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One-pot synthesis of functional poly(amino ester sulfide)s and utility in delivering pDNA and siRNA

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ABSTRACT

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. Herein, 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 or acrylamide addition in one-pot. The polymer platform was evaluated for pDNA and siRNA delivery to HeLa cells *in vitro*. 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. Hydrophobically modified 5S and 2B grafted HEMATL and 5S grafted ATL polymers exhibit capability for siRNA delivery that approaches the efficacy of commercially available transfection reagents. Due to tunable functionality and scalable preparation, this synthetic approach may have broad applicability in the design of delivery materials for gene therapy.

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1. Introduction

Gene therapy has shown great potential for the treatment of a wide range of serious acquired and inherited diseases during the past three decades [1–3]. Polymer-based carriers are an essential component of this treatment strategy. Between 1990 and 2013, numerous gene therapy clinical trials commenced worldwide [4,5], of which >60% were related to cancer therapy. The first commercial gene therapy product was approved for the treatment of squamous cell carcinoma in China in 2003 [6]. The delivery of pDNA encoding for functional proteins to replace mutated or down-regulated genes remains a promising strategy to treat a variety of diseases [7]. More recently, delivery of shorter nucleic acids that invoke the RNA Interference (RNAi) pathway has been shown to be a powerful way to regulate gene expression post-transcriptionally. In particular, strategies that silence oncogenes using synthetic short interfering RNA (siRNA) or restore endogenous microRNA (miRNA) that function as tumor suppressors, represent next generation therapeutic approaches to treat cancer [8].

One of the practical challenges for both classical gene therapy and RNAi therapeutics is to efficiently deliver DNA or RNA into cells. A number of difficult barriers must be overcome to facilitate effective delivery. These barriers include protection from degradation, localization to the diseased tissue or tumor, cellular uptake into targeted cells, and intracellular release [9–12]. Viral vectors are established carriers for gene delivery because of their high efficiency [13]. However, safety concerns and production costs have restricted their utility. Non-viral vehicles for gene delivery have attracted much attention because of reduced immune response, low cost, and highly tunable diversity in structure [13–15]. Typically, cationic materials are used to bind negatively charged nucleic acids and facilitate cellular uptake. Cationic lipids represent one of the most extensively investigated non-viral vectors [16–18], and are commercially available for use as *in vitro* transfection reagents (e.g. Lipofectamine 2000 and RNAiMax). Cationic lipid nanoparticles have been used in human clinical trials [19]. The other representative non-viral delivery carrier, cationic polymers, have attracted increasing attention because of the flexibility in their

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synthesis and structural modifications, as well as the relatively higher stability of polyplexes (spherical complex of nucleic acids and cationic polymers) [15,20,21].

Some commonly used cationic polymers for non-viral gene delivery, such as poly(ethylene imine) (PEI), can generate high cytotoxicity because polymers with strong positive charges can induce hemolysis, apoptosis, or autophagy [22,23]. Therefore, more biocompatible and biodegradable cationic polymers have been prepared for gene delivery in the past two decades. A large number of carriers have been synthesized and summarized in various reviews [14,24–31]. In the context of research reported herein, we were particularly interested in degradable cationic polymers for gene delivery. For example, (oligo)PEI or poly((2-dimethylamino) ethyl methacrylate) (PDMAEMA) was grafted to degradable polymer backbones (e.g. poly(carbonate)s and poly(caprolactone)s) to utilize the pH buffering amino groups on PEI and PDMAEMA and increase the biocompatibility of the copolymers [32–34]. Polyesters, including poly(amino ester)s have been extensively studied as degradable polymer carriers for gene delivery [20,35–45]. Polyamides, e.g. poly(ketal amidoamine) or polypeptides [46–50], polycarbonate-based polymers [25,51–53], polyphosphoesters [54,55], and bio-reducible poly(disulfide)s [33,56–60] were also reported as gene delivery materials with varying physicochemical properties and distinct preparation procedures. However, classical synthesis of amino functional polyesters, polycarbonates, or polyamides sometimes involves multi-step protection/deprotection and/or ring-closure [25,61,62]. It has been shown that the development of combinatorial libraries of polymers is an effective way to discover efficacious nucleic acid carriers [63]. Because stringent polymerization conditions can limit the diversity of functional group incorporation, we aimed to synthesize a clinically-translatable platform with high structural diversity using a one-pot method.

