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Degradable terpolymers with alkyl side chains demonstrate enhanced gene delivery potency and nanoparticle stability

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Keywords

Polymer; Lipid; DNA; Nanoparticle; Gene therapy

Non-viral gene delivery vectors are attractive due to their potential safety and ease of manufacture, but low transfection potency and poor formulation stability have limited their application. Among the numerous materials that have been studied for gene delivery, poly(β-amino ester)s (PBAEs) represent one promising class of degradable delivery materials. Here, we develop PBAE terpolymers incorporating hydrophobic alkyl side chains that exhibit significantly enhanced transfection potency and resistance to aggregation. Top-performing PBAE terpolymers demonstrated significantly higher transfection potencies in HeLa cells than the commercial reagent, Lipofectamine 2000. Inclusion of alkyl side chains promoted the stability of polymer-DNA complexes under physiological conditions. Furthermore, at high DNA concentrations, PBAE terpolymers with alkyl side chains, but not PBAEs lacking them, could support functionalization with PEG-lipid conjugates to yield particles that maintained their size and transfection activity after incubation at high ionic strength for several hours. The marked improvements in nanoparticle transfection potency and stability offered by these degradable PBAE terpolymers may facilitate the *in vivo* translation of these materials.

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Gene therapy is a promising treatment strategy for a variety of inherited and acquired diseases, but safe and efficient delivery remains a challenge. Though gene therapy mediated by viral vectors has recently made great clinical progress, limitations associated with their use persist, such as the possibility of adverse immune reactions, the difficulty of repeat dosing, and small DNA loading capacities.[1, 2] Non-viral vectors, meanwhile, continue to suffer from generally low DNA delivery efficiency.[3] Nonetheless, the diversity of synthetic materials offers potential for the identification and incorporation of functional motifs that confer not only efficient gene transfection, but also formulation stability and biocompatibility.[4]

Poly(β-amino ester)s (PBAEs) are a class of cationic gene delivery polymers that have been studied pre-clinically for applications including local cancer therapy and the genetic modification of stem cells for treatment of ischemia[5, 6]. Synthesis is relatively simple and versatile, and is based on the Michael-type conjugate addition of a primary or secondary amine to a diacrylate. Using this approach, combinatorial library synthesis and high-throughput screening methods have been developed to identify polymers that deliver DNA with high efficiency and low cytotoxicity[7-9]. Through these experiments, structural features associated with highly active gene delivery polymers have emerged, such as the presence of hydroxyl groups in the side chains and the conjugation of certain primary amines to the chain ends[10-13]. In addition to high transfection efficiency, the degradability of the polyester backbone offers the possibility of reduced toxicity and rapid clearance, a feature that distinguishes PBAEs from polyethylenimine (PEI), the most widely used gene delivery polymer[14].

Cationic polymers can form polymer-DNA polyplexes that may aggregate under physiological conditions[15, 16]. This potential aggregation in the blood represents a serious barrier to the systemic delivery of nucleic acids, since large aggregates can in some cases lead to embolism or otherwise may be quickly cleared by the reticuloendothelial system[16, 17]. Colloidal stability is a complex phenomenon influenced by many factors including concentration, surface charge, pH, ionic strength, and the presence of serum proteins, but a common approach to improve formulation stability is to introduce a polymer shield comprising polyethyleneglycol (PEG), poloxamers, or other non-fouling polymers[18]. Although attachment of PEG can be covalent or non-covalent, the latter may be preferable to avoid potential issues resulting from direct PEGylation of gene delivery polymers, such as impaired DNA condensation and decreased uptake[19, 20]. For nanoparticles with sufficiently hydrophobic surfaces, one simple non-covalent approach entails coating the particles with PEGylated phospholipid conjugates[21]. Therefore, we hypothesized that the inclusion of long, linear alkanes in PBAE side chains might facilitate non-covalent, hydrophobic interaction with PEG-lipid conjugates, resulting in stable particle formulations upon nanoprecipitation with DNA at high concentration. Because the modification of cationic polymers with hydrophobic groups has been reported to increase the physical encapsulation of nucleic acids, promote cellular adsorption, and reduce the positive surface charge associated with cytotoxicity and aggregation, we further hypothesized that hydrophobic PBAE terpolymers might deliver DNA more efficiently than PBAEs lacking alkyl side chains and might condense DNA into polyplexes with greater aggregation resistance[22-24].

