

Poly(Beta-Amino Ester)s as High-Yield Transfection Reagents for Recombinant Protein Production

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Purpose: Transient transfection is an essential tool for recombinant protein production, as it allows rapid screening for expression without stable integration of genetic material into a target cell genome. Poly(ethylenimine) (PEI) is the current gold standard for transient gene transfer, but transfection efficiency and the resulting protein yield are limited by the polymer's toxicity. This study investigated the use of a class of cationic polymers, poly(beta-amino ester)s (PBAEs), as reagents for transient transfection in comparison to linear 25 kDa PEI, a commonly used transfection reagent.

Methods: Transfection efficiency and protein production were assessed in human embryonic kidney 293F (HEK) and Chinese hamster ovary-S (CHO) cell suspensions using PBAE-based nanoparticles in comparison to linear 25 kDa PEI. Production of both a cytosolic reporter and secreted antibodies was investigated.

Results: In both HEK and CHO cells, several PBAEs demonstrated superior transfection efficiency and enhanced production of a cytosolic reporter compared to linear 25 kDa PEI. This result extended to secreted proteins, as a model PBAE increased the production of 3 different secreted antibodies compared to linear 25 kDa PEI at culture scales ranging from 20 to 2000 mL. In particular, non-viral gene transfer using the lead PBAE/plasmid DNA nanoparticle formulation led to robust transfection of mammalian cells across different constructs, doses, volumes, and cell types.

Conclusion: These results show that PBAEs enhance transfection efficiency and increase protein yield compared to a widespread commercially available reagent, making them attractive candidates as reagents for use in recombinant protein production.

Keywords: non-viral gene transfer, transient transfection, mammalian cell expression systems, DNA delivery

Introduction

Current research into chemical-based transfection methods focuses largely on optimizing agents for use in the development and production of recombinant proteins. Transient transfection, in which introduced genetic material is not incorporated into the host genome, is especially useful during the high-throughput design and screening of proteins (eg, candidate biologics) wherein stable expression is not needed. While culture conditions and plasmid design have been popular targets for optimization in transient transfection workflows,^{1,2} many proteins remain difficult to express via transient transfection, and thus development of improved transfection reagents is needed to boost protein yields for applications in biomedical research and pharmaceutical design. In particular, discovery and development of next-generation antibody fusion proteins and multispecific antibodies are often limited by protein production challenges.^{3,4} Chemical-based transient transfection relies on condensation and either encapsulation or complexation of plasmid DNA by a biocompatible material into particles, which are taken up by target cells; differences in particle size can affect the method of cellular uptake, leading to differences in transfection efficiency.⁵ Particles must then escape

the endosome and the encapsulating material must degrade to allow for DNA release, nuclear translocation, transcription and subsequent export, and finally translation and processing into fully formed protein.⁶

Transfection reagent structure and buffering capacity have been demonstrated to influence DNA uptake and escape, making these properties particularly consequential in reagents for transient transfection workflows.⁷ Maximizing protonability, for example, facilitates endosomal swelling and consequent rupture via the “proton sponge” effect.^{8,9} Cationic polymers have typically been among the most promising transfection reagents; their charge-based association with DNA into particles offers protection from degradation and offers sufficient buffering capacity to facilitate endosomal escape following cellular uptake.⁷

Poly(ethyleneimine) (PEI) is a commercially available cationic polymer used extensively as a transfection reagent that has a high density of protonable amines, giving rise to high buffering capacity and efficient endosomal escape.^{8,10} PEI is available in both linear and branched forms, each of which is produced at various molecular weights, allowing for optimization of the production of individual protein constructs. PEI of average molecular weight 25 kDa is frequently used in transfection workflows, but its toxicity limits transfection efficiency and, consequently, protein yield.^{11,12} Previously, PEI has been conjugated to polyethylene glycol¹³ and arginine modified oligo-(alkylaminosiloxane) [P(SiDAAr)n]¹⁴ to mitigate cytotoxicity.

An alternative to PEI, poly(beta-amino ester)s (PBAEs), are a class of cationic polymers used to facilitate efficient gene transfer in vitro.¹⁵ PBAEs are composed of an acrylate backbone monomer, an amine sidechain, and a terminal end-capping group, each of which can be varied to create a vast library of materials.¹⁶ Hydrolyzable ester linkages allow for degradation of the PBAEs in transfection conditions, which allow for use of the polymers at high weight ratios relative to other non-biodegradable materials, maximizing density of buffering amines to facilitate endosomal escape.⁷ Their biodegradability also obviates the need for medium replacements or additions, themselves contributors to cell death, which are common where PEI is utilized.¹ Moreover, these linear polymers are synthesized from inexpensive, commercially available reagents using a two-step polymerization method (Figure S1A) and are stable long term when stored dry at -20°C .¹⁷

Given the high transfection efficacy observed with PBAEs in various in vitro contexts, we sought to investigate the use of PBAE nanoparticles for transient transfection of suspension cultures for the production of cytosolic and secreted proteins (Figure 1). We chose linear 25 kDa linear to compare to PBAEs, based on the common use of this form of PEI in protein production workflows.^{2,18–20} Whereas previous work with PBAEs has focused on DNA introduction into cells for therapeutic applications, this work instead focuses on use of PBAEs for transient transfection of cells in suspension at different scales to enhance protein production workflows.

