Characterization of Long-term stability of PgP/pGFP Polyplexes with Varying Cryoprotectants

[1, 2]Joshua Woo, [1]KiTaek Kim, PhD, [1]Christian Macks, [1]JeoungSoo Lee, PhD
[1]Drug Design, Development, and Delivery (4D) Laboratory, Bioengineering, Clemson University, Clemson, SC
[2]Johns Hopkins University, Baltimore, MD

Statement of Purpose: Genetic disorders affect more than 350 million people globally, and can have significant physical, social, and psychological impacts. Gene therapy can treat disorders through intracellular delivery of genetic material into patients' cells; however, a carrier is needed to transport this material into cells. We previously reported the synthesis and characterization of a cationic, amphiphilic copolymer, poly (lactide-co-glycolide)-graft-polyethylenimine (PgP), and demonstrated PgP as a pDNA carrier in normal rat spinal cord models[1], as well as a therapeutic siRNA carrier in rat spinal injury models[2]. In this study, we investigated the long-term stability of PgP/pGFP polyplexes after lyophilization with various cryoprotectants for 6 months at -20°C, important features for commercial and clinical applications.

Methods: PgP was synthesized using three segments of PLGA (4kDa) and a bPEI (25kDa) as previously described [1, 2]. PgP/pGFP (1 µg pGFP/well) polyplexes were prepared by mixing PgP with pGFP (MonsterGreen Fluorescent Protein phMGFP Vector [Prometga]) at N/P (Nitrogen atoms in PgP/Phosphorus atoms in pGFP) ratios of 30/1, and then allowed to incubate at 37°C for 30 minutes. At this point, in-solution aqueous samples were stored at 4°C for various time points. For lyophilized samples, after incubation, varying cryoprotectants (5% Sucrose, 5% Glucose) were added to the polyplex solutions, gradually cooled to -80°C over 24 hours, and then lyophilized for 24 hours. After lyophilization, polyplexes were stored at -20°C for various time points. At predetermined time points (0, 1W, 2W, 1, 2, 4, and 6M), lyophilized polyplexes were reconstituted with sterile water. The stability of both lyophilized and in-solution polyplexes were evaluated by heparin competition assay using 1% agarose gel. Both in-solution aqueous polyplexes and reconstituted lyophilized polyplexes were transfected into C6 (rat glioma) cells in media containing 10% serum. At 24 hours post-transfection, media was replaced with fresh media, and cells were incubated for an additional 24 hours. GFP expression was measured by flow cytometry (Guava easyCyte, Millipore), and imaged using an inverted epifluorescent microscope (Zeiss Axiovert 200, Göttingen, Germany). The cytotoxicity of polyplexes were measured by MTT assay.

Results: When PgP/pGFP polyplexes are stored in solution, polyplexes were stable and heparin competition assay showed that DNA was protected up to 2 months (Fig. 1A and C). In case of lyophilized PgP/pGFP polyplexes, polyplexes with 5% sucrose as a cryo - protectant showed intact DNA up to 6 months, whereas PgP/pGFP polyplexes with 5% glucose showed intact DNA up to 4 months (Fig 1B and D). PgP/pGFP polyplexes without cryoprotectants was not stable after lyophilization (Fig 1B) and intact DNA was not shown

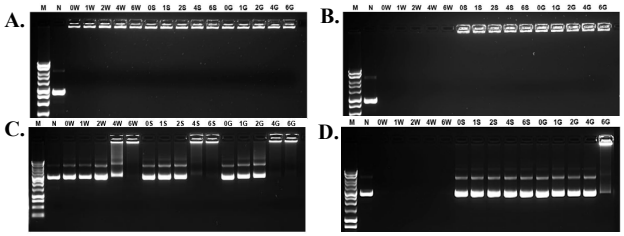

Figure 1. Characterization of polyplex stability in-solution and after lyophilization by gel electrophoresis (**A-B**) and heparin competition assay (**C-D**). **A and C**: polyplexes stored in-solution, **B and D**: lyophilized polyplexes. M: Marker, N: Naked DNA, 0W-6W: Polyplexes with water only, 0S-6S: Polyplexes with sucrose, 0G-6G: Polyplexes with glucose at 1, 2, 3, 4, and 6 months.

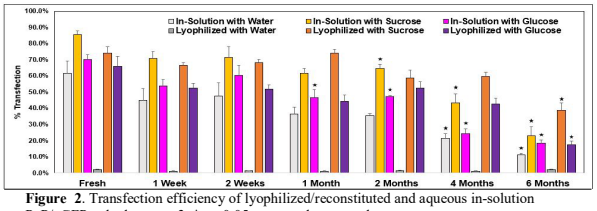

Figure 2. Transfection efficiency of lyophilized/reconstituted and aqueous in-solution PgP/pGFP polyplexes: n=3, *p < 0.05 compared to controls

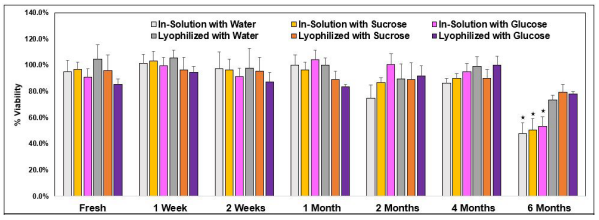

Figure 3. Cell viability of lyophilized/reconstituted and aqueous in-solution PgP/pGFP polyplexes: n=3, *p < 0.05 compared to controls

(Fig. 1D). As a result, lyophilized/reconstituted PgP/pGFP polyplexes with both 5% sucrose and glucose maintained transfection efficiencies up to 6 months, while transfection efficiency of PgP/pGFP polyplexes without cryoprotectants were not observed. Transfection efficiency of polyplexes stored in-solution started to decrease after 1 month. In both lyophilized and in-solution polyplexes, 5% sucrose protected DNA the most and maintained transfection efficiency up to 6 months (Fig. 2). In the case of cytotoxicity, both lyophilized/reconstituted and in-solution polyplexes were not cytotoxic up to 4 months, but polyplexes in-solution showed significant cytotoxicity at 6 months compared to freshly prepared polyplexes (Fig. 3).

Conclusions: We demonstrated that lyophilized PgP/pGFP polyplexes with 5% sucrose as a cryoprotectant was the most stable and maintained transfection efficiency without significant cytotoxicity up to 6 months at -20°C. In the future, we will evaluate the transfection efficiency and cytotoxicity of lyophilized PgP/pGFP polyplexes in vivo animal models.

Acknowledgements: This research was supported by NIGMS of the National Institutes of Health under grant number 5P20GM103444-07

References: [1] Gwak, SJ. Acta Biomat. 2016;35:98-108 [2] Gwak, SJ. Biomat. 2017;121:155-166 [3] Gwak, SJ. Sci. Rep. 2017;7:11247