In this study, we synthesized random PBAE terpolymers by step-growth polymerization of three starting monomers: a diacrylate, a hydrophobic alkylamine, and a comparatively hydrophilic amine. Previous work has shown the importance of terminal amine groups[11, 12]. Therefore, we used a two-step reaction scheme involving co-polymerization of the amine monomers with excess diacrylate to yield acrylate-terminated base polymer, followed by reaction with excess diamine to produce amine end-modified PBAE terpolymer (Fig. 1a). Because it is not easily predicted which PBAEs would benefit from the inclusion

of alkyl side chains, we used combinatorial library synthesis and screening as a tool to accelerate their development. Our initial library (Fig. 1b) consisted of 80 amine-end modified PBAE terpolymers synthesized using 8 diacrylates, 10 hydrophilic amines, one hydrophobic amine (dodecylamine), and one end-capping diamine ("122"). Based on pilot studies, we chose a monomer molar feed ratio of 1.2:0.7:0.3 diacrylate:hydrophilic amine:hydrophobic amine for the polymerization, and the reactions were carried out in DMSO. Under these conditions, eight of the 80 polymers precipitated out of reaction, while the remaining library members were soluble.

Using these 72 polymers, we formed complexes with GFP-encoding plasmid DNA and incubated them with cultured HeLa cells in serum-containing growth medium for 4 h. As a screen for transfection efficiency, we performed fluorescence-activated cell sorting (FACS) 48 h later to quantify the proportion of cells expressing GFP. As shown in the heat map (Fig. 2a), the top five PBAE terpolymers transfected over 70% of HeLa cells, and four of these five, D90-C12-122, D60-C12-122, D21-C12-122, and D24-C12-122, shared a common diacrylate structure based on bisphenol A ethoxylate ("D"). The top two polymers from this screen outperformed one of the best previously identified PBAEs lacking alkyl side chains, C32-122, and rivaled the commercially available lipid reagent Lipofectamine 2000 in transfection efficiency (Fig. 2b).

To investigate the structural space surrounding these polymers, we synthesized a second library of hydrophobic PBAE terpolymers focused on bisphenol diacrylate monomers (Fig. 1c). When we screened this set of polymers for transfection efficiency at half the DNA dose used in the first library, five polymers emerged in addition to D60-C12-122 and D90-C12-122 that transfected HeLa cells with greater than 85% efficiency (Fig. 2c). After resynthesizing these polymers on a larger scale, and transfecting cells at reduced DNA doses, we observed that these five polymers, based on DD24, DD90, DD60, DF90, and DF60, exhibited transfection potencies in HeLa cells superior to Lipofectamine 2000 (Fig. 2d, Fig. S1). These polymers were generally short, ranging in weight-average molecular weight (M_w) from 2.40 to 2.94 kDa with polydispersity indices (PDI) from 1.7 to 2.3 (Table S1). DD24-C12-122, in particular, appeared to be the most potent terpolymer to emerge from these screens, yielding ~80% transfection efficiency at the 50 ng dose without producing significant toxicity (Fig. 2e). This polymer was purified by preparative gel permeation chromatography (GPC) using an HPLC system (Fig. S2). The resulting polymer (M_w = 4.37 kDa, PDI = 1.43) was characterized by ^{1}H-NMR spectroscopy, which confirmed the incorporation of the hydrophobic amine as well as the end-capping amine (Fig. S3).

When we used dynamic light scattering (DLS) measurements to compare the stabilities of D60-C12-122, D90-C12-122, and C32-122 polyplexes formed at low DNA concentration, we observed that all three polymers formed stable, sub-100 nm complexes with plasmid DNA under conditions of reduced pH and low ionic strength (Fig. 3a). However, when the polyplexes were diluted in phosphate-buffered saline (PBS) at physiological pH and ionic strength, only the complexes formed from the PBAE terpolymers remained stable, with effective diameters below 100 nm (Fig. 3b).