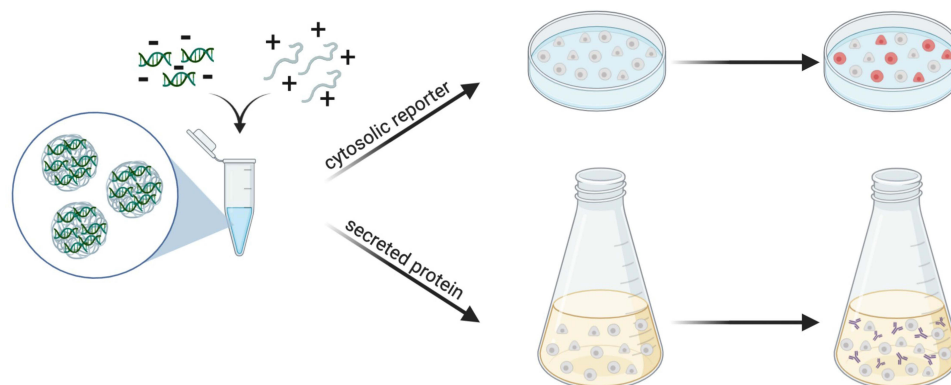


Figure 1 Schematic of transient transfection workflow for cytosolic and secreted proteins. Plasmid DNA and polymer were combined to allow for nanoparticle self-assembly. Cells were transfected with plasmid DNA encoding a fluorescent reporter or a secreted protein. Created with BioRender.com.

Materials and Methods

Tissue Culture

HEK 293F (HEK) cells (Thermo Fisher Scientific) were cultured in FreeStyle 293 Expression Medium (Thermo Fisher Scientific) supplemented with penicillin-streptomycin (0.2 U/mL) (Thermo Fisher Scientific). CHO-S cells (Thermo Fisher Scientific) were cultured in FreeStyle CHO Expression Medium (Thermo Fisher Scientific) supplemented with L-glutamine (8 mM, Thermo Fisher Scientific) and penicillin-streptomycin (0.2 U/mL). All cells were grown at 37°C and 5% CO₂ with rotation at 125 rpm in a humidified atmosphere. Transfections were incubated in the same growth conditions. HEK cells were not subject to more than 25 passages, whereas CHO cells were studied below 10 passages to mitigate cell clumping. All cells were grown to a density of 1.2×10^6 cells/mL and diluted to 1.0×10^6 cells/mL on the day of transfection.

PBAE Synthesis

1,4-Butanediol diacrylate (B4), 3-amino-1-propanol (S3), 5-amino-1-pentanol (S5), 1-(3-Aminopropyl)-4-methylpiperazine (E7) (Alfa Aesar), 2-(3-Aminopropylamino)ethanol (E6), 1-(2-Aminoethyl)piperazine (E39) (Sigma Aldrich), 1,5-Pentanediol diacrylate (B5) (SantaCruz Biotechnology), and 4-amino-1-butanol (S4) (Fisher Scientific) were purchased and stored as directed. Base monomer (B4 or B5) was mixed with sidechain monomer (S3, S4, or S5) at a 1.08:1 ratio, and the reaction was allowed to proceed for 24 h with stirring at 85°C. The neat acrylate terminated B-S polymer was then resuspended in tetrahydrofuran (THF, final concentration 200 mg/mL) and combined with 0.5M end-capping monomer (E6, E7, or E39) for 2 h with stirring at room temperature (see [Table S1](#)). The resulting polymer was precipitated twice in a 10X volume of diethyl ether and dried under vacuum for 48 h, then resuspended to 100 mg/mL in DMSO and stored at -20°C in Silica gel orange desiccant (Fisher Scientific). Yields of synthesized polymers following precipitation and drying range from 90% to 95%.

