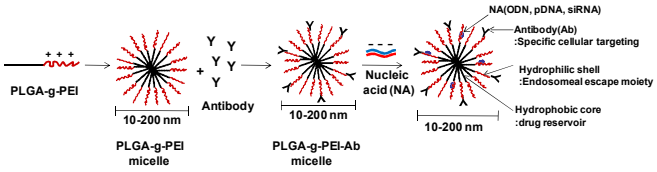
Neuron-specific polymeric micelle nanotherapeutics for CNS regeneration

Jeoung Soo Lee, Jeremy Zhang, and Ken Webb
Clemson University, Bioengineering Department, Clemson, SC

Statement of Purpose

Adult CNS axons retain the intrinsic capability for growth, but fail to do so effectively following injury due to the presence of native and injury-induced growth inhibitors in the extracellular environment. Recently, three molecules present in CNS myelin termed Nogo A, myelin associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) have been identified as inhibitory molecules for axon growth and function in CNS. All three molecules have been shown to bind to a common receptor present on the axon called the Nogo-66 receptor (NgR)[1]. The long-term objective of this project is to develop neuron-specific polymeric micelles as a carrier for simultaneous delivery of two therapeutic agents (NgR antibody and NgR siRNA) directed at reducing the activity of the Nogo 66 (NgR) receptor. This dual strategy is based on interference with the function of existing NgR receptors at the protein level and inhibition of further production of new NgR receptors at the genetic level[2]. In this study, we synthesized and evaluated the feasibility of an amphiphilic co-polymer, poly (lactide-co-glycolide)-graft-polyethylenimine (PLGA-g-PEI), as a nucleic acid carrier using pGFP and primary E8 chick forebrain neurons (Scheme 1).



Scheme 2. Antibody functionalized polymeric micelle (PLGA-g-PEI-Ab)/NA complex

Materials and Methods

1.Synthesis and characterization of PLGA-g-PEI (PGP): To synthesize PGP, the carboxylic acid group of PLGA (MW=4kDa) was activated by DCC and NHS in DMF and added to a solution of branched PEI (MW=25kDa). The PGP was purified by dialysis and freeze-dried. Following synthesis and purification, the structure of PGP was determined by ^{1}H-NMR and FTIR. The CMC of PGP was determined by a dye solubilization method using DPH. The particle size and ζ potential of PGP/pGFP complexes at various N/P (nitrogen atoms of polymer/ phosphorus atoms of nucleic acid) ratios was determined by dynamic light scattering and electrophoretic analysis, respectively.

2. Gel retardation assay: The complexes of PGP micelle/pGFP were prepared at N/P ratios ranging from 5 to 30. The complexes were electrophoresed on a 1% agarose gel for 90min at 80 V and visualized by UV illuminator.

3. Transfection efficiency and cytoxicity of PGP/pGFP complex nanoparticle in vitro: E8 chick forebrain neuron (CFN) were transfected with varying N/P ratios of PGP/pGFP complexes. Forty-eight hours following transfection, the transfected cells were counted using fluorescence microscopy and neuron cells were stained for β-III tubulin. Cytoxicity were evaluated by MTT assy.

Results and Discussion

1.Synthesis and characterization of PGP: The molecular weight of PGP was determined to be 37,000 from ^{1}H-NMR and the IR spectrum showed a typical amide bond formed at 1665 cm^{-1} and a typical ester carbonyl peak from PLGA at 1765 cm^{-1}. The CMC of PGP micelle was determined to be 0.69 mg/ml. The mean particle size of various PGP/pGFP complexes was in the range of 146.8±1.4 to 177.6±1.1 nm at above 10/1 N/P ratio. The surface charge of PGP/pGFP prepared at various N/P ratios was determined and positive above N/P ratio of 5/1 and increased with N/P ratio.

2.Gel retardation assay: To demonstrate the stable complex formation, a gel retardation assay was performed and the complete retardation of complexes of PGP/pGFP was observed above N/P ratio of 5/1.

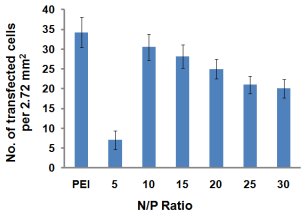


Figure1. GFP expression in primary E8 CFN cells after transfection with PGP/pGFP complexes at various N/P ratios. PEI/pGFP complex (N/P:5/1) was used as positive control. Each column represents the mean ± STD (n=12) from four independent experiments.

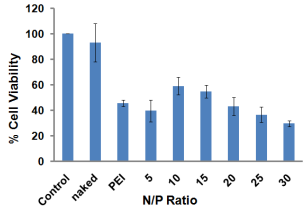


Figure2. Cell viability assay of primary E8 CFN cells after transfection with PGP/pGFP complexes at various N/P ratios. No treatment control, naked DNA, and PEI/pGFP complex at N/P ratio of 5/1 was used for comparison. Each column represents the mean ± STD (n=3).

3.Transfection efficiency and cytoxicity of PGP/pGFP complex: The average number of transfected cells of PGP /pGFP complexes was assessed in CFN cells at various N/P ratios and PGP/pGFP complexes at N/P ratios of 10/1 showed similar transfection efficiency compared to PEI and higher cell viability (~59 %) than that of PEI(~45.5%) (Fig 1 and 2). Figure 3 shows images of GFP transfected E8 CFN cells and neurites.

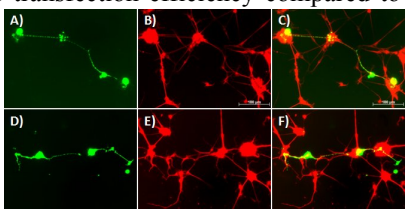


Figure 3. GFP expression in primary E8 CFN cells after transfection with PEI/pGFP complex (A-C;N/P;5/1) and PGP/pGFP complexes (D-F;N/P;10/1). Neurons were stained with beta-III tubulin (red). Original magnifications, 200X.

Conclusions

We demonstrated this novel PLGA-g-PEI micelle as a nucleic acid delivery carrier in primary CFN cells with similar transfection efficiency and lower cytotoxicity compared to PEI. Currently, we are studying the gene silencing efficiency of PGP/GFP siRNA complex after co-transfection of pGFP using PEI. In the future, we will synthesize PLGA-g-PEI-Ab (Ab:NgR antibody) and evaluate the feasibility of PLGA-g-PEI-Ab as a neuron-specific nucleic acid (ODN or siRNA of NgR) carrier for CNS regeneration in rat DRG/astrocyte co-culture system.

References

1. Fournier et al. *Nature*, **409**, 341-6 (2001) 2. Ahmed et al. *Molecular and Cellular Neuroscience*, **28**, 509-23 (2005)