To examine the effect of alkyl side chain content on PBAE terpolymer transfection efficiency and complex stability in greater depth, we synthesized D60-C12-122 using a range of molar feed ratios for which the dodecylamine (C12) feed varied from 0 to 50 mol% of the total amine feed, while the diacrylate:amine ratio was kept constant at 1.2:1.0. An increase in the alkylamine feed ratio up to 30 mol% C12 generally corresponded to an increase in transfection potency in HeLa cells, with a rough plateau in efficiency between 30 and 50 mol% C12 (Fig. 3c). Interestingly, the alkylamine feed ratio also appeared to correlate positively with polyplex stability in PBS (Fig. 3d). One hour after dilution in PBS,

polyplexes formed from D60-122 polymer lacking alkyl side chains grew 50% in diameter, in contrast to <10% growth for those formed from D60-122 terpolymers synthesized with C12 feed ratios at or above 20 mol%.

To determine how the alkyl side chain length affects the gene delivery efficiency of PBAE terpolymers, we synthesized C32-122 and DD24-122 using alkylamines ranging from 8 to 16 carbons in length. We also varied the alkylamine molar feed from 0 to 40 mol% of the total amine feed, with the diacrylate:amine molar ratio fixed at 1.2:1.0. We observed a positive association between the alkyl side chain length of C32-122 terpolymers and transfection efficiency of HeLa cells (Fig. 3e). As was true for D60-122, there was also a positive correlation between alkylamine feed ratio and transfection activity of the C32-122 terpolymers. At this DNA dose, though, varying the alkyl side chain length and content of DD24-122 terpolymers did not yield much variation in observed transfection efficiencies of HeLa cells (Fig. 3f). Nonetheless, for both C32-122 and DD24-122, the best-performing hydrophobic terpolymers produced a marked enhancement in transfection activity relative to the polymers synthesized without alkylamine (C32-122: 0.22% vs. 78.1%; DD24-122: 4.2% vs. 87.5%).

Because polymer terminal group modification has been previously shown to affect transfection efficiency[11, 12], we also synthesized C32-C12 and DD24-C12 polymers with end-capping diamines other than 122 (Fig. S4a). For the C32 terpolymers, amine end-modification dramatically influenced transfection efficiency, but for the DD24 terpolymers, there was less variation at this dose (Fig. S4b). We expect that at lower DNA doses, we would observe greater variation in transfection activity as a result of changes to the alkylamine feed ratio and terminal groups of DD24 terpolymers.

Due to their low molecular weights and high polydispersities, the polymers resulting from the step-growth polymerization of the three starting monomers likely represent a mixture of three species: copolymers of the diacrylate and the hydrophilic amine, copolymers of the diacrylate and the hydrophobic amine, and finally terpolymers incorporating all three monomers. To elucidate which species is responsible for the observed enhancements in transfection potency and nanoparticle stability, and whether there might be a synergistic interaction between the relatively hydrophilic and hydrophobic copolymer species, we prepared four PBAE variants containing the DD diacrylate: one synthesized with only the hydrophilic 24 amine (DD24-122, DD:24 = 1.2:1.0 mol/mol, M_W = 2.48 kDa); another synthesized with only the hydrophobic C12 amine (DDC12-122, DD:C12 = 1.2:1.0, M_W = 5.38 kDa); a third synthesized with 70 mol% of the hydrophilic amine and 30% of the hydrophobic amine (DD24-C12-122, DD:24:C12 = 1.2:0.7:0.3, M_W = 3.03 kDa); and a fourth comprising a 70%:30% v/v mixture of DD24-122 to DDC12-122 (M_W = 3.44 kDa). To avoid polymer crosslinking that was observed at high molar ratios of the C12 amine, these end-capping reactions were performed at room temperature rather than at 40°C. When we examined polyplex stabilities in PBS, only the relatively hydrophilic DD24-122 was unstable, whereas DDC12-122, DD24-C12-122, and the polymer mixture all resisted aggregation (Fig. S5a). When we compared their transfection efficiencies, the polymer synthesized using all three monomers (DD24-C12-122) was significantly more potent than the mixture of the DD24-122 and DDC12-122 polymers, as well as the hydrophobic DDC12-122 polymer alone (Fig. S5b). These data suggest that although a synergistic effect between the hydrophilic and hydrophobic polymers may contribute to polyplex stability, the presence of terpolymer species incorporating both the hydrophilic and hydrophobic amines is most likely responsible for the enhanced potency of the alkane-containing PBAEs.

