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Influence of molecular weight upon mannosylated bio-synthetic hybrids for targeted antigen presenting cell gene delivery

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ABSTRACT

Given the rise of antibiotic resistant microbes, genetic vaccination is a promising prophylactic strategy that enables rapid design and manufacture. Facilitating this process is the choice of vector, which is often situationally-specific and limited in engineering capacity. Furthermore, these shortcomings are usually tied to an incomplete understanding of the structure–function relationships driving vector-mediated gene delivery. Building upon our initial report of a hybrid bacterial-biomaterial gene delivery vector, a comprehensive structure–function assessment was completed using a class of mannosylated poly(beta-amino esters). Through a top-down screening methodology, an ideal polymer was selected on the basis of gene delivery efficacy and then used for the synthesis of a stratified molecular weight polymer library. By eliminating contributions of polymer chemical background, we were able to complete an in-depth assessment of gene delivery as a function of (1) polymer molecular weight, (2) relative mannose content, (3) polymer-membrane biophysical properties, (4) APC uptake specificity, and (5) serum inhibition. In summary, the flexibility and potential of the hybrid design featured in this work highlights the ability to systematically probe vector-associated properties for the development of translational gene delivery candidates.

1. Introduction

The emergence of multidrug resistant pathogens highlights the inadequacies of current therapeutic options, thus prompting a shift from the use of active treatment regimens towards prophylactic alternatives. Of these, genetic vaccines are ideal candidates, as this vaccine variation offers the most flexibility in terms of antigen design, production, and storage potential [1]. Furthermore, genetic vaccines are predicated on the controlled introduction of genetic material into immune effector cells for the eventual induction of an adaptive immune response [2]. Driving this process is the choice of delivery vector that, unfortunately, is often associated with situationally-specific properties that do not permit ubiquitous usage. Accordingly, to address these and other concerns, we previously developed a hybrid bio-synthetic gene delivery vector that combined the vector-specific advantages and toolsets of bacteria and cationic polymers [3]. These normally separate vectors were also selected, in part, due to their independent records for stimulating adaptive immune responses [4–6].

The hybrid vector utilized the combination of an engineered *Escherichia coli* (*E. coli*) strain and a class of biodegradable cationic polymers, poly(beta-amino esters) (PBAEs). The polymer component was then further modified by the terminal conjugation of mannose to improve antigen presenting cell (APC) uptake specificity through CD206 stimulation. Although preliminary results indicated improved APC uptake and gene delivery, the analysis of a single polymer did not permit a comprehensive investigation of structure–function relationships or the effect of mannose inclusion. Thus, in the current study a library of structurally unique mannosylated PBAEs (PBAE-Mans) were prepared and analyzed for their potential use as a hybrid constituent. Upon identification of an optimal polymer, this specific polymer chemical background was further modified to elucidate the dependency of hybrid gene delivery improvements upon molecular weight and relative mannose content (independent of chemical composition). The results provide the first assessment of structure–function

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relationships for the development of next-generation hybrid biosynthetic gene delivery vectors.

2. Materials and methods

2.1. Materials

Monomers were purchased from Sigma–Aldrich (St. Louis, MO). Acetone (HPLC), DMF (HPLC), and DMSO (≥99.7%), and cell culture components were purchased from Fisher Scientific (Pittsburgh, PA). Allyl alcohol (≥99%), D-(+)-mannose (cell culture grade) and 4-toluenesulfonyl chloride (p-TsCl) were purchased from Sigma–Aldrich. Phosphate buffered saline (PBS) and 3 M sodium acetate were purchased from Life Technologies (Grand Island, NY).

2.2. Cell lines, strains, and plasmids

A murine RAW264.7 macrophage cell line kindly provided by Dr. Terry Connell (Department of Microbiology and Immunology, University at Buffalo, SUNY) was used for gene delivery assays. The cell line was maintained in medium prepared as follows: 50 mL of fetal bovine serum (heat inactivated), 5 mL of 100 mM MEM sodium pyruvate, 5 mL of 1 M HEPES buffer, 5 mL of penicillin/streptomycin solution, and 1.25 g of D-(+)-glucose were added to 500 mL phenol red-containing RPMI-1640 and filter sterilized. Cells were housed in T75 flasks and cultured at 37 °C/5% CO_2.

YWT7-*hly* (BL21[DE3] background strain containing a chromosomally located copy of listeriolysin O [*hly*]) was selected as the bacterial vector for all gene delivery experiments [3,7–9]. A luciferase reporter plasmid driven by a cytomegalovirus promoter (pCMV-Luc; Elim Biopharmaceuticals) was utilized during positive control transfection experiments and introduced to YWT7-*hly* via electroporation prior to bacterial or hybrid vector transfections. To assess bacterial membrane shearing (to be described below), a plasmid expressing blue fluorescent protein (BFP) (pB18cmBFPAzurite [10]) was transformed into YWT7-*hly* using electro-transformation.

