Supporting Information

Vitamin E-Labeled Polyethylenimine for \textit{in vitro} and \textit{in vivo} Gene Delivery

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Synthesis of PEI-vitE conjugates

Synthesis of vitE-OH. vitE-OH was synthesized via a simple substitution reaction between vitamin E (vitE) and 2-bromoethanol according to the literature\(^1\) with minor modification. Briefly, vitE (5.0 g, 1.0 eq), 2-bromoethanol (1.1 mL, 1.25 eq) and NaOH (0.7 g, 1.5 eq) were mixed in 25 mL anhydrous DMF, and stirred at 90°C overnight. The mixture was cooled to room temperature, then poured into water (100 mL). The solution was extracted with methyl tert-butyl ether (50 mL × 3). The organic layers were combined and evaporated in vacuum to leave the residue that was further purified by silica gel column chromatography using a mixture of petroleum ether (PE) and ethyl acetate (EA) as eluent (PE:EA = 5:1). Yield: 4.5 g (81.7%). \(^1\)H NMR (\(\delta/\text{ppm}, 400 \text{ MHz, CDCl}_3\)) 4.04-3.93 (t, 2H, OC\(\text{H}_2\)CH\(\text{CH}_2\)OH), 3.85-3.80 (t, 2H, OCH\(\text{CH}_2\)CH\(\text{CH}_2\)OH), 2.61 (t, J = 6.7 Hz, 2H, PhCH\(\text{CH}_2\)), 2.35 (brs, 1H, OH), 2.22 (s, 3H, CH\(\text{CH}_3\)), 2.18 (s, 3H, CH\(\text{CH}_3\)), 2.13 (s, 3H, CH\(\text{CH}_3\)), 1.80 (m, 2H, PhCH\(\text{CH}_2\)), 1.68-1.02 (m, 24H), 1.00-0.80 (m, 12H, 4 × CH\(\text{CH}_3\)). \(^{13}\)C NMR (\(\delta/\text{ppm}, 101 \text{ MHz, CDCl}_3\)): 147.99, 147.63, 127.72, 125.74, 123.00, 117.63, 74.86, 73.71, 67.99, 62.43, 40.11, 39.40, 37.44, 32.76, 31.24, 28.01, 25.63, 24.84, 24.47, 23.89, 22.70, 21.07, 20.67, 19.69, 12.74, 11.84. MS: calculated: 474.41, found: 474.69 [M]+, 497.64 [M + Na]+.

Synthesis of vitE-COOH. vitE-COOH was synthesized by coupling adipic acid to vitE-OH through carbodiimide chemistry. Briefly, DCC (2.6 g, 2.0 eq), DMAP (8.0 mg, 0.1 eq) were added to a solution of vitE-OH (3.0 g, 1.0 eq) and adipic acid (2.8 g, 3.0 eq) in 45 mL anhydrous THF at 0 to 5°C, and stirred at room temperature for 6 h. The white precipitate was filtered and the filtrate was evaporated in vacuum. The residue was dissolved in EA (90 mL), washed by water (50 mL×2). The organic layers were dried over anhydrous Na\(\text{SO}_4\). After filtration and evaporation in vacuum, the residue was purified by silica gel column chromatography using a mixture of dichloromethane (DCM) and methanol (MeOH) as eluent (DCM:MeOH = 200:1, 0.5% AcOH). Yield: 2.6 g (68.3%). \(^1\)H NMR (\(\delta/\text{ppm}, 400 \text{ MHz, CDCl}_3\)) 4.51-4.36 (t, 2H, OCH\(\text{CH}_2\)CH\(\text{CH}_2\)OH), 3.97-3.83 (t, 2H, OCH\(\text{CH}_2\)CH\(\text{CH}_2\)OH), 2.59 (t, J = 6.7 Hz, 2H, PhCH\(\text{CH}_2\)CH\(\text{CH}_2\)), 2.53-2.36 (m, 4H, COCH\(\text{CH}_2\)CH\(\text{CH}_2\)), 2.20 (s, 3H, CH\(\text{CH}_3\)), 2.16 (s, 3H,
CH₃), 2.11 (s, 3H, CH₃), 1.89-1.01 (m, 28H), 0.97-0.78 (m, 12H, 4 × CH₃). $^{13}$C NMR (δ/ppm, 101 MHz, CDCl₃): 179.06, 173.32, 148.01, 147.65, 127.71, 125.75, 122.98, 117.62, 74.86, 70.47, 63.76, 40.09, 39.39, 37.44, 33.83, 33.61, 32.75, 31.26, 28.00, 24.82, 24.46, 24.18, 23.86, 22.70, 21.05, 20.65, 19.69, 12.65, 11.78. MS: calculated: 602.45, found: 601.80 [M-H].