Nanoparticle Formulation and Characterization

PBAE and plasmid DNA were each dissolved in sterile 25 mM magnesium acetate buffer, pH 5.2 (4 µg DNA and 240 µg 4-4-6 in a total volume of 80 µL for HEK cells; 8 µg DNA and 480 µg 4-4-6 in a total volume of 80 µL for CHO cells) then combined by vigorous pipetting or vortexing to yield a weight ratio of 60:1 PBAE:DNA and incubated at room temperature for 15 minutes to allow for nanoparticle self-assembly. Linear PEI of average molecular weight 25 kDa (Polysciences) was prepared according to manufacturer's instructions and stored at -80°C. To prepare particles, PEI was thawed, diluted in OptiPRO SFM (4 µg DNA and 8 µg PEI in a total volume of 80 µL for HEK cells; 8 µg DNA and 24 µg PEI in a total volume of 80 µL for CHO cells), and incubated for 15 minutes at room temperature before being combined with plasmid DNA, also diluted in OptiPRO SFM, for resulting weight ratios of 2:1 PEI:DNA for HEK cell transfections or 3:1 PEI:DNA for CHO cells. The PEI:DNA mixtures were then incubated at room temperature for 15 minutes to allow for nanoparticle self-assembly. To characterize the resultant particles, 1:20 dilutions were prepared in FreeStyle 293 Expression Medium (HEK cells) or FreeStyle CHO Expression Medium (CHO cells). Size, as measured by hydrodynamic radius determined via dynamic light scattering, was measured using a Malvern Zetasizer Pro (Malvern Panalytical). Surface charge, as measured by Zeta potential, was determined via electrophoretic light scattering (Malvern Zetasizer Pro), using 50 µL of particles formulated in 25mM magnesium acetate buffer diluted into 450 µL of either 10% PBS diluted in water, FreeStyle 293 Expression Medium, or FreeStyle CHO Expression Medium. Measurements were reported as mean values from n = 3 independently prepared replicates. To determine the molecular weight of the PBAEs, polymers were purified in ether as described above, resuspended in tetrahydrofuran, and subjected to gel permeation chromatography (GPC) analysis relative to polystyrene standards using a refractive index detector (Agilent). To confirm structures, polymers were purified in ether as described above and 10mg PBAE was resuspended in 700 µL DMSO-d₆. ¹H nuclear magnetic resonance (NMR) was run on a Bruker 500 MHz NMR followed by analysis in TopSpin4.1.4 (Bruker).

Cytosolic mCherry Protein Expression and Transfection Efficiency

4-4-6 and PEI nanoparticles delivering the CAG-mCherry plasmid (Addgene #108685²¹) were formed as described above and added to HEK or CHO cells diluted for transfection (2 mL culture at 1.0×10^6 cells/mL in a 6-well plate).

Optimized DNA concentrations and weight ratios were determined based on mean fluorescence over 5 days (580 ± 20 nm excitation; 630 ± 20 nm emission) using a BioTek SynergyMX plate reader (BioTek Instruments, Inc.) by reading a 3×3 grid distributed across the well surface area. For transfection efficacy studies, HEK or CHO cells were dosed with PBAE or PEI nanoparticles formed as described above, delivering 2 μ g/mL (HEK) or 4 μ g/mL (CHO) mCherry DNA ($n = 5$). Cells were collected on day 5, and CHO cells were treated with Accutase (Sigma Aldrich), according to manufacturer's instructions. Cells were then resuspended in phosphate-buffered saline (PBS, pH 7.4) containing 2% fetal bovine serum (FBS) and stained with LIVE/DEAD™ Fixable Green Dead Cell Stain Kit (Invitrogen), according to manufacturer's instructions. mCherry expression in live cells was assessed using an Attune NxT Flow Cytometer (ThermoFisher Scientific). Experiments were performed a minimum of twice with consistent results.

Cellular Viability

Viability was assessed via MTS CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega) 24 h and 5 d following transfection, according to the manufacturer's instructions. PBAE and PEI-treated conditions were normalized to an untreated control sample. Experiments were performed with $n = 5$ and conducted twice with similar results.

Fluorescence Microscopy

10 $\times$ images were acquired with a Zeiss Axio Observer with Axiovision 5 software (Zeiss United States), and composite images were generated with ImageJ.

Secreted Protein Expression and Purification

The 10H2 and BS2 antibodies²² were expressed via transient co-transfection of gWiz plasmids (Genlantis) encoding their respective heavy and light chains (1:1 ratio) in HEK cells (1 μ g DNA/mL culture). The 602 antibody²³ was expressed in CHO cells (4 μ g DNA/mL culture). The optimized DNA concentration for transfection was determined by dose titration in small-scale transfections by adding 4-4-6- and PEI-based nanoparticles (formulated as described above) to 2 mL cells in 6-well plate format. Cells were incubated as described above for 96 to 120 h. Cell supernatants were then incubated with Protein G agarose beads (Thermo Fisher Scientific) for 3 hours at room temperature. Beads were washed, and bound protein was eluted using 0.1M glycine, pH 2, and measured by SDS-PAGE analysis. Band quantification was performed using ImageJ software. For larger scale (>2 mL) transfections, 4-4-6- and PEI-based nanoparticles were formed as described in [Tables S2](#) and [S3](#), added to cells in a shaking flask, and incubated as described above for 96 to 120 h. Cell supernatants were then incubated with Protein G agarose beads either at room temperature for 3 hours or at 4°C overnight. Secreted protein was harvested from cell supernatant by Protein G affinity chromatography, and 10H2 and BS2 samples were further purified using a Superdex 200 sizing column equilibrated in PBS on a fast protein liquid chromatography (FPLC) instrument (Cytiva). Yield was quantified by measuring absorbance at 280 nm using a Nanodrop spectrophotometer (Thermo Fisher Scientific).

