Peptide-Nanoparticle (PNP) Uptake and Localization Pathways when loaded with mRNA and Co-Assembled with Lipids

TED ROGERS CENTRE FOR HEART RESEARCH
Translational Biology and Engineering Program

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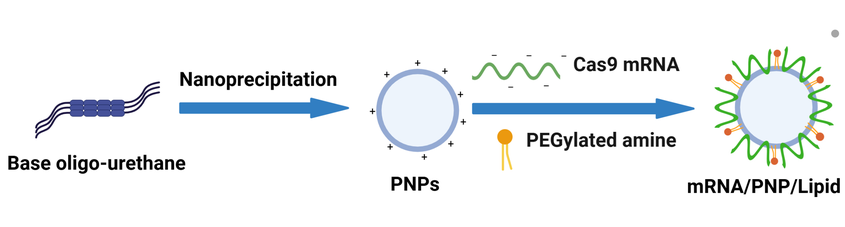
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Background

- Messenger RNA (mRNA) therapies hold great potential for treating diseases such as Duchenne muscular dystrophy and cardiovascular disease[1]
- However, mRNA is large, unstable, and negatively charged, requiring effective delivery systems[1]

Table 1. Delivery systems for gene therapy[2,3]

Delivery System	Attribute
Viral Vector	• Efficient • Limits on size • Risk immune response
LNPs	• Cytotoxicity • Storage at low temperature • High cost
Self-assembled oligo-urethane peptide nanoparticles (PNPs)	• Biocompatible • Immunomodulation • Co-assembly with PEGylated lipids



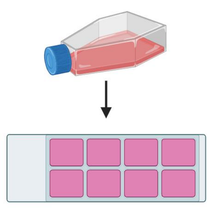
mRNA transfects into cells via endocytosis of PNPs, however the specific uptake pathway has yet to be defined[4]

Objective

To investigate:

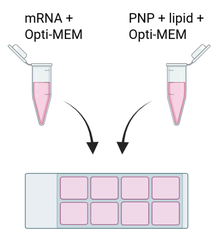
1. The transfection of mRNA using PNP/lipid co-assemblies in C_2C_{12} myoblasts
2. The cellular pathway responsible for uptake

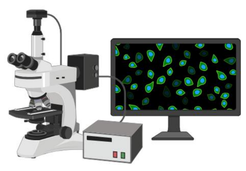
Methods



C_2C_{12} mouse myoblasts, model muscle tissue cells were seeded onto an 8-chambered slide.

Cells were pre-incubated with endocytosis inhibitors and treated with mRNA, FITC-PNPs, and PEGylated amine





Hoechst, Lysotracker red, and Wheat Germ Agglutinin (WGA) staining. Characterize the PNP uptake.