Finally, to test our hypothesis that the incorporation of hydrophobic side chains in PBAEs can facilitate their interaction with PEG-lipid conjugates, we used a nanoprecipitation

approach to formulate particles comprised of polymer and DNA in the presence or absence of PEG-lipid. Working with C32-122 and D60-122-based polymers, we dissolved either the copolymers lacking alkyl side chains (C:32 = 1.2:1.0 mol/mol; D:60 = 1.2:1.0) or the terpolymer variants (C:32:C12 = 1.2:0.7:0.3; D:60:C12 = 1.2:0.7:0.3) in acetonitrile with or without PEG-lipid and mixed them with DNA at high concentration in sodium acetate buffer at pH 5.2. To remove the organic solvent, we then dialyzed the formulations against PBS for 3 h. Comparing particle sizes before and after dialysis, we found that only the formulations employing both the terpolymers and the PEG-lipid conjugate gave rise to well-defined and stable nanoparticles (Fig. 4a). At this high DNA concentration, D60-122, for instance, produced large particles ~1 μm in size whether or not PEG-lipid was present. In contrast, when PEG-lipid was present, the terpolymer version, D60-C12-122, produced particles that remained stable after dialysis at ~250 nm in size; in the absence of PEG-lipid, the particles grew to >700 nm in diameter after dialysis, a size which seemed poorly defined given the large variation between replicate measurements. When we then transfected HeLa cells using identical volumes of formulation per well, corresponding to approximately 75 ng and 150 ng of DNA, we found that only the terpolymer formulations including PEG-lipid yielded significant transfection (Fig. 4b, Fig. S6). Further support for non-covalent interaction of the PEG-lipid conjugate was provided by ζ-potential measurements, which showed a reduction in ζ-potential upon inclusion of PEG-lipid for particles formed from hydrophobic C32-122 terpolymers, but not for particles formed from regular C32-122 (Table S2). Taken together, these data suggest that at high DNA concentration, PBAE terpolymers incorporating alkyl side chains, but not polymers lacking them, are capable of interacting with PEG-lipid conjugates and DNA to form stable nanoparticles, and that these particles retain the ability to mediate gene delivery.

Due to the commercial availability of functionalized PEG-lipid conjugates, the capacity of the hydrophobic PBAE terpolymers to support formulation with PEG-lipid represents a straightforward non-covalent method for attaching targeting ligands such as peptides to the polymer/DNA nanoparticles. Although the particles produced using a combination of PBAE terpolymers and PEG-lipid were rather large here, with effective diameters of ~250-350 nm, there is likely significant potential for optimization of particle size through variation of the PBAE alkyl side chain length and content, the ratio of PBAE:DNA:PEG-lipid, the lengths of the lipid tails and PEG polymer in the PEG-lipid conjugate, and the use of additional excipients such as cholesterol or poloxamers. Furthermore, increasing the mixing rate with the aid of a microfluidic mixing device should enable the formulation of smaller nanoparticles[25].

In summary, we employed parallel synthesis and screening as a tool to accelerate the development of degradable alkane-containing PBAE terpolymers for gene delivery, guided by our hypothesis that they might provide superior transfection potency and particle stability over polymers lacking hydrophobic side chains. The top-performing PBAE terpolymers exhibited transfection potencies in HeLa cells significantly higher than that of Lipofectamine 2000. Transfection efficiency was generally positively correlated with increasing hydrophobicity, as defined by either increasing the feed ratio of alkylamine monomer or increasing its chain length. Increasing the hydrophobic content of the polymers also appeared to be associated with greater polyplex stability at low DNA concentration. At high DNA concentrations under conditions of physiological pH and ionic strength, these hydrophobic PBAE terpolymers were better able to facilitate interaction with PEG-lipid and DNA to yield stable, well-defined nanoparticles capable of transfecting cultured cells *in vitro*. This simple yet powerful approach to improving the transfection potency and formulation stability of degradable cationic polymers may facilitate the development of multifunctional, nanoparticulate gene delivery systems suitable for *in vivo* application.