Statistics

Statistical analysis was performed in Prism 8 (GraphPad). Differences between multiple groups were determined via one-way ANOVA with Dunnett post-test comparing all treatment groups to PEI treatment. Differences between two groups were calculated using Student's *t* test. Significance designated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Results

PBAE Nanoparticle Characterization

We selected four PBAEs with varying backbone (B), sidechain (S), and end-cap (E) structures to evaluate in comparison with linear 25 kDa PEI (henceforth denoted PEI): B4-S4-E6 (4-4-6); B4-S5-E7 (4-5-7); B4-S5-E39 (4-5-39); and B5-S3-E6 (5-3-6) ([Figure 2A](#) and [B](#), [Figure S1B](#)). Physiochemical characterization of PBAE and PEI nanoparticles in serum-free transfection media indicated that PBAE nanoparticles maintained a smaller size in transfection conditions (approximately 200–350 nm), whereas PEI nanoparticles were prone to aggregation, resulting in sizes over 1 μ m ([Figure 2C](#),

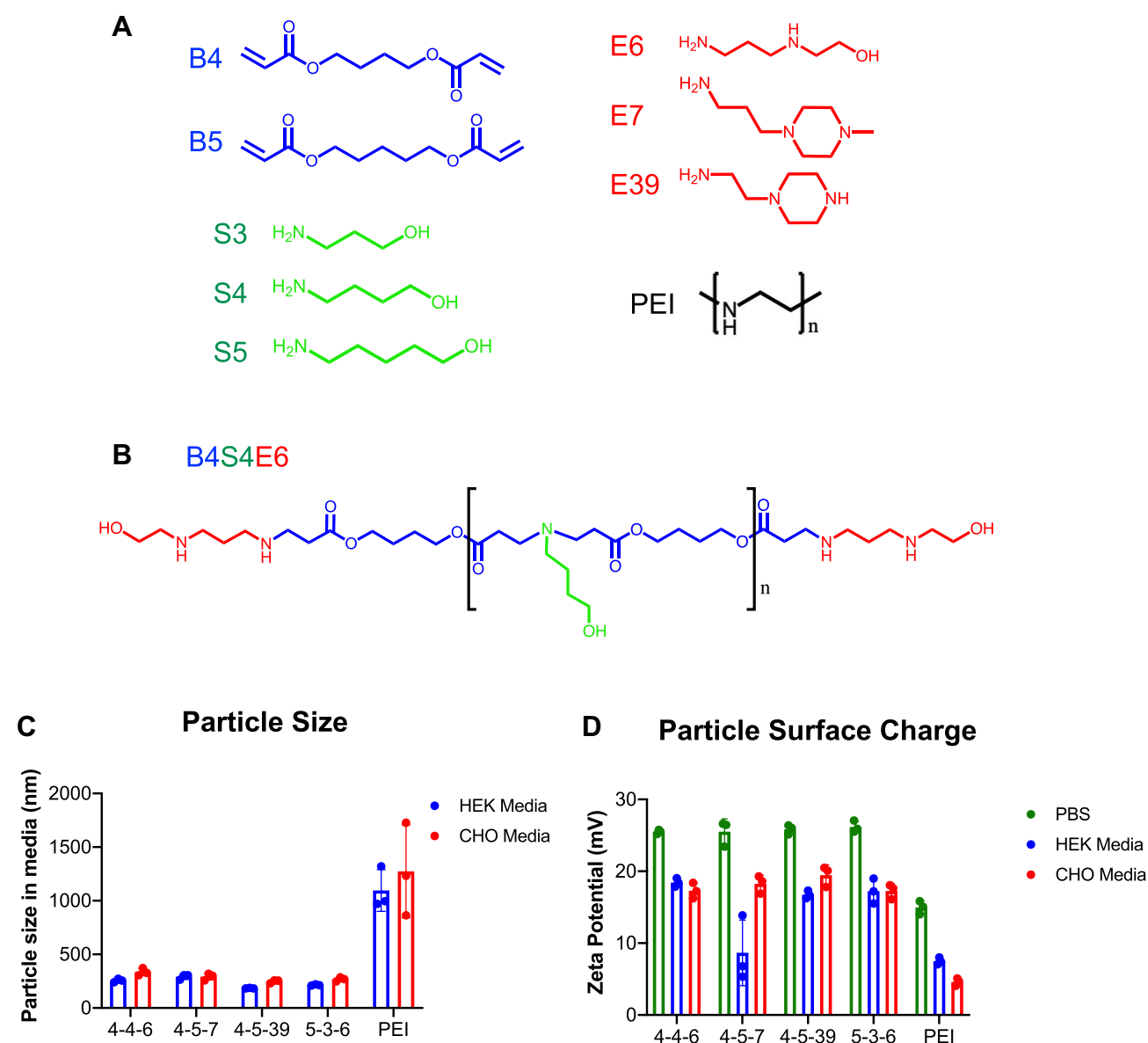


Figure 2 Nanoparticle structures and characterization. **(A)** PBAE and PEI monomer structures. Backbone monomers B4 and B5, side chain monomers S3-S5, endcap monomers E6, E7, and E39 used to synthesize PBAE polymers, and PEI 25 kDa. **(B)** Structure of B4S4E6 (4-4-6) polymer. Additional polymer structures are shown in [Figure S1](#). **(C)** Nanoparticle size determined via dynamic light scattering (DLS) in HEK media (blue) or CHO media (red). **(D)** Zeta potential of nanoparticles in PBS (green), HEK media (blue), or CHO media (red). Error bars represent standard deviation (SD).

