Laser Induced Sonoporation using Optically Triggered Perfluorohexane Droplets

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Background, Motivation and Objective

Microbubbles in tandem with focused ultrasound have been shown to create pores in cellular membranes allowing delivery of drugs and other therapeutics in a process called sonoporation. However, this approach is limited to vascular targets due to the size of the microbubble, and the use of focused ultrasound prevents real-time ultrasound monitoring due to interference from the focused ultrasound. In this study, we examine the use of optically vaporizable perfluorohexane nanodroplets (PFHnDs) as a drug delivery agent. PFHnDs are nanometer sized emulsions that can be vaporized upon laser irradiation and become transient microbubbles that provide ultrasound/photoacoustic (US/PA) signal and contrast. This additional contrast can allow for real-time monitoring of the delivery process and an estimation of delivery dosage. We hypothesize that the vaporization of PFHnDs can mimic the focused ultrasound induced microbubble oscillations, allowing for extravascular delivery and real-time US/PA monitoring for spatial and dosage information.

Statement of Contribution/Methods

PFHnDs were synthesized with a lipid shell (DSPE-PEG2k, DSPC), a perfluorohexane core, and a near infrared absorbing dye (IR 1048). PFHnDs were incubated with Hek 293t cells in a glass bottom dish and cultured in complete DMEM with 100 μM of calcein green. The PFHnDs were then lased (1064 nm, 10 Hz, 300 pulses, varying fluences up to 66 mJ/cm^2; Vibrant, Opotek). The cells were then washed three times with PBS, the nuclei were stained using Hoechst 33342, and imaged using a laser scanning confocal microscope (Zeiss LSM 700B). No signs of cell death were observed.

Results/Discussion

We showed that optical vaporization of PFHnDs is capable of delivering calcein green into cells (Fig. 1A). Since the dye is only fluorescent when cleaved by intracellular esteraces, fluorescence indicates successful intracellular transport. Negative controls without lasing (Fig. 1B) or without PFHnDs (Fig. 1C) show no fluorescence. Our results suggest that the optical triggered vaporization of the PFHnDs interact with cells similarly to that of focused ultrasound and microbubbles. Current and future work will focus on translating laser induced sonoporation using optically triggered PFHnDs to *in vivo* US/PA image-guided therapeutic delivery.

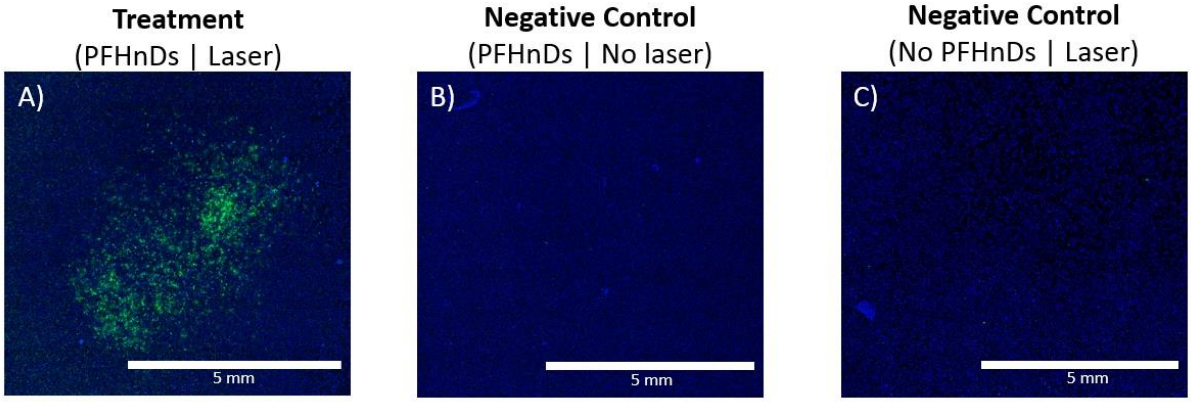

Figure 1. Confocal images of cells stained with Hoechst that were incubated with **A)** PFHnDs/ calcein and were exposed to pulsed laser irradiation, **B)** PFHnDs/calcein but no laser irradiation, and **C)** only calcein (i.e. no PFHnDs) and were exposed to pulse laser irradiation. No evidence of cell death was observed in any of the conditions.