Experimental

Materials

Diacrylate and amine monomers, as well as end-capping reagents, were purchased from Sigma-Aldrich (St. Louis, MO, USA), Alfa Aesar (Ward Hill, MA, USA), TCI America (Portland, OR, USA), and Monomer-Polymer & Dajac Labs (Trevose, PA, USA). $(PEO)_4$-*bis*-amine ("122") was acquired from Molecular Biosciences (Boulder, CO, USA). All reagents were used without further purification. Plasmid DNA encoding green fluorescent protein (gWiz-GFP) was purchased from Aldevron (Fargo, ND, USA). PEG-lipid conjugate (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-5000], or 18:0 PEG5000 PE) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). Slide-A-Lyzer MINI dialysis devices (20 kDa MWCO, 0.1ml) were purchased from Pierce Biotechnology (Rockford, IL, USA). HeLa cells (ATCC, Manassas, VA, USA) were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen).

Polymer synthesis

The monomers were dissolved in DMSO (Sigma-Aldrich) to a concentration of 200 mg ml^{-1}. Alkylamines generally required heating to 60°C to enable complete dissolution. Library scale reactions were performed in glass shell vials (1 mL) with polyethylene snap caps (Waters, Milford, MA, USA) in a 96-well reaction block (Symyx, Santa Clara, CA, USA). To each vial equipped with stir bar, diacrylate monomer, hydrophobic amine monomer, and hydrophilic amine monomer were added such that their molar ratio was 1.2:0.3:0.7 and the total mass of monomers was 100 mg. After heating and stirring at 90°C for 48 h, the reactions were allowed to cool to RT, and to each vial, end-capping amine (0.2 mmol in 0.5 mL DMSO) was added. The reactions were stirred at 40°C for 24 h, divided into aliquots, and then stored frozen at −20°C. Top-performing polymers were resynthesized by scaling up the reactions tenfold.

Analytical Gel Permeation Chromatography (GPC)

GPC was performed using a Waters system equipped with a 2400 differential refractometer, 515 pump, and 717-plus autosampler. The flow rate was 1 ml min^{-1} and the mobile phase was tetrahydrofuran (THF). The Styragel columns (Waters) and detector were thermostated at 35°C. Linear polystyrene standards were used for calibration.

Preparative GPC/HPLC

DD24-C12-122 was subjected to GPC using a Phenogel 5 μm MXL gel filtration column (300 mm × 7.8 mm, Phenomenex, P/No. 00H-3087-KO) with THF as the mobile phase at a flow rate of 1 ml min^{-1}. The separation was performed on a 1200 Series Agilent HPLC system equipped with a UV diode array detector and a 1260 Infinity analytical scale fraction collector. The column compartment was kept at 40°C during fractionation. Based on the absorption of the polymer at 260 nm (Fig. S2), polymer was collected between 5.2 and 7.7 min. The fractionated polymer was transferred to a tared vial and dried until further analysis.

NMR

The HPLC-purified DD24-C12-122 polymer, along with the monomers and end-capping reagent, was characterized on a Varian mercury spectrometer by ^{1}H-NMR spectroscopy (500 MHz, DMSO-d_6).