[Figures S1C](#) and [S2A–E](#)). Analysis of surface charge in PBS, HEK, and CHO media revealed that PBAE nanoparticles have a higher zeta potential in PBS compared to PEI, and exhibit less charge shielding in transfection conditions ([Figure 2D](#), [Figure S1C](#)).

Optimization and Comparison of PEI- and PBAE-Mediated Transfection Using a Cytosolic Reporter

To determine the optimal DNA dose for the production of cytosolic mCherry using various polymer-based transfection agents, we selected a representative PBAE, 2-(3-aminopropylamino)ethanol end-capped poly(1,4-butanediol diacrylate-co-4-amino-1-butanol) (referred to here as 4-4-6) and compared this PBAE to 25 kDa PEI at doses ranging from 0.5 to 4 $\mu\text{g/mL}$ DNA. The polymers were compared in two mammalian cell lines frequently employed for protein expression: human embryonic kidney 293F (HEK) cells and Chinese hamster ovary-S (CHO) cells. Evaluation of mCherry fluorescence over a span of 5 days

indicated that peak mCherry expression occurred using the 4-4-6 polymer at 2 $\mu\text{g/mL}$ and 4 $\mu\text{g/mL}$ DNA doses in HEK and CHO cells, respectively (Figure S3A). Screening various DNA:polymer w/w ratios for both 4-4-6 and PEI revealed that the optimal ratios in HEK cells were 60:1 and 2:1, respectively (Figure S3B). DNA:polymer w/w ratio titration in CHO cells identified the optimal PEI:DNA ratio to be 3:1 (S6E-F). This represents an N:P ratio of ~ 68 for 4-4-6 and N:P ratios of ~ 15 (HEK) and ~ 23 (CHO) for PEI. Notably, peak mCherry expression in PEI-based transfections was not comparable to that attained by 4-4-6 at any dose or polymer:DNA w/w ratio (Figure S3A and B). Subsequent time course studies using the optimized DNA dose and DNA:polymer ratio, which compared additional PBAE structures, demonstrated significantly increased mCherry expression using 4-4-6, 4-5-7, and 4-5-39 in HEK cells, and using 4-4-6 and 4-5-7 in CHO cells, compared to PEI-mediated transfection (Figure 3A and C). Transfection efficiency, as measured by mCherry-positive cells on day 5, was significantly increased with all PBAEs tested in HEK cells and with 4-4-6, 4-5-7, and 4-5-39 in CHO cells (Figures 3B, D and S5A). Fluorescence microscopy confirmed the increase in mCherry expression (Figures 3E and S5B). Cell viability, assessed via MTS assay 24 h following transfection, indicated that PBAEs (especially 4-5-39 and 5-3-6) showed greater toxicity than PEI, though notably at a 20- to 30-fold higher weight ratio (Figure S4A). Further investigation into cellular viability demonstrated decreased viability with increasing PEI ratio in HEK cells, whereas viability remained relatively constant with increasing PBAE ratio (Figure S4B). Importantly, at optimized weight ratios, this reduced viability did not result in inferior mCherry expression relative to PEI, with 4-4-6 and 4-5-7 demonstrating superior expression in both HEK and CHO cells (Figure 3A and C).

Comparison of PEI- and PBAE-Mediated Transfection for Production of Secreted Antibodies

To demonstrate that the results of these fluorescent protein expression experiments were replicable at scales relevant to the development and production of secreted proteins, we transfected HEK cell cultures of varying volumes with DNA encoding the recombinant antibody 10H2^{22,24} using either 4-4-6- or PEI-based particles. Based on SDS-PAGE image analysis of small-scale dose titrations (Figure S6A–D), DNA was dosed at 1 $\mu\text{g/mL}$ for secreted proteins in HEK cells, with polymer weight adjusted accordingly. At volumes ranging from 20 to 200 mL, transfection with 4-4-6 yielded between 4.5-fold and 8.2-fold more protein than did transfection with PEI (Figure 4A). To further demonstrate the scalability of enhanced protein expression using PBAEs, 2 L cultures of HEK cells were transfected with DNA encoding either 10H2 or the bispecific antibody BS2²² using either 4-4-6 or PEI. Both the 10H2 and BS2 antibodies were recovered in significantly higher quantities (6.2-fold and 4.3-fold higher, respectively) when 4-4-6 was utilized compared to PEI (Figure 4B–C). Superiority of PBAE nanoparticles was reproducible across cell lines; transfection of CHO cells with DNA encoding the recombinant antibody 602^{23,25,26} at an optimized dose of 4 $\mu\text{g/mL}$ resulted in 3.4-fold more protein recovered when 4-4-6 was used compared to PEI (Figure S6G–I).

