^{19}F Magnetic Resonance Quantification of siRNA Delivery via Perfluorocarbon Nanoparticle Emulsions

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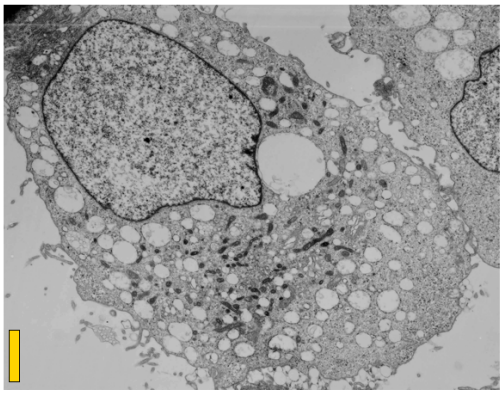
RNA interference mediated by the introduction of exogenous siRNAs has been a powerful tool for the modulation of gene expression. The ability to track siRNA delivery would be advantageous for future *in vivo* studies.

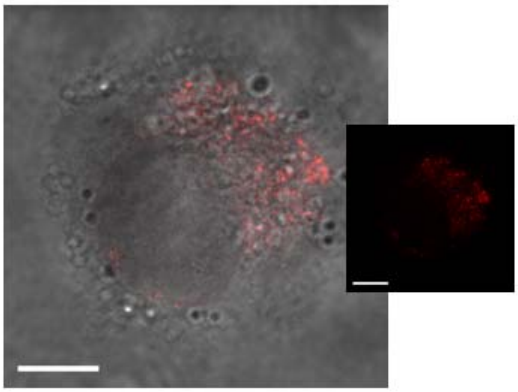
Methods: To design a synthetic vehicle to serve as both an siRNA delivery agent and an imaging agent, perfluorocarbon nanoparticles (PFC NP) were loaded with the cationic lipid 1,2-Dioleoyl-3-Trimethylammonium-Propane DOTAP in the lipid monolayer to form transfection complexes with siRNA for the house keeping gene LaminAC. siRNA loading onto nanoparticles was measured via PAGE gel. Human C32 melanoma cells were incubated with transfection complexes (PFC-NP/siRNA) for 4h. Cell viability was assessed via flow cytometry using a nuclear stain. Transmission electron microscopy (TEM) verified the uptake of PFC-NPs in cells after incubation with PFC-NP/siRNA. Confocal imaging was used to determine localization of fluorescently labeled siRNA. ^{19}F MR spectroscopy (MRS) was performed at 11.7 T to determine the number of NP bound to each cell. mRNA levels were measured via reverse transcription quantitative PCR 48h after transfection to assess siRNA-mediated knockdown.

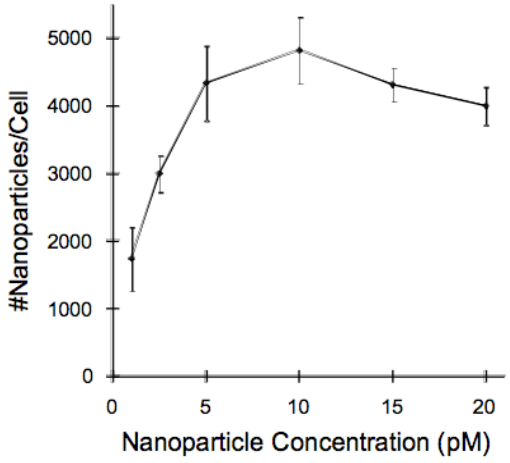
Results: The selected loading conditions resulted in 645 siRNA molecules per nanoparticle. TEM showed surface binding and vesicular uptake of PFC-NPs in C32 cells (Fig 1). Confocal imaging showed internalization of siRNA in C32 cells (Fig 2). As determined by ^{19}F MRS, incubation with C32 cells led to a roughly sigmoidal increase in bound nanoparticles over the range of concentrations tested (Fig 3). Leveling of transfection correlated with cell viability (Fig 4). For one condition that resulted in a 53% reduction in LaminAC mRNA levels, ^{19}F spectroscopy data reported 1,837 particles per cell.

Conclusion: For future *in vivo* studies, a trackable delivery agent would aid in the determination of localization of siRNA delivery to specific tissues. To this end, we have developed a nanoparticle which is able to deliver siRNA to human melanoma cells and whose signal can be detected by ^{19}F MR spectroscopy and imaging.

Summary: Perfluorocarbon nanoparticles were functionalized to deliver VCAM-1 siRNA to human melanoma cells. Delivery was quantified via ^{19}F magnetic resonance spectroscopy.







Nanoparticle Concentration (pM)	Viability (%)
control	91.89 ± 1.51
1	93.86 ± 0.80
2.5	90.02 ± 1.25
5	82.81 ± 5.26*
10	68.04 ± 3.58*
15	57.57 ± 3.12*
20	36.27 ± 3.09*

* denotes $p < 0.05$ vs control