Lyophilized Poly(ethylene glycol-b-(dimethylaminoethyl methacrylate-co-butyl methacrylate))-DNA Nanoparticles for Nonviral Gene Therapy

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Statement of Purpose: Nonviral gene therapy has great potential for use in tissue regeneration and treatment of diseases, but many of the traditional transfection reagents such as polyethylenimine (PEI) suffer from instability and aggregation, especially when lyophilized for storage and/or incorporation into biomaterial scaffolds. Here, a novel library of poly(ethylene glycol-b-(dimethylaminoethyl methacrylate-co-butyl methacrylate)) [poly(EG-b-(DMAEMA-co-BMA))] polymers were developed and screened for improved stability and nucleic acid transfection following lyophilization. The studies were motivated by the desire to prepare a plasmid DNA (pDNA) nanocarrier that is stable through lyophilization and fabrication of tissue engineering scaffolds in order to improve bioactivity *in vivo*. These polymers were designed so that DMAEMA initiates nucleic acid electrostatic interactions to trigger formation of polyplexes that are further stabilized by the hydrophobic interactions of the BMA in the core and the steric shielding by the PEG corona. The BMA content was also finely tuned so that the polyplexes exert pH-dependent membrane disruption finely tuned to promote endosome escape. In this study, the stability and transfection efficiency of poly(EG-b-(DMAEMA-co-BMA))-pDNA polyplexes were investigated in order to determine their suitability for delivery from tissue engineering scaffolds *in vivo*.

Methods: Poly(EG-b-(DMAEMA-co-BMA)) polymers (40S, 40L, 50S, 50M, 50L) were synthesized by reversible addition fragmentation chain transfer polymerization. The abbreviated names indicate the percentage of BMA in the DMAEMA-co-BMA block (40% or 50%) and the relative length of the DMAEMA/BMA block (short [S] = 13,000 Da; medium [M] = 18,000 Da; long [L] = 23,000 Da). The PEG block length was held constant at 5,000 Da. Polyplexes were initially formed by mixing luciferase pDNA and polymers in pH 4 buffer with amine to phosphate (N/P) ratios of 5, 10, and 20. After 15 min, the pH was raised to 7.4. The size and zeta potential of fresh and lyophilized complexes in water and phosphate-buffered saline (PBS) were measured using dynamic light scattering. Polyplexes with a dose of 150 ng pDNA were delivered to MDA-MB-231 tumor cells in 96-well plates, and bioluminescence was measured after 24 h using a Xenogen IVIS 200.

Results: Hydrodynamic diameters of fresh poly(EG-b-(DMAEMA-co-BMA))-pDNA complexes ranged from 130 – 180 nm. After lyophilization, the sizes of PEI lipofectamine2000 (lipo) complexes and many 50% BMA poly(EG-b-(DMAEMA-co-BMA))-pDNA polyplexes increased significantly (indicated with asterisks in Fig. 1A, $p < 0.05$). In contrast, the sizes of 40S and 40L polyplexes with N/P ratio of 20 remained below 200 nm and did not significantly increase after lyophilization. Furthermore, polyplex stability over time in PBS was assessed (Fig. 1B). Lipofectamine2000 and PEI complexes were less stable than the novel polymers, forming aggregates >1000 nm within 24 h. In contrast, poly(EG-b-(DMAEMA-co-BMA))-pDNA complexes remained <400 nm for more than 60 hours. Previous studies on colloid aggregation have shown that for slow Brownian flocculation with high stability ratio, the diameter grows exponentially with time.[1] The time constants for aggregation were determined from the exponential model, which fit well for all complexes investigated (R^2 ranged from 0.85 – 0.98). The transfection efficiency of lyophilized polyplexes was investigated. Lyophilized poly(EG-b-(DMAEMA-co-BMA))-pDNA complexes with N/P ratios of 10 and 20 (except 50L) had significantly higher transfection than PEI (indicated with asterisks in Fig. 2, $p < 0.05$).

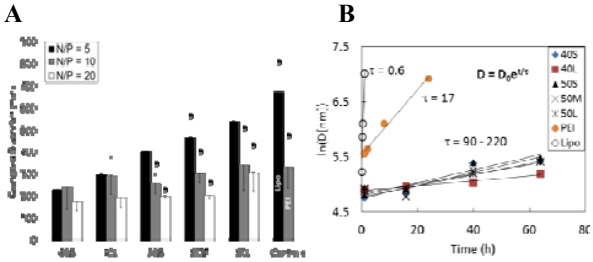

Figure 1. (A) Hydrodynamic diameter of lyophilized complexes. (B) Exponential model of aggregation for complexes with N/P ratio of 10 in PBS.

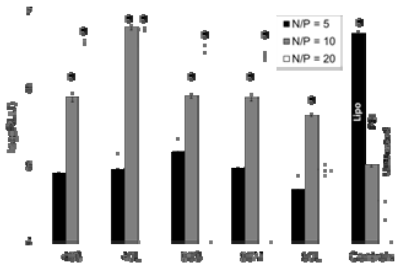

Figure 2. Relative luminescence signal from MDA-MB-231 cells transfected with lyophilized complexes.

Conclusions: Novel poly(EG-b-(DMAEMA-co-BMA))-pDNA nanoparticles were shown to be stable after lyophilization and incubation in PBS and efficiently transfect cells *in vitro*. Except for 50L, complexes with N/P ratios of 10 and 20 had smaller sizes after lyophilization than lipofectamine2000 and PEI and higher transfection efficiency than PEI. The time constants for aggregation determined from the exponential fits provide quantitative evidence for the increased stability of poly(EG-b-(DMAEMA-co-BMA)) complexes compared to lipofectamine2000 and PEI. Future work will involve incorporating lyophilized poly(EG-b-(DMAEMA-co-BMA))-pDNA nanoparticles into tissue engineering scaffolds for *in vivo* gene therapy applications.

Reference: 1. Lin MJ. Phys Rev A. 1990;41:2005-2020.