Histone-Mimetic Conjugates as Self-Activating & Tailorable Non-Viral Gene Delivery Vehicles

Meghan J. Reilly, Millicent O. Sullivan
Department of Chemical Engineering, University of Delaware, Newark, DE 19716

Statement of Purpose: A novel solution to address ineffective non-viral DNA delivery is the development of biomimetic materials capable of regulating DNA accessibility. Recognition of the histone H3 tail trimethylated at lysine 4 by nucleosome remodeling factors has been implicated in mechanisms for chromatin activation at the transcription start sites of essentially all active genes. The presented study examines H3 tail peptides both with and without a trimethylated K4 for their potential as gene delivery materials. The H3 tail peptides, in combination with the helper cationic polymer polyethylenimine (PEI), were analyzed for their ability to bind, protect, and release DNA. The presented studies will provide the framework for further development of our system into a more complex synthetic conjugate for efficient gene delivery.

Methods: The gWIZ mammalian expression vector coding for green fluorescent protein (GFP) was amplified in *E. coli* and purified using anion exchange chromatography *via* a QIAGEN Plasmid Mega Kit (Valencia, CA). PEI was purchased from Sigma-Aldrich (St. Louis, MO). H3 tail peptides and ethidium homodimer-1 (EthD-1) were obtained from AnaSpec (San Jose, CA).

Polycation-gWIZ complexes were prepared by adding a polycation solution dropwise to an equal volume of pDNA while gently vortexing. The mean hydrodynamic diameters and zeta-potentials of particles were determined using a Brookhaven Instruments light scattering instrument (Holtsville, NY). Complexes were formed and analyzed over a range of N:P ratios. The DNA fraction bound to the complexes was observed using agarose gel electrophoresis and compared to a naked plasmid control. The gel was stained with ethidium bromide solution from Fisher (Fairlawn, NJ) and then imaged under UV illumination on a Bio-Rad Molecular Imager (Hercules, CA).

The cell lines used for *in vitro* cell transfection and viability studies were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and cultured following ATCC protocols. Complex solutions were added dropwise to the cells 20 hr post-seeding. After a 4 hr incubation with the transfection reagents, the complexes were removed for the remaining 24 hr incubation period. Cells were imaged on a Leica DM600 B microscope (Bannockburn, IL). EthD-1 was used to study the cytocompatibility of the complexes. Transfection efficiencies were determined using a Becton Dickinson FACSCalibur Flow Cytometer (Franklin Lakes, NJ).

Results: The formation of positively-charged nanoscale particles (diameter ~ 100 – 300 nm) that were stable in the presence of serum nucleases was achieved for both H3 peptides. The H3-PEI hybrid vehicles were observed to transfect a substantially higher number of CHO-K1 cells *in vitro* compared to both complexes that were formed with only the H3 peptides and those formed with only PEI at the same total charge ratio (Figure 1a). This synergistic effect was further explored by looking at the cytocompatability of the conjugates (Figure 1b). The H3-PEI hybrid vehicles were found to have no significant effect on cell viability, whereas PEI was shown to increase the amount of cell death.

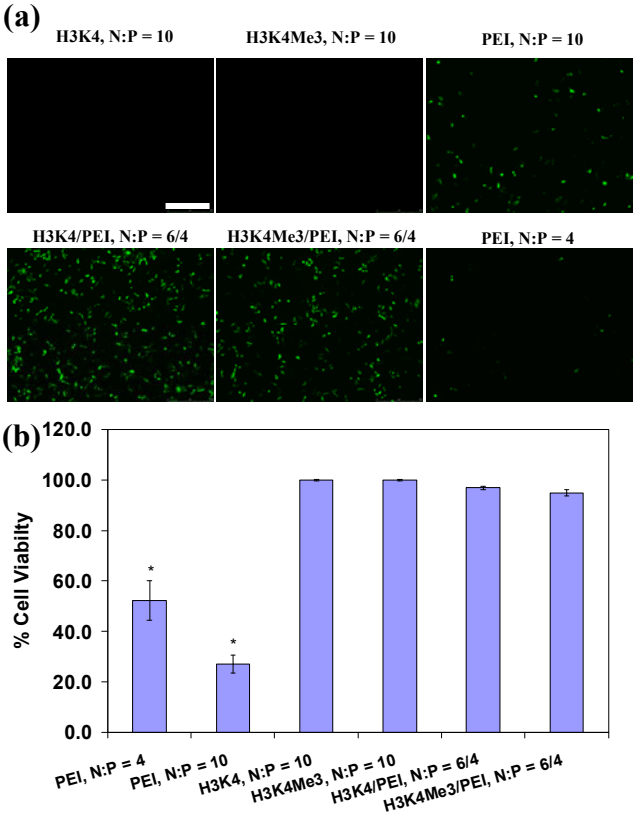

Figure 2.
(a) Fluorescence images taken 24 hr after the addition of the appropriate conjugate to CHO-K1 cells. Cells were transfected with pDNA encoding for GFP. Scale bar = 500 μm. **(b)** Cell viability was determined by staining dead cells with 4 μM EthD-1. (*) indicates samples with statistically significant cell death relative to autofluorescent controls ($p < 0.5$, $n = 3$).

Conclusions: The H3-PEI vectors developed have been validated as delivery vehicles that can successfully bind, protect, and release DNA. These hybrid conjugates improved cell transfection and eliminated the cytotoxic effects associated with PEI. These effects will be further explored by investigating the intracellular trafficking of the complexes.

The presented studies provide the framework for further development of our system. Based on these results, H3 tail peptides appear to be viable candidates for incorporation in a conjugate with greater complexity that will be aimed at achieving more efficient cell transfection.