One-pot Synthesis of Functional Poly(amino ester sulfide)s and Utility in Delivering pDNA and siRNA

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Statement of Purpose: The development of efficacious carriers is an important long-standing challenge in gene therapy. In the past few decades, tremendous progress has been made toward non-viral vectors for gene delivery including cationic lipids and polymers. However, there continues to be a need for clinically translatable polymer-based delivery carriers because they offer tunable degradation profiles and functional groups, diverse structures/morphologies, and scalability in preparation.[1] Here, we developed a library of 144 degradable polymers with varying amine and hydrophobic content via a facile method that involves thiobutyrolactone aminolysis and consequent thiol-(meth)acrylate addition in one-pot (**Figure 1**). The polymer platform was evaluated for pDNA and siRNA delivery in vitro. Due to tunable functionality and scalable preparation, this synthetic approach may have broad applicability in the design of delivery materials for gene therapy.[2]

Methods: Polymer synthesis. The polymer library with varying amines and octylamine percentage was prepared at optimized conditions. The concentration of monomers was 0.5 M in all polymerizations. As a typical example, 17.1 mg (0.1 mmol) ATL, 1.1 equivalent dimethylamino-1-propylamine (DMP) (11.4 mg, 0.11 mmol) and 0.05 equivalent DPP (0.7 mg, 0.005 mmol) were added to 0.2 mL of DMSO, and the mixture was kept stirring at r.t. for 24 h. **pDNA delivery with functional poly(amino ester sulfide)s.** pDNA polyplexes were prepared at a polymer/pGFP ratio of 30:1 (wt/wt) in 10 mM phosphate buffer (pH 6.8) by vigorous pipette mixing. HeLa cells were incubated with polyplexes for 24 h, after which GFP fluorescence intensity was measured on a plate reader (Tecan Infinite M200 Pro). **siRNA delivery with functional poly(amino ester sulfide)s.** siRNA polyplexes were prepared at a polymer/siLuc ratio of 30:1 (wt/wt) in 10 mM citric acid/trisodium citrate buffer (pH 4.2) by vigorous mixing. HeLa cells stably expressing luciferase were incubated with polyplexes for 24 h, after which the cell viability and luciferase activity was analyzed using ONE-Glo + Tox assay kits (Promega).

Results: The one-pot polymerization method involving thiobutyrolactone aminolysis and consequent thiol-(meth)acrylate addition is an attractive route towards functional, degradable polymers.[3] We designed new monomers that contained both (meth)acrylate and thiolactone groups to produce polymers containing ester, urethane, and sulfide linkages in the polymer backbone. Hydrophobically modified 5S, 2E1, 6CY1, 5CY2, and 2M1 grafted HEMATL polymers are capable of delivering pDNA depending on the chemical composition and the size of the polyplexes (**Figure 2**). Binding assays and DLS measurements indicate that the polymer series with similar

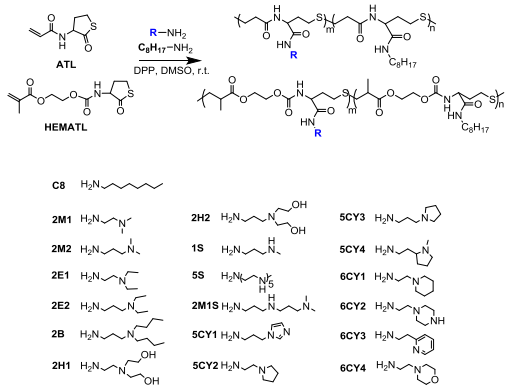

Figure 1. Functional poly(amino ester sulfide) polymerization via combination of thiolbutyrolactone aminolysis and thiol-(meth)acrylate addition.

chemical structure have identical binding efficacy but different polyplex sizes with pDNA which affects delivery. Small polyplexes (typically <300 nm in diameter) are favorable for delivery. But only small size is not sufficient for successful pDNA delivery; certain chemical composition (pKa and hydrophobicity) is also required.[4] In addition, hydrophobically modified 5S and 2B grafted HEMATL and 5S grafted ATL polymers exhibit capability for siRNA delivery that matches the efficacy of commercially available transfection reagents (RNAiMax).

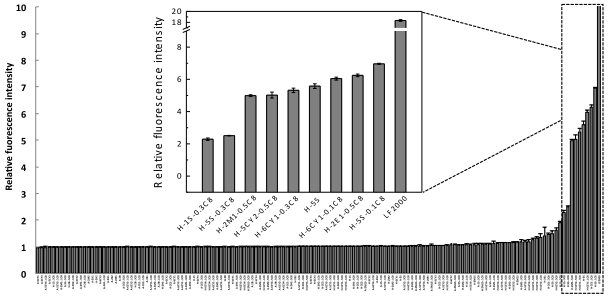

Figure 2. Relative fluorescence intensity after treatment with pDNA (GFP)-polymer polyplexes for 24 h. LF2000 was used as a positive control for pDNA delivery. The fluorescence intensity was normalized to untreated cells.

Conclusions: 144 poly(amino ester sulfide)s were synthesized by the one-pot combination of thiolbutyrolactone aminolysis and the consequent thiol-(meth)acrylate addition at room temperature. Due to tunable functionality and scalable preparation, this synthetic approach may be useful in the design of biomaterials for a wide range of applications.

References: 1) Tian HY et al., Jing XB. Prog Polym Sci **2012**, 37, 237. 2) Yan, Y et al., Siegwart DJ. In revision. 3) Espeel P et al., Du Prez FE. Polym Chem **2013**, 4, 2449. 4) Siegwart, DJ et al. PNAS **2011**; 108, 12996.