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Synthesis and Optimization of Novel Poly-(β-amino ester) Polymer for Gene-Delivery

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Synthesis and Optimization of Novel Poly-(β-amino ester) Polymer for Gene-Delivery.

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Abstract

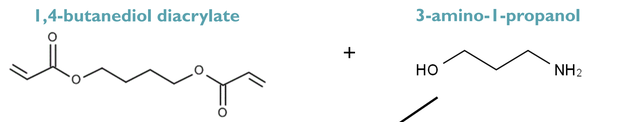
Gene delivery is one of the most promising methods to treat multiple diseases through the alteration of a genetic code to enhance or inhibit gene expression (NIH). The potential for gene delivery to be used as a treatment for cancerous cells is the beginning for advanced personalized healthcare. Current methods for cancer are costly, nonspecific, and come with major side effects that lowers quality of life in cancer patients. Previous studies have demonstrated Poly-(β-amino ester) (PBAE) to be biodegradable, non-toxic, and capable to deliver payloads in a targeted manner. For this study, PBAE was synthesized and characterized by Nuclear Magnetic Resonance, Dynamic Light Scattering, and Zeta-Potential. Cell experiments were conducted to determine transfection efficiencies. Also, conjugation of PBAE with a cell nucleus penetrating peptide (WTAS) will be investigated to develop a more effective gene delivery system, composed of two nanocarriers. Specifically, focus is emphasized on the advancement of non-viral gene delivery systems that can have a general application towards resolving current barriers in gene therapy.

Objective

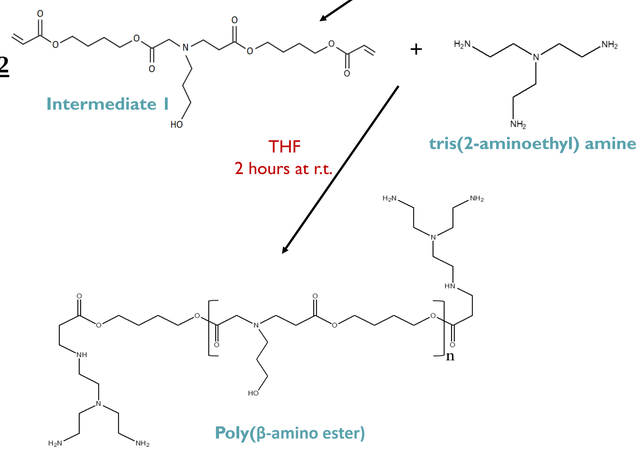
Synthesis of PBAE-cationic polymer and optimization of molecular weight for linkage of PBAE polymer to cell penetrating peptide for further cell transfections.

Polymer Synthesis

Step 1



Step 2



Transfection experiments

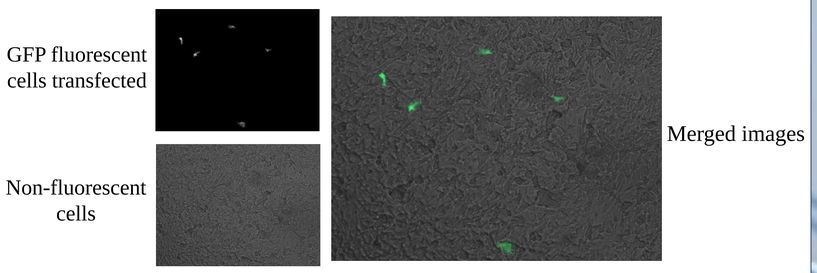
Incubation of PBAE with DNA at various concentrations and time

Concentration (μM)	Time (min.)	Zeta Potential Charge
1.05:1		
50	240	0.28
200	60	9.17
200	240	1.69
1.10:1		
200	60	1.80

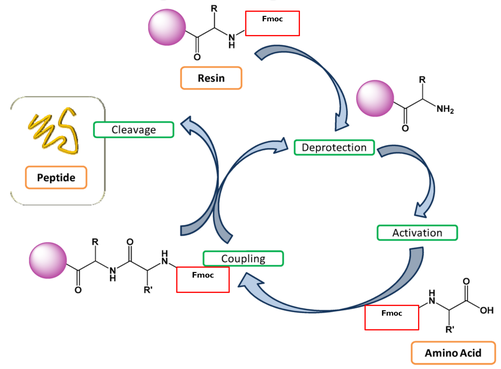
Positive zeta potential charge is ideal for cellular uptake through endocytosis, which was obtained only these PBAE concentrations and incubation time with GFP plasmid DNA

Cell transfection experiments in Neural stem cells (NSC)

150μM PBAE (1.05:1) incubated with 500 ng pGFP DNA for 30 min



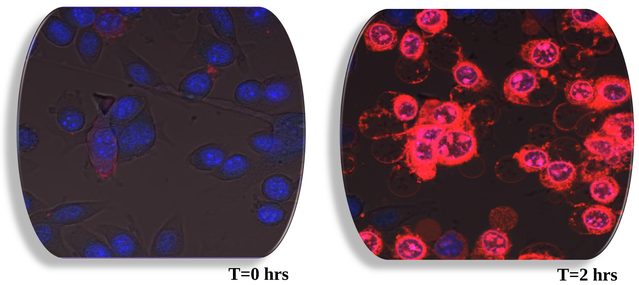
Peptide Synthesis



(W)TAS : PLKWPGKKKKGKPGKRKEQEKKKRRTR

WTAS Live Confocal Studies

50 μM WTAS loaded into B16F10 cells



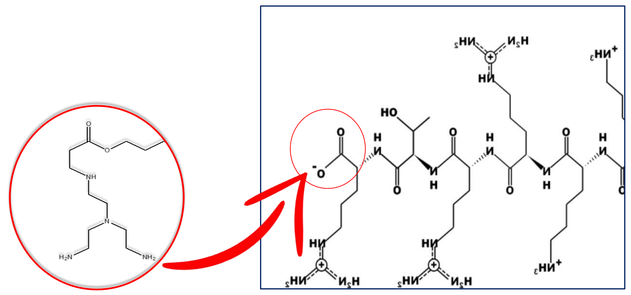
- Non-toxic,
- Cell penetration to cell nuclei within a couple minutes after exposure.

Conclusion & Next Steps

- Polymer was successfully synthesized and characterized.
- Zeta potential experiments demonstrated optimal conditions to obtain a positive zeta potential charge, which is ideal for cellular uptake during a transfection.
- Cell experiments demonstrated successful transfection for 150μM PBAE incubated for 30 min with GFP plasmid DNA.
- WTAS successfully synthesized and loaded onto cells to determine cell penetration and toxicity.

Linking PBAE to WTAS

PBAE Polymer : WTAS : EDC : DMAP
1 : 4 : 4 : 4



PBAE polymer WTAS C-terminal side

References

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Acknowledgements

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NIH National Institutes of Health

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