Discussion

Recombinant protein expression via transient transfection is a cornerstone of scientific research as well as drug discovery and development workflows. PBAEs are promising as transient transfection reagents for protein production as they can self-assemble with nucleic acids into small nanoparticles due to their primary and secondary amines, promote endosomal escape due to their titratable tertiary amines, and facilitate nucleic acid release and low cytotoxicity due to their ester linkages.^{6,15} Here, we evaluate the potential for this class of polymers in protein production workflows and compare their performance to a standard transient transfection reagent, the commonly employed linear 25 kDa PEI.

With respect to physiochemical properties, we found that PBAE nanoparticles maintained a small size whereas PEI particles tended to aggregate. Previous studies indicated that PEI nanoparticles were prone to aggregation in serum-free media due to interactions with salts and a lack of adsorbed proteins that can help stabilize discrete particles and prevent clustering.^{27,28} PEI particles also exhibited a lower positive surface charge in HEK and CHO media compared to PBS, which may limit interactions with a charged cell membrane, thus hindering cellular uptake and decreasing overall transfection efficiency and protein production. In contrast, PBAE nanoparticles exhibited highly positive surface charges that were more robust to media conditions. PBAE nanoparticle binding, uptake and escape have been previously

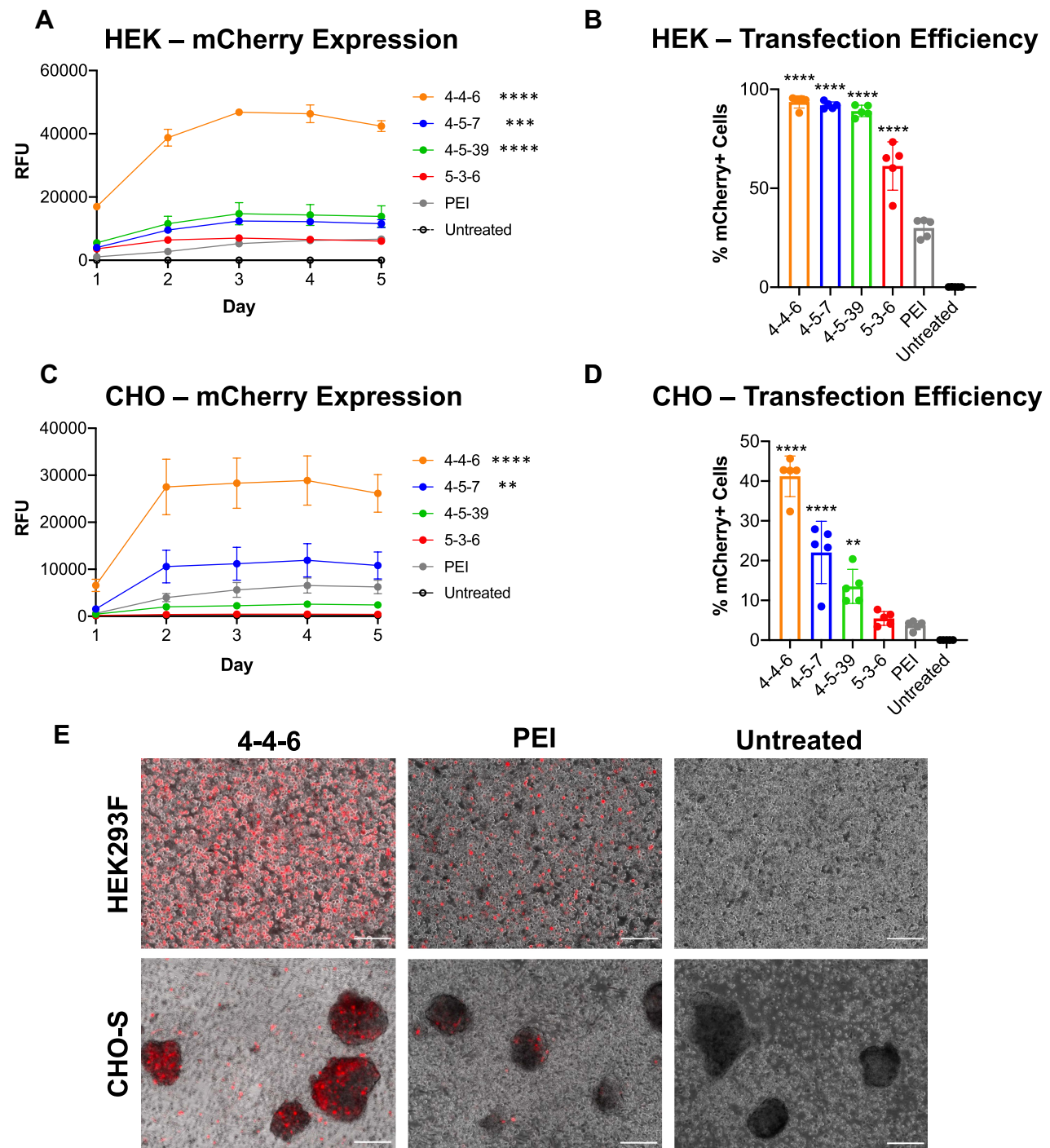


Figure 3 Comparison of polymers for transient transfection of cytosolic mCherry in HEK and CHO cells. In HEK cells, PEI was used at a 2:1 polymer:DNA w/w ratio, whereas in CHO cells, PEI was used at a 3:1 polymer:DNA w/w ratio. All PBAEs were used at a 60:1 polymer:DNA w/w ratio in both cell lines. **(A)** HEK cells were transfected with 2 μ g/mL mCherry-encoding DNA via PBAE or PEI nanoparticles (n=5). mCherry fluorescence was assessed via plate reader each day, and significance was calculated on day 5. **(B)** mCherry transfection efficiency was determined via flow cytometry 5 days following transient transfection of HEK cells with 2 μ g/mL mCherry-encoding DNA via PBAE or PEI nanoparticles (n=5). **(C)** CHO cells were transfected with 4 μ g/mL mCherry-encoding DNA via PBAE or PEI nanoparticles (n=5). mCherry fluorescence was assessed via plate reader each day, and significance was calculated on day 5. **(D)** mCherry transfection efficiency determined by flow cytometry 5 days following transient transfection of CHO cells with 4 μ g/mL mCherry-encoding DNA via PBAE and PEI nanoparticles (n=5). **(E)** Representative fluorescence microscopy images of HEK and CHO cells 5 days following transient transfection of mCherry-encoding DNA (2 μ g/mL for HEK cells; 4 μ g/mL for CHO cells) with 4-4-6 or PEI nanoparticles. Scale bars are 200 μ m. Additional images are shown in Figure S5. For all panels, error bars represent SD. Significance on day 5 was calculated using one-way ANOVA with Dunnett post-test, comparing all conditions to treatment with PEI. Increases relative to PEI are designated: **p < 0.01, ***p < 0.001, ****p < 0.0001.