In this report, we synthesized a poly(amino ester sulfide) library of 144 polymers with varying pKa and hydrophobic content using a one-pot method involving the reaction of amines with thiobutyrolactone (meth)acrylates or acrylamides. We were inspired by a recent report on an amine-thiol-ene polymerization strategy by Du Prez and coworkers [64], and decided to use a similar polymerization method to construct a polymer library. In this way, the resulting polymer library possesses a degradable backbone, diverse amine structures (modular apparent pKa), tunable hydrophobicity, and scalable preparation. *In vitro* screening of these polymers for pDNA and siRNA delivery to HeLa cells was performed to identify efficacious carriers. The binding of pDNA/siRNA with hit polymers and the size of the resulting polyplexes were comprehensively investigated in this paper to understand structure–function relationships. Due to tunable functionality and scalable preparation in one pot, this approach is a useful strategy to prepare polymer libraries for nucleic acid delivery.

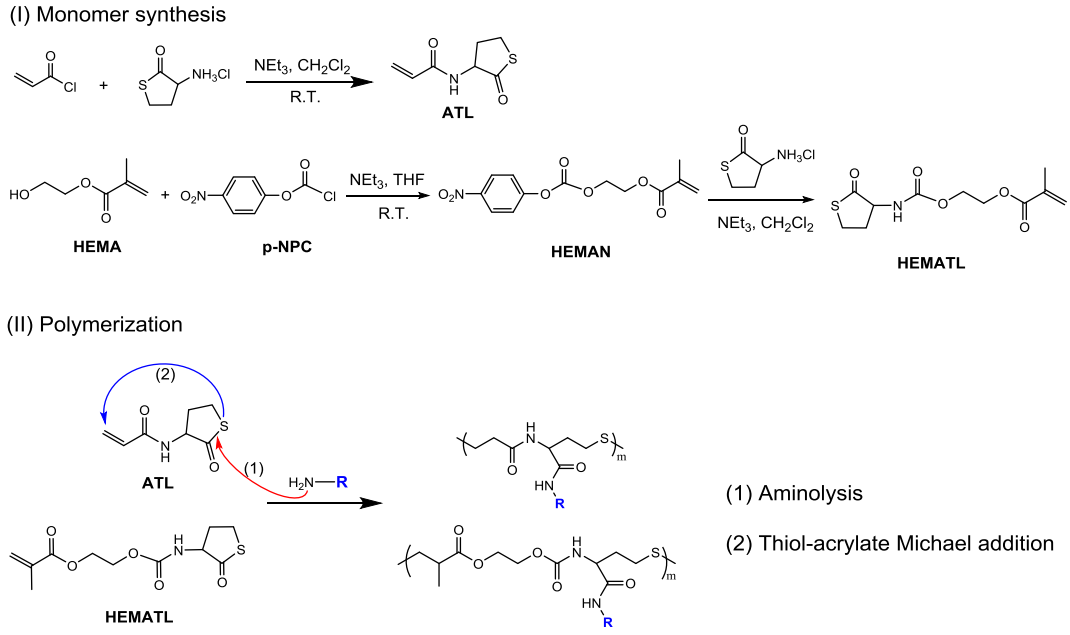
2. Experimental

2.1. Materials

DL-Homocysteine thiolactone hydrochloride (CTH), acryloyl chloride, methacryloyl chloride, 2-hydroxyethyl methacrylate (HEMA), 4-nitrophenyl chloroformate (p-NPC), dimethylphenylphosphine (DPP) and all amines were purchased from Sigma–Aldrich and used as received. All organic solvents were purchased from Fisher Scientific and purified with a solvent purification system (Innovative Technology). Milli-Q water was used throughout the experiments. siRNA against luciferase (sense strand: 5′-GAUUAUGUCCGGUUAUGUA[dT][dT]-3′; anti-sense strand: 3′-UACAUAACCGGACAUAAUC[dT][dT]-5′), Dulbecco's Modified Eagle Media (DMEM), and fetal bovine serum (FBS) were purchased from Sigma–Aldrich. gWiz pDNA (GFP) was purchased from Aldevron. OptiMEM was purchased from Life Technologies. Lipofectamine 2000 (LF2000) and RNAiMax was purchased from Invitrogen and used following the supplier's recommended protocols.

2.2. Methods

The molecular weight of polymers was measured by Gel Permeation Chromatography (GPC) (Viscotek) equipped with RI detection and ViscoGEL I-series columns (Viscotek I-MBLMW-



Scheme 1. Synthesis of monomers ATL and HEMATL and preparation of functional poly(amino ester sulfide)s via combination of thiolbutyrolactone aminolysis and thiol-(meth) acrylate addition.