2.3. Mannosylated poly(beta-amino ester) synthesis

Polymers were synthesized using a previously developed three-step reaction [3,4]. Briefly, diacrylate-capped polymers were first synthesized in DMSO at various diacrylate/amine molar ratios (D:A ratio) for 5 days at 60 °C. The initial polymer library was synthesized using a 1.2 D:A ratio (Table 1); whereas, the expanded polymer set utilized a wider range (Table 2). Variance of molar ratios in the expanded polymer set permitted tunable control of the base polymer molecular weight (Table 2). All acrylate-terminated polymers were then reacted with excess ethylenediamine to amine-cap the terminal ends. Specifically, acrylate-terminated polymers were dissolved in DMSO at 167 mg/mL and reacted with 5 M ethylenediamine (in DMSO) at room temperature for 24 h. Amine-capped polymers were purified by dialysis followed by evaporation under vacuum. Dialysis procedures were conducted against acetone using molecular porous membrane tubing (Spectra/Por Dialysis Membrane, Spectrum Laboratories Inc.) with an approximate molecular weight cut off at 3500 Da. As a last step, amine-capped PBAEs were then reacted with allyl-α-D-mannopyranoside (ADM) at a 1:2 M ratio in DMSO at 90 °C for 24 h and then purified via dialysis. Structure and purity of polymers were confirmed using ^{1}H NMR spectroscopy (reported elsewhere [4]).

ADM was synthesized by dissolving 3 g of D-(+)-mannose and 18 mg p-TsCl in allyl alcohol (20 mL) at 90 °C under reflux for 24 h. The reaction solution was then concentrated by vacuum distillation at 35 °C.

Table 1
Polymer synthesis and characterization summary of base polymers.

Polymer	PDI^{GPC}	M_n^{GPC} (Da)	M_w^{GPC} (Da)	Zeta potential (mV) PBS (pH 7.4)	Zeta potential (mV) NaOAc (pH 5.15)
D3mA4	1.342	7839	10,520	−19.4	28.8
D3mA4-Man	1.696	6996	11,863	−6.4	20.1
D3A5	1.418	8214	11,643	−18.6	37.5
D3A5-Man	1.640	5083	8337	−4.1	31.7
D3mA5	1.206	8848	10,667	−19.9	33.1
D3mA5-Man	1.574	8128	12,794	−12.6	28.7
D4A3	1.405	9617	13,511	−12.5	34.5
D4A3-Man	1.680	4403	7397	−9.9	28.6
D4A4	1.629	8571	13,961	−19.5	25.9
D4A4-Man	1.377	4967	6839	−12.4	20.6
D4A5	1.453	8520	12,380	−16.7	28.5
D4A5-Man	1.542	4762	7345	−11.2	34.2
D5A4	1.377	8927	12,294	−12.7	21.2
D5A4-Man	1.622	8701	14,109	−8.4	26.3
D5A5	1.319	9577	12,632	−26.0	31.1
D5A5-Man	1.501	7040	10,567	−15.5	25.5
D6A5	1.472	8286	12,198	−21.8	34.2
D6A5-Man	1.664	7517	12,508	−18.4	33.4

2.4. Preparation of hybrid vectors

Bacterial and hybrid vectors were prepared from bacterial cultures inoculated at 2% (v/v) from overnight starter cultures. Plasmid selection antibiotics were used as needed during bacterial culture within lysogeny broth (LB) medium. Following incubation at 36 °C and 250 rpm until 0.4 to 0.5 OD_{600}, samples were induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 30 °C for 1 h. Bacterial vectors were then washed once and standardized to 0.5 OD_{600} in PBS; whereas, bacterial strains to be used in hybrid vector formation were washed once and standardized to 1.0 OD_{600} in 25 mM NaOAc (pH 5.15). Polymer doses dissolved in chloroform were desiccated and resuspended in 25 mM NaOAc (pH 5.15) prior to equal volume addition to 1.0 OD_{600} bacterial strains. Hybrid vectors (final 0.5 OD_{600}) and bacterial vectors in PBS were allowed to incubate at 22 °C for 15 min.

2.5. Hybrid vector characterization

Zeta potential of bacterial, polymer, and hybrid vectors was measured by dynamic light scattering (DLS). To measure surface

Table 2
Synthesis and characterization summary of molecular weight variants of D4A4-Man.

Polymer designation	D4:A4 feed ratio	PDI^{GPC}	M_n^{GPC} (Da)	M_w^{GPC} (Da)	Zeta potential (mV) PBS (pH 7.4)	Zeta potential (mV) NaOAc (pH 5.15)
P1	1.025	2.477	13,623	33,745	−8.6	32.6
P2	1.0375	1.641	13,425	22,031	−5.3	34.0
P3	1.05	1.536	11,961	18,372	−6.2	33.6
P4	1.0625	1.370	11,848	16,232	−8.3	32.4
P5	1.075	1.629	10,474	17,063	−7.7	32.7
P6	1.1	1.642	10,130	16,633	−8.8	34.6
P7	1.125	1.833	7902	14,484	−8.0	31.9
P8	1.15	1.468	7312	10,734	−11.1	27.5
P9	1.175	1.699	6589	11,195	−12.1	28.6
P10	1.2	1.549	5966	9241	−11.2	24.9
P11	1.225	1.590	5510	8761	−11.4	26.4
P12	1.25	1.600	5112	8180	−12.7	24.0
P13	1.275	1.638	4673	7654	−12.9	20.9
P14	1.3	1.615	4479	7234	−11.2	21.4