Polymeric Micelle Delivery System for Neural Regeneration

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Statement of Purpose: Physical trauma or ischemia results in significant damage to the central nervous system (CNS). The regenerative capacity of the injured adult CNS is extremely limited, due to both extrinsic microenvironmental factors and intrinsic, age-related changes in neuronal biochemistry[1]. There are many strategies for improving axonal regeneration. While all these strategies have achieved varying degrees of improvement in plasticity, regeneration, and function; it is clear that no single therapeutic will achieve adequate functional recovery. Recently, combinatorial strategies incorporating two or more therapeutic modalities have achieved synergistic increases in growth and recovery. The long term goal of our work is to develop neuron-specific multi-functional polymeric micelle nanotherapeutics for combinatorial delivery of multiple bioactive molecules targeting different barriers to plasticity and axonal regeneration. Our approach is based upon amphiphilic copolymers (poly (lactide-co-glycolide)–g-polyethylenimine: PgP) that spontaneously form polymeric micelles in aqueous solution[2]. This material offers three important capabilities: 1) loading of hydrophobic drugs in the PLGA hydrophobic core, 2) complexation of siRNAs with the PEI hydrophilic shell, and 3) cell-type specific targeting through surface conjugation of cell-type specific ligands or antibodies.

Methods: To synthesize PgP, the carboxylic end group on the PLGA (MW: 4,000) was activated by NHS and DCC and then conjugated to branched PEI (MW: 25kDa). Following synthesis and purification, the structure and molecular weight of PgP were determined by ^{1}H- NMR. The Monster Green Fluorescent Protein phMGFP Vector (pGFP) was used as a reporter gene to evaluate the feasibility of PgP as nucleic acid carrier. First, we evaluated particle size and zeta-potential of PgP/pGFP complexes at various N/P (no. of nitrogen atom in PGP/no. of phosphorous atom in pGFP) ratio using Zeta PALS and the stability of PgP/pGFP complex by gel retardation assay. The transfection efficiency and cytotoxicity of PgP/pGFP complexes at various N/P ratio were evaluated at 48hrs post-transfection of PgP/pGFP complexes in C6 neuroglioma cells and primary CFN (E8 Chick forebrain neuron) cells in non-serum and 10% serum condition. Transfection efficiency was measured by flow cytometry and cytotoxicity of PgP/pGFP complexes was evaluated by MTT assay. PEI/pGFP at N/P of 5/1 was used as positive control.

Results and Discussion: The molecular weight of PgP was approximately 37,000 Da. Particle size of PgP/pGFP complexes was 146.8±1.4 to 177.6±1.1 nm at N/P ratios above 10/1 and surface charge of complexes was positive above N/P ratio of 5/1 and increased with N/P ratio. Complete gel retardation of complex was observed at N/P ratios greater than or equal to 5/1, demonstrating that PgP can form stable complex with pGFP. Figure 1 shows % transfection efficiency of PgP/pGFP complexes at various N/P ratio in C6 cells (Fig 1A) and CFN cells (Fig 1B). In the case of C6 cells, PgP/pGFP complex at N/P of 15/1 or above showed higher transfection efficiency than PEI control in both 10% serum and non-serum condition without significant cytotoxicity. In case of CFN cells, transfection efficiency of PEI/pGFP complex at N/P of 5/1 in 10% serum condition was dramatically decreased relative to non-serum condition, while transfection efficiency of PgP/pGFP complex increased with increasing N/P ratio. In non-serum condition, both PgP/pGFP and PEI/pGFP complex were very cytotoxic with cell viability about 50%. In 10% serum condition, the cell viability of PgP/pGFP complex decreased with increasing N/P ratio, while PEI/pGFP complex was not cytotoxic. Figure 2 shows representative images of GFP-transfected C6 cells in the 10% serum condition.

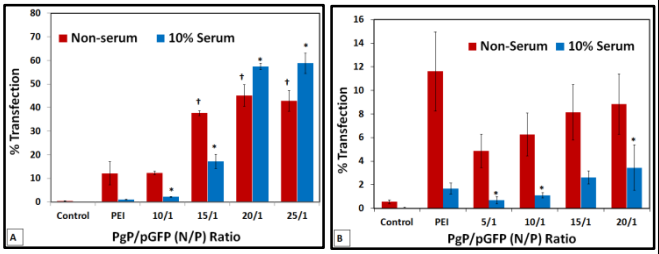

Fig 1. GFP expression after transfection of PgP/pGFP complexes in C6 cells (A) and CFN cells (B). Data represent mean ±SED (n=6). *:P<0.05 (serum), ł:P<0.05 (non-serum) compared to PEI

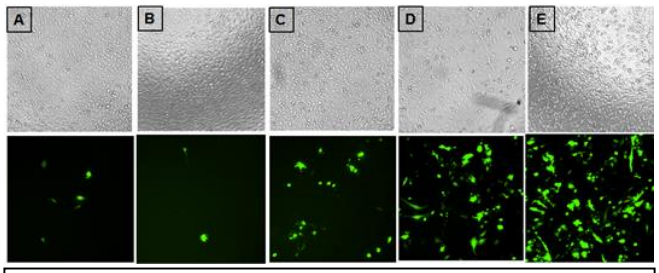

Fig 2. Representative images of transfected C6 cells with PgP/pGFP complexes in 10% serum condition. Magnification 100X, top: Phase contrast, bottom: GFP transfected cells A)PEI/pGFP:5/1, B~E) PgP/pGFP: 5/1, 10/1, 15/1, and 20/1.

Conclusion and Future Studies: We demonstrated that the PgP polymeric micelle is a promising nucleic acid carrier for plasmid DNA_and is capable of transfecting neuroglioma (C6) cells and primary CFN cells with low cytotoxicity in 10% serum condition, which dramatically reduces the efficacy of the branched PEI control. Currently, we are preparing PgP-Ab (Ab:NgR antibody) and evaluating the feasibility of PgP-Ab as a neuron-specific nucleic acid (NgR siRNA) carrier for CNS regeneration. **Acknowledgements:** This research was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103444.

References:1. C. Bandtlow. GLIA 2000; 29: 175-181
2. N. Nishhiyama. Pharm. & Therap. 2006; 112(3) 630-648