temperature on release. In this study eLiposome release at 24° and 37°C was measured. Fluorometery was used to measure the stability of eLiposomes and their ability to sequester and release calcein (as a model drug).

Methods: Small liposomes were made by the hydration method; then small emulsion droplets were added to the liposome suspension, and the mixture was sonicated at 0°C. The resulting eLiposomes were extruded through a 200-nm or 800-nm filter to create small and large sizes. Non-encapsulated emulsions were separated from eLiposomes using a pillow density method.[1] The eLiposome suspension was diluted in 2 mL of buffer solution. The fluorescence intensity was measured before and after insonation for 0.1 s at 24° or 37°C, and again after the sample was lysed with Triton X-100.

Results: Fig. 1 shows release from 200-nm eLiposomes with small perfluorohexane (PFC6) (blue triangles) or perfluoropentane (PFC5) (red squares) emulsion droplets inside, after 20-kHz insonation at 37°C. Green circles are conventional liposomes without any emulsion droplets inside. PFC5 eLiposomes produced more release than PFC6 eLiposomes and control liposomes.

Fig. 2 shows the results of several experiments comparing large and small eLiposomes at 24° and 37°C. Release from large vesicles is greater than smaller ones, and release with PFC5 is greatest. However, there was no increase in release at 37°C except with large PFC5 eLiposomes.

As temperature increases from 24° to 37°C, the vapor pressure increases about 60% for PFC5 and about 79% for PFC6. Theoretically at the 37°C, less US intensity should be required to overcome the Laplace pressure and induce vaporization. It is possible that the lack of increase in release is due to the size distribution of droplets. Threshold calculations assume a homogeneous size, but there are larger and smaller emulsion droplets. These droplets would therefore have different thresholds for vaporization and could result in the observed lack of expected thresholds in the data. Gas nucleation is also a potential limiting factor.

Conclusions: It is interesting that calcein release did not increase at the higher temperature, because increasing temperature increases the vapor pressure of PFCs, making these vesicles more susceptible to rupture by ultrasound. However, it is very good that the eLiposomes containing PFC5 do not rupture prematurely at 37°, which is above the normal boiling point of PFC5.

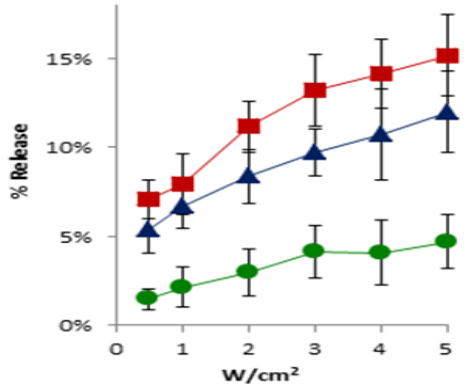

Figure 1. Calcein release from 200-nm vesicles exposed to 1 W/cm^2 ultrasound for 0.1 seconds at 37°C. Exposure intensity was varied from 0.5 to 5 W/cm^2 Experiments were performed on PFC5 eLiposomes (■), PFC6 eLiposomes (▲) and conventional liposomes (●). Error bars represent ± 1 standard deviation (n=3).

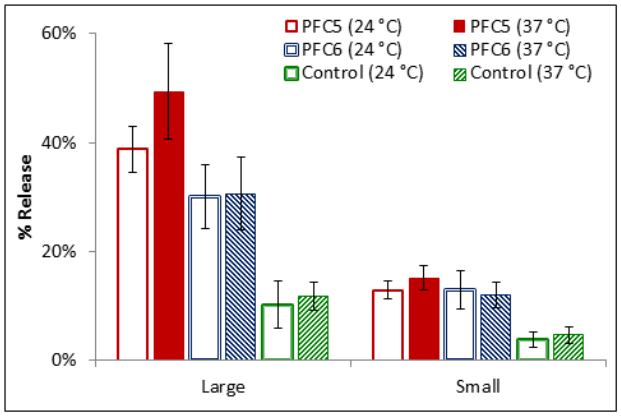

Figure 2. Calcein release from eLiposomes and control liposomes at 24°C (empty bars) and at 37°C (filled bars). Large (800 nm) vesicles with 450-nm droplets and small (200 nm) vesicles with 100-nm droplets were exposed to 5 W/cm^2 ultrasound for 0.1 s. Red, blue and green represent PFC5 eLiposomes, PFC6 eLiposomes, and conventional liposomes, respectively. Error bars represent ± 1 standard deviation.

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References:

[1] M. Javadi, W.G. Pitt, D.M. Belnap, N.H. Tsosie, J.M. Hartley, Encapsulating Nanoemulsions Inside eLiposomes for Ultrasonic Drug Delivery, Langmuir, 28 (2012) 14720-14729.

[2] J.R. Lattin, D.M. Belnap, W.G. Pitt, Formation of eLiposomes as a drug delivery vehicle, Colloid Surface B, 89 (2012) 93-100.