Transfection experiments

One day before transfection, 12,500 HeLa cells (100 μl) were seeded into each well of a 96-well polystyrene tissue culture plate. In a typical example, for a 150 ng/well DNA dose, gWiz-GFP plasmid DNA (5 mg ml^{-1}) was diluted to 15 μg ml^{-1} in 25 mM sodium acetate (NaOAc) buffer at pH 5.2. Polymers (100 mg ml^{-1}) were thawed immediately prior to transfection and diluted in NaOAc buffer to a concentration of 300 μg ml^{-1} (20:1 w/w polymer:DNA). To form DNA-polymer nanoparticles, polymer solution (25 μl) was added to DNA (25 μl) in a half-area 96-well plate, mixed by repeated pipetting using a multichannel pipette, and allowed to incubate for 10 min at room temperature. Polymer-DNA complexes (30 μl) were then gently mixed with fresh medium (195 μl) pre-warmed to 37°C. Conditioned medium was removed using a 12-channel aspirating wand and replaced with the complexes diluted in medium (150 μl). Following a 4-h incubation, complexes were removed with the aid of a multi-channel aspiration wand and replaced with fresh medium (100 μl). Lipofectamine 2000 (Invitrogen) was used according to the protocol provided by the vendor.

Fluorescence-activated cell sorting (FACS)

GFP expression was assessed 48 h after transfection. After aspirating conditioned medium, cells were washed with PBS and detached with 0.25% trypsin-EDTA (25 μl, Invitrogen). FACS running buffer (50 μl), consisting of 98% PBS and 2% FBS, was added to each well. Cells were mixed thoroughly and then transferred to a 96-well round-bottom plate. GFP expression was measured using FACS on a BD LSR II (Becton Dickinson, San Jose, CA, USA). To determine the viabilities of treated cells relative to non-treated control cells, propidium iodide stain (Invitrogen) was added to the FACS buffer (1:200 v/v). The relative viability was calculated as the ratio of live treated cells per well to the mean number of live non-treated cells per well. 2D gating was used to separate increased auto-fluorescence signals from increased GFP signals to more accurately count positively expressing cells. Gating and analysis were performed using FlowJo v8.8 software (TreeStar, Ashland, OR, USA).

Particle formulation with PEG-lipid at high DNA concentration

Polymer in DMSO (100 mg ml^{-1}) and 18:0 PEG5000 PE in ethanol (10 mg ml^{-1}) were co-dissolved in acetonitrile to yield final concentrations of 8 mg ml^{-1} and 10 mol%, respectively. gWiz-GFP plasmid DNA (5 mg ml^{-1}) was diluted to 0.4 mg ml^{-1} in 25 mM NaOAc buffer. The polymer and PEG-lipid in acetonitrile (25 μl) was then added to DNA (25 μl) and mixed by repeated pipetting. After incubation for 10 min, the formulations were diluted in PBS (50 μl) and dialyzed against PBS (3 l) for 3 h at RT.

Dynamic light scattering (DLS) measurements

Particle sizes and ζ potentials were measured using a ZetaPALS DLS detector (Brookhaven Instruments Corp., Holtsville, NY, USA, 15-mW laser, incident beam 676 nm). Correlation functions were collected at a scattering angle of 90°, and particle sizes were obtained from the MAS option of BIC's particle sizing software (v. 2.30) using the viscosity and refractive index of water at 25°C. Particle sizes are expressed as effective diameters (z-average or hydrodynamic diameters) calculated using the Stokes-Einstein relationship from the diffusion coefficient obtained by cumulant analysis.Average electrophoretic mobilities were measured at 25°C using BIC PALS ζ-potential analysis software, and ζ-potentials were calculated using the Smoluchowski model for aqueous suspensions. For polyplex sizing, particles were prepared in NaOAc buffer as for DNA transfection, except volumes were scaled up by a factor of five. Once formed in NaOAc buffer, complexes were diluted fourfold in either additional NaOAc buffer or PBS and then sized at the indicated times. For

