Ultrasonically Activated Delivery to the Cytosol using Acoustic Droplet Vaporization

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Statement of Purpose: Our main objective in developing biomaterials for controlled drug delivery is to produce a secure container that does not release its drug payload outside of cells. We have placed emulsion droplets that change phase when insonated inside a phospholipid vehicle. The vehicle is labeled with folate for active targeting and endosome uptake. Triggered targeting by ultrasound causes acoustic droplet vaporization which lyses the phospholipid vehicle and the endosome, thus releasing drug to the cytosol. The purpose of this research is to show that emulsion vaporization is responsible for ultrasonically triggered delivery to the cytosol.

Methods: Folate was attached to DSPE-PEG2000-amine using a technique that has previously been reported.[1] perfluoropentane (PFC5) emulsion droplets were prepared by adding 0.2 g of PFC to 1.5 mL of water and 167 µL of hydrated DPPC suspension (30 mg/mL).[1] The sample was chilled on ice sonicated at 20-kHz at 1 W/cm^2 on ice for 5 min. In some samples folate was added to the surface of emulsion droplets by mixing DSPE-PEG2000-folate with the DPPC at 1.2 mol% prior to emulsion formation by sonication. Samples were prepared in concentrated (30 mM) calcein. The 200-nm eLiposomes used in this study were formed using a "sheet-refolding" method.[1] Briefly, DPPC liposomes were formed into sheets with 3M EtOH. After the EtOH was removed, calcein and emulsions were added and the sheets reformed into liposomes (with calcein and emulsions inside) by heating to 50°C. The eLiposome surface was folated by incubating in a 1.2mol% micellar suspension of DSPE-PEG2000-folate. eLiposomes were purified on a density cushion.[2]

HeLa cells were maintained in DMEM with 10% fetal bovine serum. 48 h prior to experiments they were grown in12-well plates in folate-free media (Sigma). 200 µL of the emulsion or eLiposome sample was added to each well and allowed to incubate for 2 h before application of ultrasound. Cells were washed thrice with PBS to remove non-endocytosed eLiposomes or droplets. 3 mL of media was added and the cells insonated with a 20-kHz ultrasonic probe tip 2 cm above the cell layer, 1 W/cm^2 for 2 sec.

The calcein used as a model drug self-quenches above 0.5 mM. Upon dilution it acquires fluorescence, and has linear fluorescence below 10 µM. Thus eLiposomes and endosomes filled with concentrated calcein show no fluorescence; but as the calcein escapes to the cytosol, the fluorescence is observed with confocal microscopy.

Results: For emulsion-only samples, the emulsion was mixed 1:1 with a 30 mM calcein solution. 200 µL of the resulting solution was added to HeLa cells that had been grown in 1.3 mL of folate free media, resulting in an approximate calcein concentration of 4 mM around the cells. This concentration of calcein is sufficiently high to be in the self-quenching range. Fig 1A and 1B show cells never insonated. No calcein was released to the cytosol and "unquenched" to fluoresce green. However, upon insonation, the green fluorescence appears in the cytosol (Fig 1C), indicating that the endosome was ruptured. The same level of insonation in the absence of emulsion produced no green in the cytosol (data not shown).

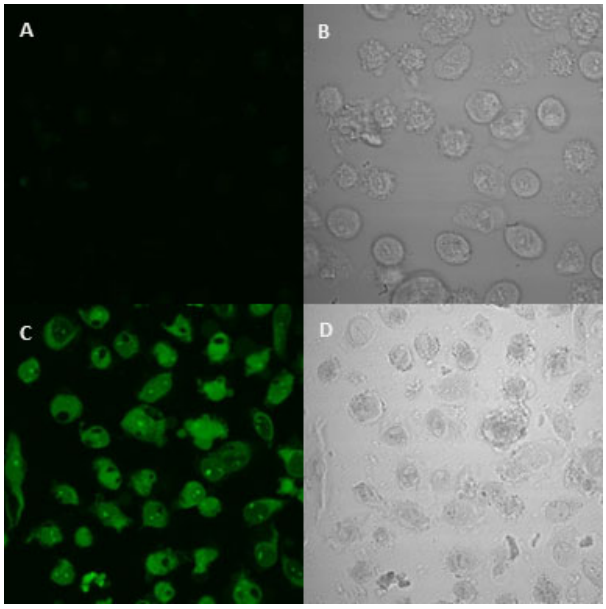

Fig1. Folated PFC5 emulsions and concentrated calcein were endocytosed into HeLa cells. When cells were not insonated (A and B) the quenched calcein was not released into the cytosol. Calcein could be observed throughout the cells that were insonated at 20 kHz for 2 seconds at 1 W/cm^2 (C and D).

When eLiposomes were incubated with cells for 2 hrs, there was no green color in the cytosol unless insonation was applied (2 s, 1 W/cm^2), which produced green color of similar intensity to Fig 1C (data not shown). We believe that insonation caused vaporization of the PFC5 droplets, which broke open the eLiposome, releasing calcein to the endosome; this expansion also broke open the endosome, releasing the calcein to the cytosol.

Conclusions: Calcein was delivered to the cytosol by ultrasonic vaporization of folated emulsion droplets by themselves or inside folated eLiposomes. The droplet expansion can rupture eLiposomes and also endosomes to deliver the released drug to the cytosol.

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References:

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