Introduction

Recent advances in genome engineering, namely Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 gene editing, have revolutionized biomedical research and hold promise for treating genetic disorders [1]. Inefficient targeted delivery and intracellular trafficking of these CRISPR constructs by nonviral nanocarriers however has vastly limited clinical applications of CRISPR therapies [2][3]. I hypothesize that interfacing polymeric nanoparticles with a cell membrane will enhance endosomal escape and that complexation of PEI to the nanoparticles will enhance the conjugation of DNA to the nanoparticles.

The goal of this project is to develop a plasma membrane cloaked nanoparticle (MCNP) to carry CRISPR based plasmid DNA to facilitate gene editing applications. This project will serve as proof of concept and evaluation of nonviral delivery vehicles for CRISPR based therapies.

Materials and Methods

Results

Characterization of Plasma Membrane Cloaked Nanoparticles (MCNP)

Figure 2. Zeta Potential of MCNP

Our zeta potential analysis shows that complexation with PEI changes the negative charge of the PLGA core to a more neutral surface charge and addition of DNA and subsequent membrane coating results in a shift to a more negative charge.

Figure 3. Size of MCNP

Our size analysis indicates that a PLGA core averages around 230nm in size and upon complexation of PEI and DNA conjugation increases to 430nm and 585nm, respectively. The final size of the particle with addition of the membrane is approximately 710nm.

Figure 4. Heterogeneity of Size of MCNP

Our size analysis indicates good particle size uniformity for PLGA and PLGA + PEI groups. Monodispersity of particle size decreases in subsequent stages of synthesis, indicating potential particle aggregation.

Figure 5. Conjugation of DNA with Increasing Amounts of PEI

Increasing amounts of PEI per fixed amount of PLGA and DNA enhances the conjugation efficiency of plasmid DNA onto the nanoparticle. Intensities of the DNA bands were quantified via ImageJ in order to calculate the percentage of DNA conjugated to the nanoparticle. Results of DNA conjugation can be observed in the graph on the right, where 75ug of PEI shows the best efficiency.

Future Work

• Preparation of cell membrane cloaked nanoparticles encapsulating CRISPR plasmids targeting GFP and evaluating gene editing efficiency

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References


Figure 6. Maximum DNA Conjugation with Fixed Amount of PEI

The DNA loading capacity of 75ug of PEI complexed onto a fixed amount of PLGA is demonstrated. Similar to the results in figure 5, 75ug of PEI can successfully conjugate over 94% of DNA to the particle per 2-3ug that are loaded.

Figure 7. 48 Hour Incubation of MCNP carrying mCherry pDNA with HEK293 Cells

MCNPs synthesized with 100ug of PLGA, 75ug of PEI, 2ug of DNA, and 100ug of membrane were incubated with HEK293 cells in completed DMEM for a period of 48 hours. The cells were imaged in bright field (left) and Texas Red (right) channels in order to observe particle uptake of the cell and estimate the transfection efficiency of the MCNP via expressed cell fluorescence.