DLS measurements of particles prepared by nanoprecipitation, particles (either freshly prepared or dialyzed) were diluted 100-fold in NaOAc buffer or PBS as indicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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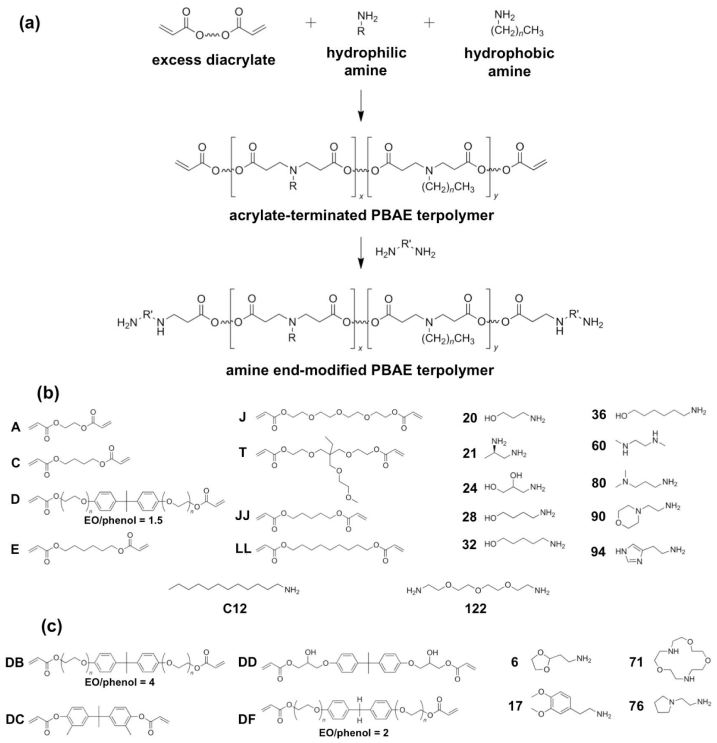

Figure 1.

a) Two-step synthesis of hydrophobic, amine end-modified poly(β-amino ester) (PBAE) terpolymers. b) Structures of diacrylates (A-LL), hydrophilic amines (20-94), hydrophobic amine (C12), and end-capping diamine (122) used for the synthesis of the initial screening library. c) Additional monomers used to synthesize the second screening library.

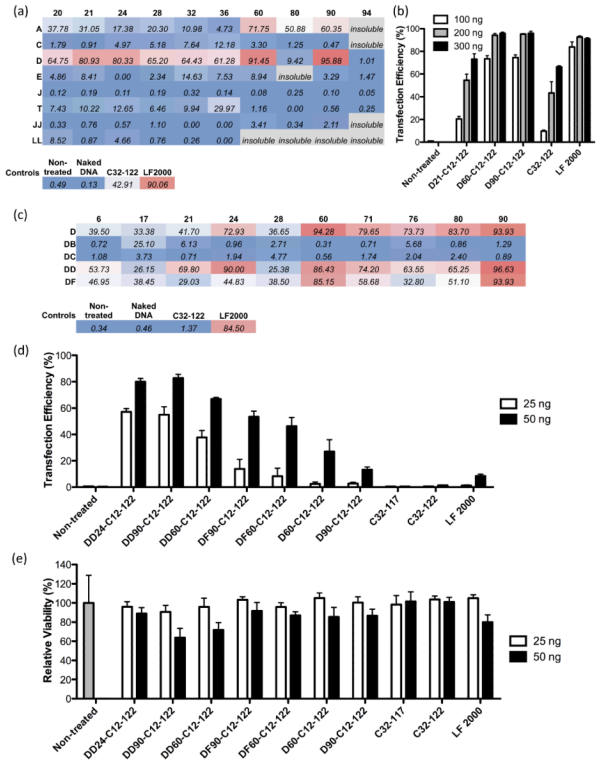

Figure 2.

Development of hydrophobic PBAE terpolymers with high DNA transfection potency. a) Heat map of DNA transfection efficiencies of HeLa cells with the initial terpolymer library (300 ng DNA/well; 20:1 polymer:DNA w/w). b) Transfection of HeLa cells with lead polymers from the initial library. c) Heat map of HeLa transfection efficiencies with the second, focused library (150 ng DNA/well). d) DNA transfection efficiency and e) relative viability of HeLa cells after treatment with the top PBAE terpolymers at reduced DNA doses.