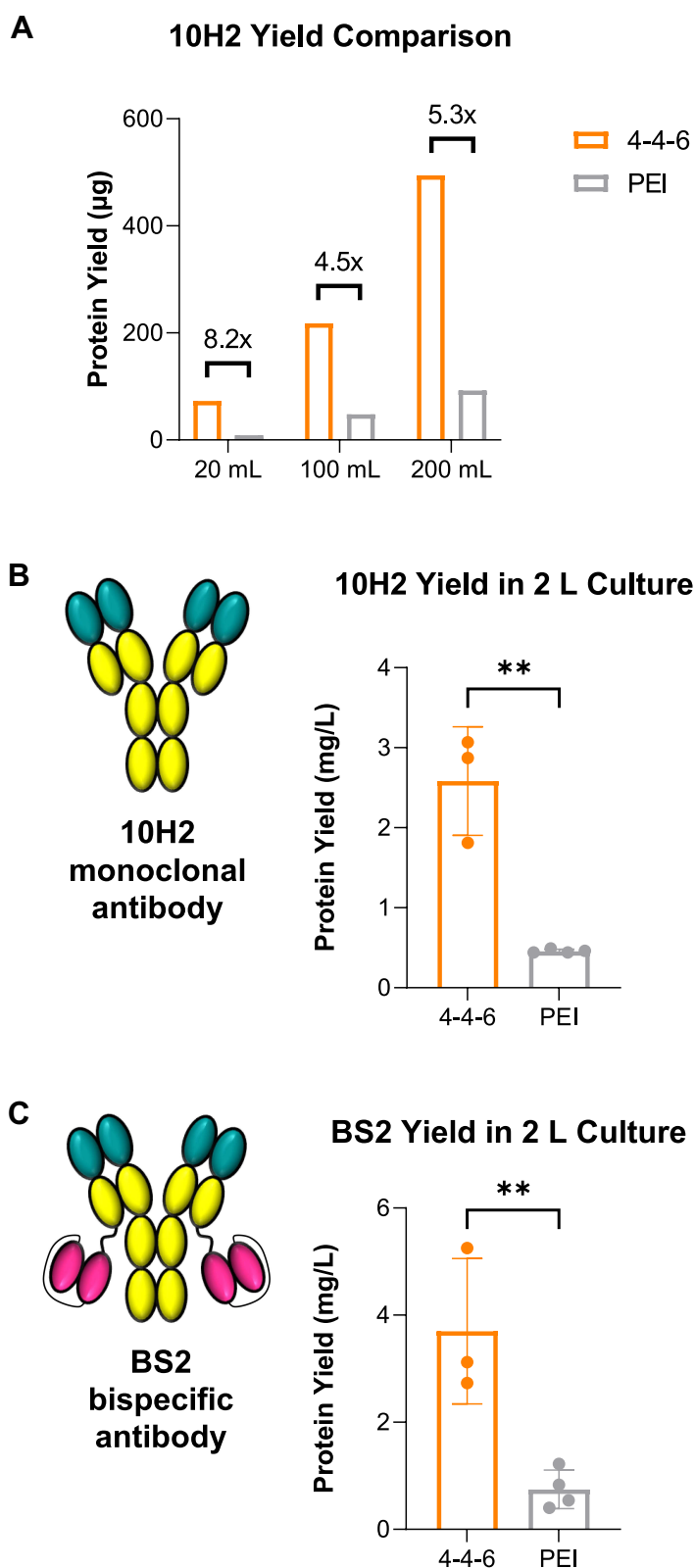


Figure 4 Comparison of 4-4-6 and PEI for transient transfection and secretion of two recombinant antibodies at various scales in HEK cells. All transfections used 1 µg/mL DNA at a 60:1 polymer:DNA w/w ratio for 4-4-6 or a 2:1 polymer:DNA w/w ratio for PEI. **(A)** Comparative yields from scaled transfections of the 10H2 monoclonal antibody utilizing 4-4-6 or PEI nanoparticles. **(B)** Comparative yields from transient transfections of 10H2 antibody utilizing 4-4-6 or PEI nanoparticles at 2 L scale. **(C)** Comparative yields from transient transfections of the BS2 bispecific antibody utilizing 4-4-6 or PEI nanoparticles at 2 L scale. Significance was determined by unpaired Student's *t*-test (***p* < 0.01). Error bars represent SD.

described in comparison to PEI,^{7,15,29} and the development of a galectin 8-based biosensor enabled detection and comparison of endosomal escape of PBAEs versus various PEI constructs.³⁰ Bishop and Kozielski et al review various structural elements, such as charge density, hydrophobicity, and molecular weight, of PBAEs, PEI, and other biomaterials that help facilitate intracellular delivery of nucleic acids.¹⁵ Karlsson et al review additional PBAE gene delivery mechanisms, giving a comprehensive description of studies investigating topics such as the binding strength of PBAEs, the effect of surface charge on toxicity, and the buffering capacity of PBAEs based on secondary and tertiary amines leading to endosomal escape.⁶

Overall, our results indicate that PBAE nanoparticles are capable of increasing the production of a cytosolic reporter and two bispecific antibodies in HEK and CHO cells compared to transfection with a commercially available linear 25 kDa PEI. This result was obtained following careful optimization of both PBAE and PEI nanoparticles with respect to DNA dose and polymer:DNA weight ratios, though other groups have obtained higher titers following optimization of cell count, polymer concentration, and other factors.^{2,31} The range of weight ratios tested in this study was similar to those tested by other groups as well as to manufacturer's protocols.^{32–34} Following optimization, we observed higher transfection efficiency with 4-4-6 compared to PEI, with an optimal DNA dose of 1–2 µg/mL observed for both polymers in HEK cells and a monotonically increasing transfection efficiency for both polymers observed in CHO cells with increasing DNA dose. We hypothesize that these results are due to toxicity resulting from the increasing amount of polymer added as DNA dose increases (at fixed N:P or weight ratio). Importantly, PBAEs are easily synthesized without any specialized equipment using inexpensive commercially available reagents and can be made in large batches and stored for several months without losing activity in transfections.