Results

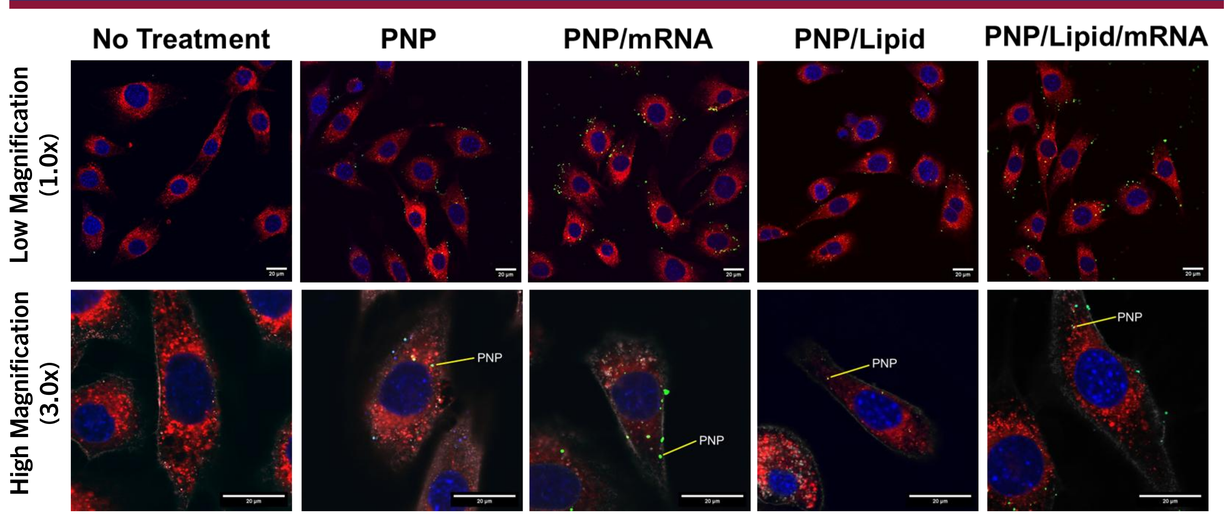


Figure 1. Representative confocal microscopy images of C_2C_{12} cells after 6 hours of treatment. The samples were stained with Hoechst (blue), Lysotracker deep red (red), Wheat Germ Agglutinin (WGA) (grey), and the PNPs are FITC-PLL labeled (green). The scale bar represents 20µm.

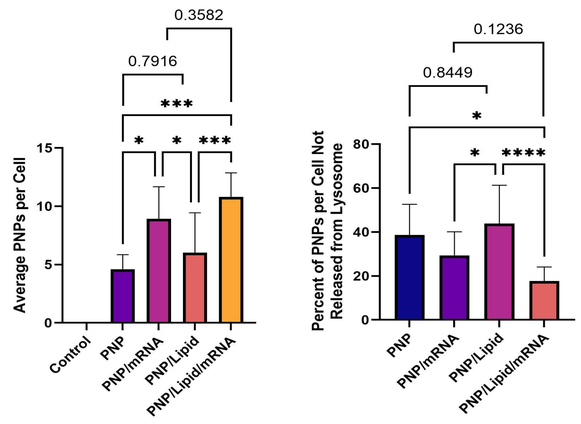


Figure 2. Average PNP uptake and percent of PNPs not released from lysosome per cell in C_2C_{12} cells following 6 hours of treatment. Data represents mean ± standard deviation (SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$.

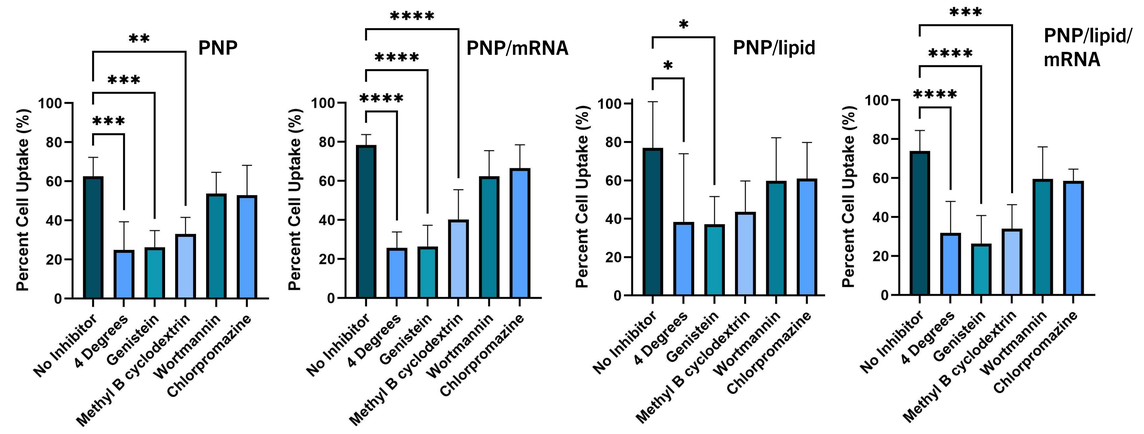


Figure 3. Percent of PNP cell uptake (%) per cell in C_2C_{12} cells. Data represent mean ± standard deviation (SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****$p < 0.0001$.