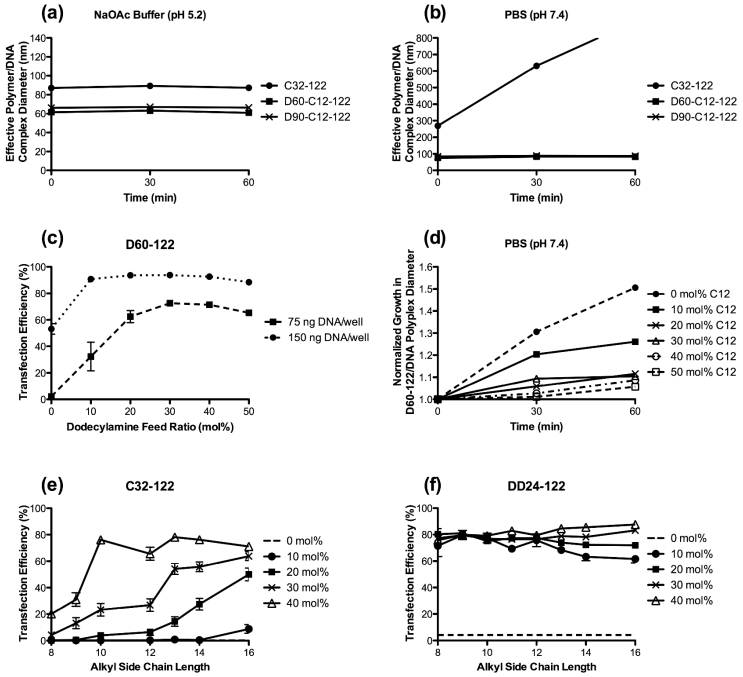

Figure 3.

Effect of alkyl side chains on PBAE transfection efficiency and polyplex stability. a-b) Stabilities of polyplexes formed from C32-122, D60-C12-122, and D90-C12-122 after self-assembly in sodium acetate (NaOAc) buffer at pH 5.2 or after dilution in phosphate-buffered saline (PBS) at pH 7.4. Polyplexes were formed at low DNA concentration (0.03 mg ml^{-1}) with a polymer:DNA w/w ratio of 20:1. c) HeLa transfection efficiencies and d) polyplex stabilities of D60-122 polymers synthesized with varying dodecylamine molar feed ratios. e-f) HeLa transfection efficiencies of C32-122 and DD24-122 polymers (100 ng DNA/well) synthesized with varying chain lengths and molar feed ratios of alkylamine. At these doses, C32-122 and DD24-122 synthesized with 0 mol% alkylamine yielded transfection efficiencies of 0.2% and 4.2%, respectively.

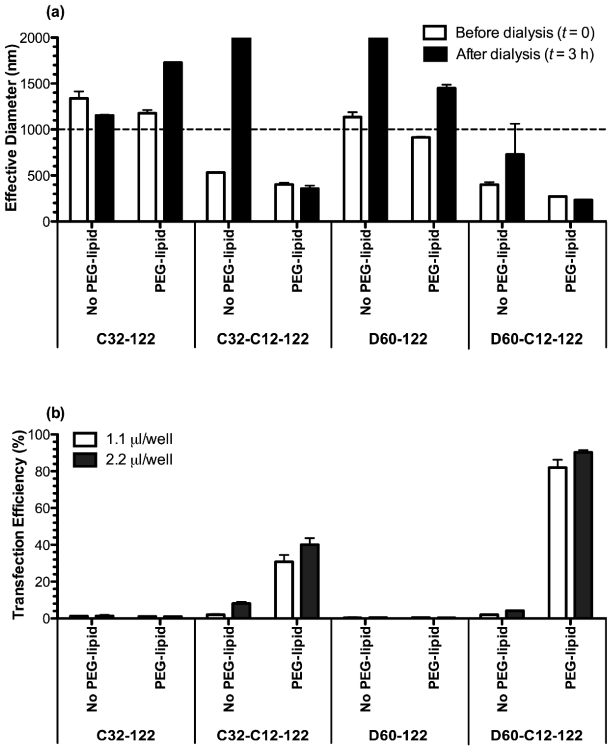

Figure 4.

Effects of PBAE alkyl side chains (C12) and the presence of PEG-lipid conjugate on nanoparticle formulation stability at high DNA concentration (0.4 mg ml^{-1}). a) Particle sizes before and after dialysis against PBS. b) Transfection efficiencies of HeLa cells using equal doses of each formulation (approximately 75 ng and 150 ng of DNA/well).