Release of Stabilized Diblock Copolymer/Plasmid DNA Polyplexes from Injectable Scaffolds

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Statement of Purpose: Nonviral gene therapy has strong potential for use in tissue regeneration if delivery barriers can be overcome. Polyurethane (PUR) scaffolds have previously been shown to support cellular infiltration and matrix remodeling in skin wounds,[1] and they have been used to deliver biologics such as proteins and drugs.[2] The goal of this study was to develop an injectable PUR delivery system for plasmid DNA (pDNA) that can be used to achieve sustained, local delivery of genes for regenerative factors. However, many traditional transfection reagents such as polyethylenimine (PEI) suffer from instability, especially when lyophilized for storage or incorporation into biomaterial scaffolds.[3] We recently developed and screened a novel library of poly (ethylene glycol-*b*-(dimethylaminoethyl methacrylate-*co*-butyl methacrylate)) [poly(EG-*b*-(DMAEMA-*co*-BMA))] polymers for transfection efficiency. These polymers were designed such that DMAEMA initiates electrostatic interactions with pDNA to trigger formation of polyplexes that are further stabilized by hydrophobic interactions of the BMA in the core and steric shielding by the PEG corona. The BMA content was tuned to achieve pH-dependent membrane disruption to promote endosomal escape. Here, the lead candidate was shown to have higher stability and transfection efficiency than PEI after lyophilization. Polyplexes released from PUR scaffolds transfected cells for up to four days. This study provides evidence that PUR scaffolds incorporating poly(EG-*b*-(DMAEMA-*co*-BMA))/pDNA polyplexes have potential for use in tissue regenerative gene therapy applications.

Methods: Poly(EG-*b*-(DMAEMA-*co*-BMA)) comprising a 5 kDa PEG block and a 21 kDa DMAEMA-co-BMA block with 40% BMA (abbreviated 40L) was synthesized by reversible addition fragmentation chain transfer. 40L polyplexes were formed by mixing luciferase pDNA and polymer in pH 4 buffer and raising the pH to 7.4 after 15 min. PEI polyplexes were formed by mixing PEI and pDNA in pH 7.4 phosphate buffer. Sizes of fresh and lyophilized polyplexes in PBS were characterized using dynamic light scattering. PUR scaffolds were fabricated by reacting a lysine triisocyanate-PEG prepolymer and a polyester triol with a backbone comprising 30% glycolide, 10% lactide, and 60% caprolactone.[1] Polyplexes were lyophilized in the presence of (2-hydroxyprolyl)-β-cyclodextrin equal to 10% of the scaffold mass and were incorporated into the scaffolds as a lyophilized powder. Scaffolds were cut into 8x1 mm discs containing ~3.5 μg luciferase pDNA. $2x10^5$ MDA-MB-231 cells were seeded onto each scaffold in 24-well plates. Bioluminescence was measured every 24 h using a Xenogen IVIS 200.

Results: The stability and transfection efficiency of 40L and PEI polyplexes after lyophilization were investigated. As shown in Fig. 1A, PEI polyplexes aggregated significantly after lyophilization, causing a more than 4-fold increase in size ($p < 0.05$). In contrast, 40L polyplexes did not aggregate significantly following lyophilization. Furthermore, fresh 40L polyplexes had greater stability against salt-induced aggregation than PEI polyplexes in phosphate-buffered saline. PEI polyplexes formed aggregates >900 nm after 5 h while 40L polyplexes remained <200 nm after 60 h (data not shown). In addition, transfection of lyophilized polyplexes at varied amine/phosphate (N/P) ratios was explored (Fig. 1B). 40L polyplexes had significantly higher transfection efficiency than PEI polyplexes at each N/P ratio ($p < 0.05$). Due to these promising results, we tested delivery of lyophilized polyplexes from PUR scaffolds *in vitro*. Cells seeded onto scaffolds containing 40L polyplexes were transfected for four days (Fig. 2) and had significantly higher transfection than PEI polyplexes at day 4 ($p < 0.05$).

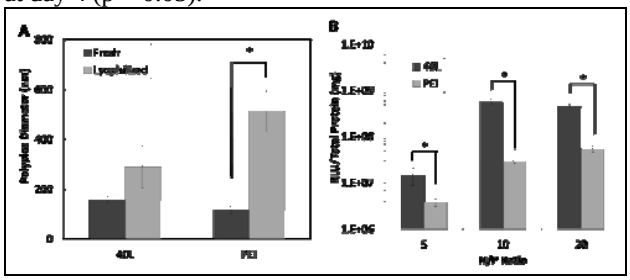

Figure 1. Comparison of lyophilized 40L and PEI polyplexes. (A) Polyplex size before and after lyophilization. (B) Transfection of MDA-MB-231 cells with lyophilized polyplexes. Data are plotted as mean ± standard deviation.

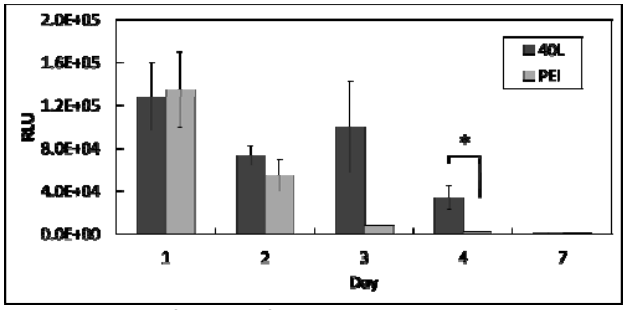

Figure 2. Transfection of MDA-MB-231 cells seeded on PUR scaffolds incorporating 40L or PEI polyplexes. Data are plotted as mean ± standard error.

Conclusions: 40L polyplexes were more stable after lyophilization and resulted in higher transfection than PEI polyplexes at a variety of N/P ratios. Cells seeded onto PUR scaffolds containing lyophilized 40L polyplexes were transfected for up to four days. Future work will involve delivering 40L polyplexes from PUR scaffolds *in vivo* for gene therapy applications.

References: [1] Adolph EJ. J Biomed Mater Res A. 2011;100A:450-61.
[2] Guelcher SA. J Orthop Trauma. 2011;25:477-82.
[3] Lei Y. Biomaterials. 2010;31:9106-16.