Ultrasonic Delivery of Doxorubicin to the Cell Cytosol

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Statement of Purpose: We have developed a potential drug-carrying vesicle called an eLiposome that consists of a liposome containing drug and perfluorocarbon (PFC) nanodroplets in its aqueous interior.(*1*) Application of ultrasound to this construct causes the PFC to expand from liquid to gas, which expansion ruptures the liposome and releases the drug.(*2*) Furthermore, folate molecules attached to the surface of the eLiposome causes binding to folate receptors (that are overexpressed on some cancer cells) and induces endocytosis. When ultrasound is applied after endocytosis, the expansion of PFC that ruptures the eLiposome also ruptures the endosome and releases drug directly to the cell cytosol.
Although we have shown delivery of calcein and plasmids to cancer cells in vitro, we have not yet described loading of doxorubicin (Dox) into the eLiposome, nor delivery to cells in a cytotoxic manner. This presentation discusses loading Dox into eLiposomes (called eLipoDox), the efficiency of loading, ultrasonic release to cells from folated eLipoDox, and cell death following insonation.

Methods: Liposomes of dipalmitoylphosphatidylcholine (DPPC), cholesterol, DSPE-PEG(2000)-amine were formed by film hydration in a 110 mM ammonium sulfate buffer, pH 4.0, and extruded through a 200-nm membrane filter. A 40 vol% emulsion of PFC5 stabilized with DPPC was formed by sonication on ice to preclude evaporation. The emulsion droplets were extruded through a 100-nm membrane filter. One mL each of emulsion and liposomes were mixed and sonicated gently at 1.25 W/cm^2 on ice for 90 seconds and extruded through a 200-nm filter. The resulting eLiposomes were separated using a pillow density technique(*3*) and resuspended in a 20 mg/mL Dox solution in a sucrose/HEPES buffer at pH 7.4 and 300 mOs. Various incubation times and temperature were used to study Dox loading efficiency. After the loading period, the Dox-loaded eLiposomes (eLipoDox) was separated from free Dox on a Dowex ion exchange column. Dox concentration was measured by absorption at 488 nm.
The release of Dox from eLipoDox and conventional liposomes was measured by fluorescence spectrometry by measuring the fluorescence of quenched Dox in eLipo-Dox, exposing to various insonations, and measuring the increase in fluorescence due to Dox release.
HeLa cells were grown in 12-well plates by standard techniques. eLipoDox and other formulations (controls) were added to the wells prior to insonation. At 6, 24 and 48 hrs post-insonation, cell viability was measured. Controls included no insonation and exposure to free Doxorubicin.

Results: Doxorubicin was successfully loaded into eLiposomes. CryoTEM images show linear crystals inside the liposome membrane, along with PFC emulsions.

The efficiency of Dox loading ranged from 35% to 95%, depending on the conditions of loading. Increased loading efficiency correlated with higher loading temperatures and longer times. However increasing these parameters is not ideal for loading in the presence of perfluorocarbons with high vapor pressure, and the optimal loading parameters may not necessarily correspond to optimal delivery by ultrasonication if PFCs evaporate during the loading process.

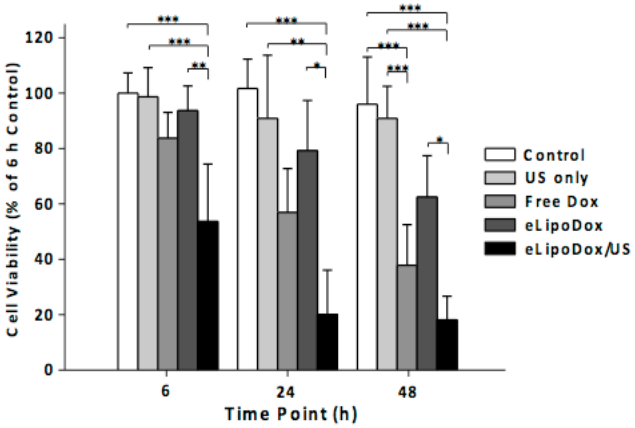

Fig. 1. HeLa cell viability after expose to eLipoDox and ultrasound (US) at 1 W/cm^2, 2 sec; and various controls. *, p<0.05; **, p<0.01; ***, p<0.001. Error bars represent standard deviations.

Cell viability is shown in Figure 1 as a function of time post insonation. Control cells and those exposed only to ultrasound maintain viability for 48 hrs. Cells exposed to free Dox decrease to 58% viability in 24 hrs (no insonation). However, cells exposed to eLipoDox without insonation only decreased to 80% viability in the same time, showing that encapsulating the Dox increased cell viability. The lowest viability was seen in cells exposed to both eLipoDox and ultrasound, with 20% viability at 24 hrs. At 48 hrs, there was no further change in viability for cells exposed to eLipoDox and ultrasound, whereas cells exposed to free Dox or eLipoDox (without ultrasound) continued to die. This suggests that exposure to eLipoDox and insonation has a much faster impact on cells that exposure without insonation.

Conclusions: We have successfully loaded Dox into eLiposomes and quantified the *in vitro* release as a function of ultrasonic parameters. High Dox loading can be achieved, but careful selection of loading parameters is required to preserve ultrasonic sensitivity. Cells exposed to eLiposomes and ultrasound are killed more rapidly than when exposed to free Dox or eLipoDox w/o insonation.

References:
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