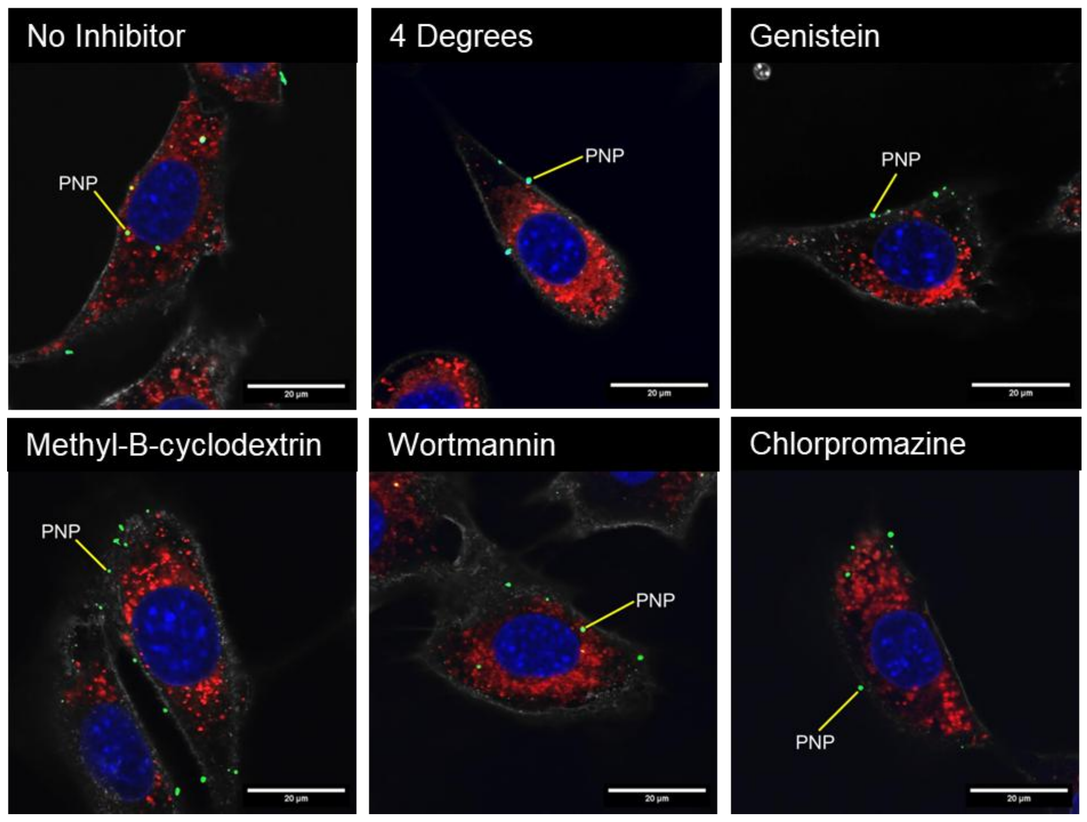


Figure 4. Representative confocal microscopy images of C_2C_{12} cells after 2 hours of treatment. The samples were stained with Hoechst (blue), Lysotracker deep red (red), Wheat Germ Agglutinin (WGA) (grey), and the PNPs are FITC-PLL labeled (green). The scale bar represents 20µm.

Discussion & Conclusions

- mRNA/PNP/lipid treatment yields higher particle uptake per cell as well as lower lysosomal entrapment per cell, suggesting the treatment increased mRNA delivery and intracellular release.
- Genistein treated groups show the most significant reduction in percent PNP uptake per cell supporting caveolar endocytosis as a key uptake pathway.
- Unlike other endocytic pathways, caveolae-mediated uptake can bypass lysosomes, organelles responsible for degradation of mRNA. This characteristics makes PNP/lipid co-assembly an attractive route for delivering therapeutic agents.

References

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Acknowledgement

Faculty of Dentistry, University of Toronto and Ted Rogers Center for Heart Research, Translational Biology and Engineering program

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