¹⁷ Though our results are very promising in highlighting PBAEs as a useful transfection reagent for recombinant protein production, we note that this study does not compare PBAEs to an exhaustive list of available PEI reagents, and additional comparisons could therefore be considered in future work.

Conclusions

Taken together, experiments with both cytosolic and secreted proteins demonstrated that PBAEs lead to significantly enhanced protein yields compared to commercial reagent linear 25 kDa PEI in two cell lines that are widely used for protein production. Storage stability and straightforward synthesis from inexpensive chemical monomers further strengthen their attractiveness for use in recombinant protein production across batch scales. Overall, the favorable properties of PBAEs combined with the results herein suggest that these polymers hold promise as effective reagents for transient transfection that can significantly improve protein production workflows.

Abbreviations

PEI, Poly(ethylenimine); PBAE, poly(beta-amino ester); HEK293-F, human embryonic kidney 293F; CHO-S, Chinese hamster ovary-S; B4, 1,4-Butanediol diacrylate; B5, 1,5-Pentanediol diacrylate; S3, 3-amino-1-propanol; S4, 4-amino-1-butanol; S5, 5-amino-1-pentanol (S5); E6, 2-(3-Aminopropylamino)ethanol; E7, 1-(3-Aminopropyl)-4-methylpiperazine; E39, 1-(2-Aminoethyl)piperazine; 4-4-6, 2-(3-aminopropylamino)ethanol end-capped poly(1,4-butanediol diacrylate-co-4-amino-1-butanol); THF, tetrahydrofuran, PBS, phosphate-buffered saline; FBS, fetal bovine serum; SD, standard deviation.

Data Sharing Statement

The data in this study are included in this article or are available from the corresponding author upon request.

Consent for Publication

The authors have declared their consent for publication.

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Disclosure

Kathryn M. Luly, David R. Wilson, and Jordan J. Green are co-inventors on patents (63/274,361 and 17/282,939) concerning the technology described in this work that has been filed by Johns Hopkins University. The authors report no other conflicts of interest in this work.

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