A Study on Gene Therapy: Using Poly (β-aminoesters)

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ABSTRACT

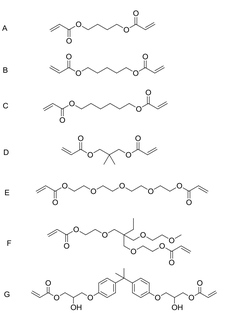
Gene delivery is the process of administering therapeutic genes to a patient's cells, where upon uptake, their modulation of the protein expression patterns in the host can result in a desired phenotype. This is especially beneficial for the treatment of a large number of diseases at the genetic level, including cancer. Cationic polymers, which are known for their ease of synthesis that can allow for a large library of polymers to be created for studies, have garnered much attention as potential gene delivery vectors. One type of cationic polymer, poly (β-aminoesters) (PBAEs), is being utilized as a potential type of vector for gene delivery. Unlike other vectors that have been observed in the past, which include viral vectors, poly-L-lysine [PLL], and poly(ethylene imine) [PEI], PBAEs are structurally diverse and non-cytotoxic. In addition, PBAEs are easily synthesized (via Michael addition), biodegradable, and capable of controlled DNA release into the cell.

INTRODUCTION

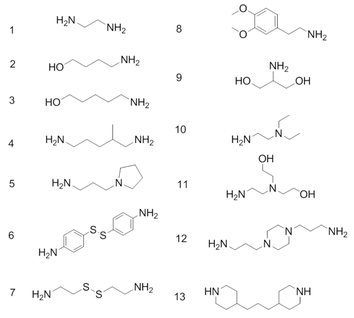
PBAE Synthesis

Monomers

Diacrylates

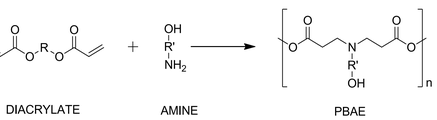


Amines

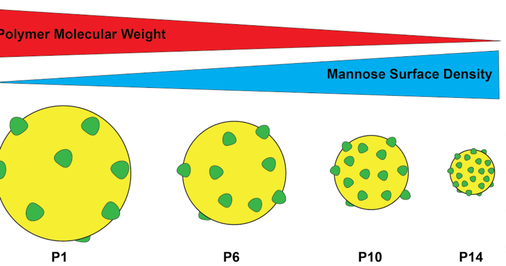


Polymers are named according to monomers used. For example: for a reaction between diacrylate A and amine 1, the resulting base polymer is called "A1"

Reaction Scheme



A diacrylate and an amine combine, via the one-step Michael Addition, to create a PBAE. The simplicity of Michael Addition allows for a large library of PBAEs..

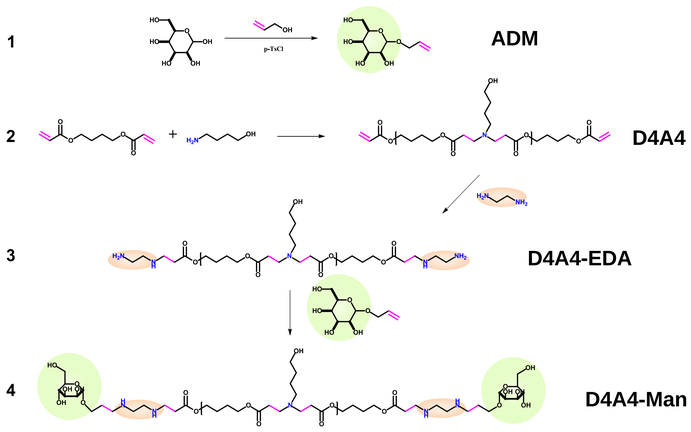


We analyzed the effects of mannosylation on PBAEs of a wide range of molecular weights. As polymer molecular weight decreased, the surface density increased.

RESULTS & DISCUSSION

Mannosylation of PBAE

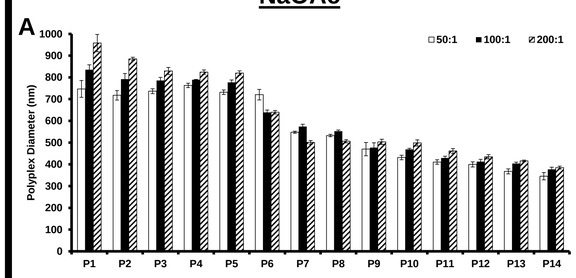
- Mannose is used as a grafting ligand.
- It has been previously shown that mannosylating PBAEs improved gene delivery efficiency.[1]
- In these studies, we examine the effects of both mannosylation and molecular weight on the gene delivery efficiency of one specific PBAE – D4A4.

