Polymeric Micelle as a Nucleic Acid Carrier for Axonal Regeneration in CNS

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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 the PLGA hydrophobic core, and 3) targeting through surface conjugation of cell-type specific ligands or antibodies. Here, we show that PgP micelle is capable of efficiently transfecting reporter genes both in the presence of 10% serum in vitro and in the rat spinal cord in vivo.

Methods: PgP was synthesized and characterized by ^{1}H-NMR and GPC as previously described[2]. Primary E8 chick forebrain neuron (CFN) were plated and transfected with PgP/pGFP prepared at various N/P ratios ranging from 5/1 to 35/1 in non-serum and 10% serum condition. GFP expression was measured after 48 hrs post-transfection by cytometry using a Millipore easyCyte flow cytometer. Cytotoxicity was analyzed by MTT assay. To evaluate gene expression in rat spinal cord in vivo, PgP/pβGal polyplexes were injected in T9 spinal cord region. Briefly, laminectomy was performed on the back of SD rats and the T9 spinal cord region were exposed. 20 µl PgP/pβ-gal polyplexes (10 µg pDNA, N/P ratio 30/1) were prepared and injected at the T9 spinal cord region. bPEI/ pβ-Gal polyplexes (N/P: 5/1) and naked pβ-Gal were used as controls. At 7 days after polyplex injection, the rats were sacrificed by perfusion with 4% paraformaldehyde and spinal cord sites were retrieved and fixed with 4% paraformaldehyde. And β-Gal expressed cells were visualized by β-Gal staining kit (Life Technologies) and Eosin staining. To study localization of PgP/siGLO-red polyplex (N/P 30/1, 10 µg siGlo-red) were injected in the T9 spinal cord region. Localization of the PgP/siGLO-red polyplex and naked siGlo-red were assessed immediately following injection by live animal flu-orescence imaging system (Quantum FX microCT Imaging System).

Results: In E8 CFN cells, transfection efficiency of PEI/pGFP complex at N/P of 5/1 in the serum condition was dramatically decreased relative to non-serum condition, while transfection efficiency of PgP/pGFP complex increased with increasing N/P ratio in 10% serum condition. To evaluate PgP as a gene carrier in vivo, we used plasmid encoding β-Galactosidase (pβ-Gal) to avoid tissue autofluoresecence interference with GFP imaging. At 7days post-injection, the PgP/pβ-gal complexes showed substantially higher β-gal positive area compared controls. (Fig 1). The PgP/siGLO-red polyplexes (N/P 30/1, 10 µg siGlo)) were injected in the T9 spinal cord lesion. Naked siGlo-red (10 µg) was used as a control. PgP/siGlo-red polyplexes were retained at the injection site for at least 2 hrs, while naked siGlo diffused away within 30 min (Fig2).

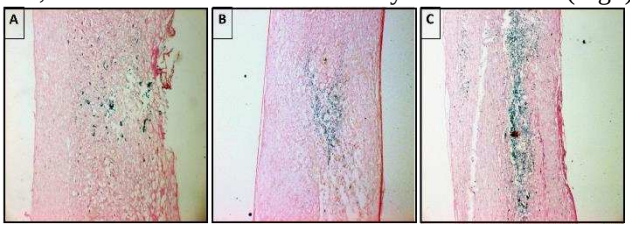

Fig.1. β-Gal expressed cells (Blue) in rat spinal cord at 7 days post-injection of PgP/ pβ-Gal polyplexes. Magnification: 40X. A) naked pβ-Gal, B) PEI/ pβ-Gal polyplex, C) PgP/pβ-Gal polyplex

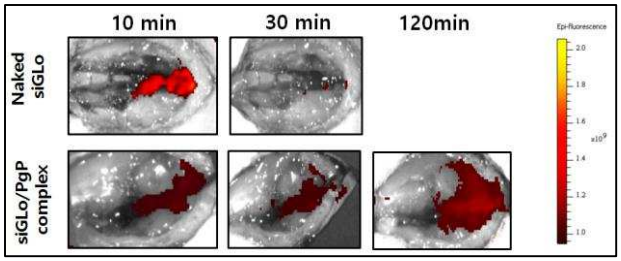

Fig 2. Localization of PgP/siGlo-red polyplexes after injection in rat T9 spinal cord region.

Conclusion: We demonstrated that PgP micelle as a nucleic acid delivery carrier in E8 CFNs in vitro and rat spinal cord in vivo. These results suggest that PgP could be useful in therapeutic gene delivery carrier for the treatment of spinal cord injury. Currently, we are evaluating the silencing efficncy and cytotoxicity of drug-loaded PgP/siRNA polyplexes in spinal cord injury model.

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References: 1. Bandtlow et al.,. GLIA 2000; 29: 175-181
2. Lee et al. Trans SFB p.917 (2010).
3. Heidemann et al., Methods Cell Biol. 2003;71:51-65