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Non-viral gene delivery using modified poly-l-lysine

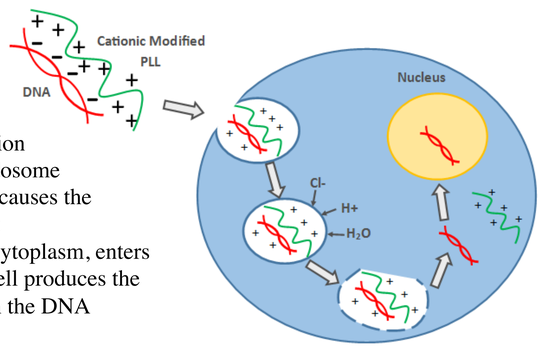
Purpose

Gene therapy has the potential to treat many diseases related to genetic defects at the cellular level. Currently, genes are primarily delivered using modified viruses. Although gene therapy using viral vectors is efficient and precise, the vectors can cause severe immunological responses and are potentially carcinogenic.

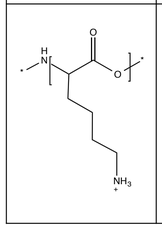
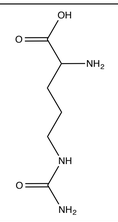
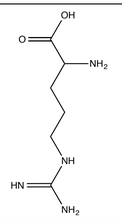
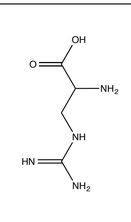
Non-viral methods are currently being investigated, but are inefficient. We synthesized three different amino acid modified poly-l-lysine (PLL) molecules to understand how material structure affects the efficiency of non-viral gene delivery vehicles. The main objectives of the project were to characterize the materials, the complexation of the materials with DNA, and the transfection efficiency of the materials.

Background

- Modified PLL has a net positive charge that forms an electrostatic complex with negatively charged DNA
- Complexes endocytosed by the cell
- Net positive charge of the complex disrupts ion balance of the endosome
- Osmotic swelling causes the endosome to burst
- DNA released in cytoplasm, enters the nucleus, and cell produces the protein encoded in the DNA

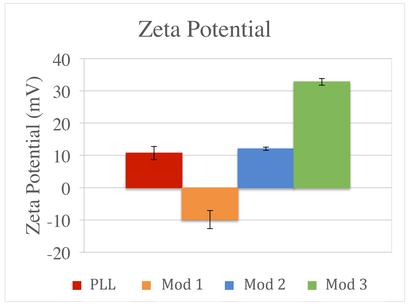


Materials

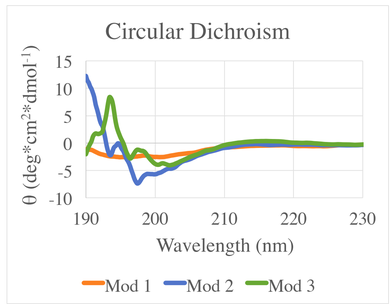
Poly-l-lysine	Citrulline (1)	Arginine (2)	2-amino-3-guanidinopropionic acid (3)
			

- Amino acid modifiers with different positive charge density and chain length were selected
- PLL was modified by creating amide bond between carboxylic acid group of the modifiers and the amine group of the PLL

Materials Characterization

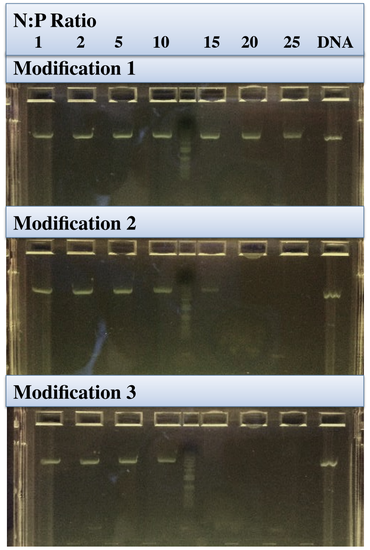


- A positive zeta potential was desired to facitlitate strong electrostatic interactions with the negatively charged phosphate backbone of DNA
- Results indicate that modification 1 has a negative zeta potential, therefore will not easily form a complex with DNA
- Results consistent with the comparatively lower positive charge density of modifier 1 (citrulline)



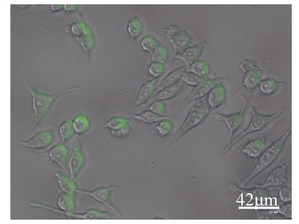
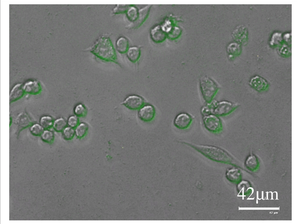
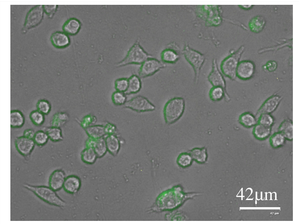
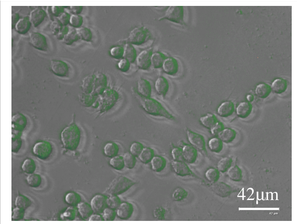
- Unmodified PLL forms an alpha helix, so it was expected that each modification would form an alpha helix
- A helical structure was hypothesized to form stronger interactions with the helical structure of DNA
- All modifications have characteristic peaks of an alpha helix

Polymer-DNA Complex Characterization



- DNA gel retardation assays used to determine the amount of modified PLL needed to form complexes with DNA
  - Free DNA appears as a band in the gel
  - When the modified PLL forms a complex with the DNA, the band is not visible
- N:P ratio indicates the ratio of amine groups in modified PLL to phosphate groups in DNA
- Modification 1:
  - Free DNA bands present at all N:P ratios
  - The band for N:P 25 is slightly dimmer, suggesting minimal complexation
- Modification 2:
  - Free DNA bands completely disappear at N:P 20
  - Complexation begins at N:P 10
- Modification 3:
  - Free DNA bands completely disappear by N:P 15
  - Complexation begins at N:P 10

Cell Transfection

	Modification 2	Modification 3
N:P 20		
N:P 25		

- Based on the results of the DNA gel retardation assays and cell viability assays, N:P ratios of 20 and 25 were selected for transfection experiments
- Complexes were incubated with HeLa cells for 8 hours, then removed
- Cells were incubated for an additional 24 hours before imaging
- Fluorescence images suggest that both modifications 2 and 3 are able to transfect cells
- Modification 3 appears to be more efficient, although it is more cytotoxic at N:P 25 than modification 2

Conclusions and Future Work

- Modified PLL molecules with a higher positive charge density were more effective at forming complexes with DNA
- Modifiers with a longer chain length are less efficient at transfection than those with a shorter chain length
- The dependence of efficiency on chain length is potentially due to structure of the molecule or the configuration of the modified PLL-DNA complex
- Transfection studies with fluorescent labels to visualize endosomal escape
- Transfection experiments with higher levels of serum in the media to test complex stability and changes in transfection efficiency
- Experiment with different modifications to further study structural effects of PLL based molecules for gene delivery

Acknowledgements: I would like to thank the members of the Bratlie lab for their collaboration and support throughout this project. This work was funded by National Science Foundation Grant Number: CBET-1227867, Roy J. Carver Charitable Trust Grant Number: 13-4265, NSF ARI-R2 (used to renovate the lab used in these studies): Grant Number: CMMI-0963224