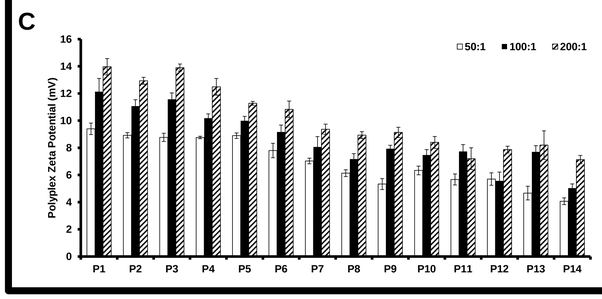


Polyplex Characterization

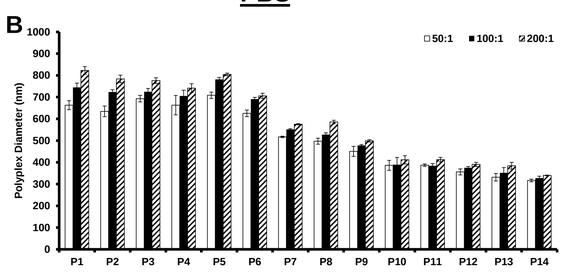
- To form polyplexes, three ratios of polymer to pDNA were used: 50:1, 100:1, and 200:1. Polyplexes are ordered on these graphs in descending molecular weight, with P1 having the highest molecular weight, and P14 having the lowest.
- Using dynamic light scattering (DLS), both the polyplex diameter (nm) and the polyplex zeta potential (mV) were measured. Sodium acetate (NaOAc) and PBS were used as solvents.
- As molecular weight decreases, both the polyplex diameter and zeta potential decrease.

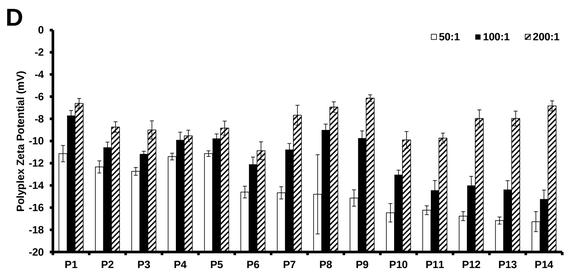
NaOAc





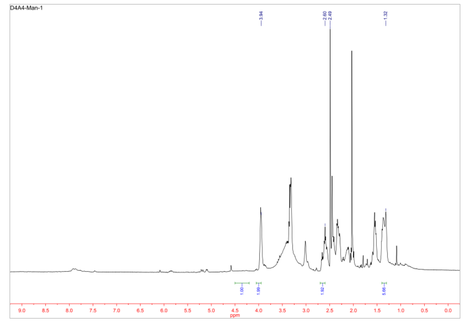
PBS





NMR Spectroscopy

- Nuclear magnetic resonance spectroscopy (NMR) allows us to determine (and confirm) the structure and purity of each polymer
- NMR was also used to evaluate polymer degradation profiles. This significantly reduced instrumentation time, as well as experimental costs.



The NMR Spectroscopy of D4A4-Man-1

- In order to determine the degradation of a polymer, the resonance intensities of the OH protons of degradants relative to the 12 CH2 protons at 3.95-4.05, 2.6-2.7, and 1.3-1.4 ppm (indicated above).
- Relative Hydroxyl Group Generation (RHGI) was then calculated:

$$RHGI = \frac{\frac{OH}{Index} - H}{H}$$

In which:

$$OH = I_{(4.5\text{-}4.2)}$$

$$H = (I_{(4.05\text{-}3.95)} + I_{(2.7\text{-}2.6)} + I_{(1.4\text{-}1.3)})/12$$

CONCLUSIONS & FUTURE DIRECTIONS

- As molecular weight increases, both zeta potential (mV) and diameter of polyplex (mm) decrease.
- NMR Spectroscopy also allowed us to determine the polymer degradation profiles at a significantly lower cost and time.
- Future experiments that can be considered include:
  1. Imaging using SEM, TEM, Confocal Microscope
  2. Antigen Presenting Cell (APC) Gene Delivery studies
  3. Infectious disease and cancer models

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2. Jones, C.H., Chen, M., Gollakota, A., Ravikrishnan, A., Zhang, G., Lin, S., Tan, M., Cheng, C., Lin, H., Pfeifer, B. A. Structure-function assessment of mannosylated poly(beta-amino esters) upon targeted antigen presenting cell gene delivery. *Under revision at Biomacromolecules*