An Investigation of a Simple, Inexpensive Apparatus for Lipid Nanoparticle Fabrication with Controllable Size and Dispersity with in vitro Applications

Wu, Susan (School: Manhasset High School)

Lipid nanoparticles are gaining increasing attention due to their intracellular drug delivering capabilities, especially with their ability to deliver gene-editing materials such as components of CRISPR/Cas9. Currently, there lacks an efficient, inexpensive method to mass- produce lipid nanoparticles that are controllable in size and dispersity. In this study, the purpose was to design and build inexpensive devices of various sizes and configurations (including a copper wire mixer) that can be coupled to inexpensive laboratory syringe pumps that can mass produce lipid nanoparticles of predictable size. Synthetic cationic lipids were used in this study to form the lipid nanoparticle vesicles, which were then loaded with GFP mRNA and delivered into HT-29 Human Colon Cancer cells to measure transfection efficiency. TEM was used to characterize lipid nanoparticle morphologies after DLS analysis of size and dispersity. Additionally, toxicity was measured via MTT Cell Proliferation Assay following mRNA delivery. The results of this study were that the different sized T-Junction devices were capable of consistently producing nanoparticles between 100-350 nm in diameter due to variations in the inner diameter of the device. Larger inner-diameter produced larger nanoparticles, and faster speeds of the syringe pumps produced smaller lipid nanoparticles. Each device produced relatively monodispersed nanoparticle solutions, with PD consistently measured under .3, with copper-wire mixer T-Junction producing the most monodisperse solutions, reaching an average polydispersity of .096. The in vitro delivery showed the smaller sized nanoparticles to have much higher GFP expression as measured by flow cytometry, displaying the size specificity of the lipid bilayer.

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