**Synthesis of PEI-vitEₙ.** The PEI-vitEₙ (n = 2, 4 and 6) conjugates were synthesized from 1.8 kDa PEI and vitE-COOH. The number of vitE modification per PEI was controlled by the feed ratio of vitE-COOH:PEI. Briefly, as shown in Table S1, vitE-COOH in anhydrous DCM (PEI-vitE₂: 3.0 mL; PEI-vitE₄: 3.75 mL; PEI-vitE₆: 4.5 mL) and TEA was activated with CDI for 1.5 h at room temperature, and the mixture was then added dropwise to a solution of 1.8 kDa PEI in anhydrous DCM (PEI-vitE₂: 13.4 mL; PEI-vitE₄: 8.4 mL; PEI-vitE₆: 6.7 mL). The solutions were stirred at room temperature for 3 h and evaporated in vacuum. The residues was then dissolved in distilled water (10 mL, pH adjusted to 8-9 with 2 M HCl) and purified by dialysis (MWCO 2000 Da) against distilled water for 24 h. The dialysate was lyophilized to give the final products as PEI-vitEₙ·xHCl, which were confirmed and analyzed by $^1$H NMR, FTIR and elemental analysis. Yield (PEI-vitE₂: 0.45 g, 90.3%; PEI-vitE₄: 0.41 g, 93.9%; PEI-vitE₆: 0.44 g, 97.9%). $^1$H NMR (δ/ppm, 400 MHz, CD₃OD) 4.36 (PhOCH₂CH₂O), 3.79 (PhOCH₂CH₂O), 3.21-2.63 (CH₂CH₂NH), 2.57 (PhCH₂CH₂), 2.49-2.23 (COCH₂CH₂), 2.21-1.94 (PhCH₃), 1.86-0.98 (characteristic peaks of vitE), 0.88 (CH₃).

Table S1. The feed ratio for the conjugation of vitE-COOH and PEI1.8. TEA, triethyl amine; CDI, carbonyldiimidazole; DCM, dichloromethane.

<table>
<thead>
<tr>
<th>n</th>
<th>vitE-COOH</th>
<th>TEA</th>
<th>CDI</th>
<th>PEI1.8</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.20 g, 1.0 eq</td>
<td>0.14 mL, 3.0 eq</td>
<td>56.5 mg, 1.05 eq</td>
<td>0.30 g, 0.5 eq</td>
<td>16 mL</td>
</tr>
<tr>
<td>4</td>
<td>0.25 g, 1.0 eq</td>
<td>0.17 mL, 3.0 eq</td>
<td>70.6 mg, 1.05 eq</td>
<td>0.19 g, 0.25 eq</td>
<td>12 mL</td>
</tr>
<tr>
<td>6</td>
<td>0.30 g, 1.0 eq</td>
<td>0.21 mL, 3.0 eq</td>
<td>84.7 mg, 1.05 eq</td>
<td>0.15 g, 0.17 eq</td>
<td>11 mL</td>
</tr>
</tbody>
</table>
FITC and TRITC labeling of PEI-vitE<sub>6</sub> and PEI25. PEI-vitE<sub>6</sub> and PEI25 were labelled with fluorescein isothiocyanate (FITC, \(\lambda_{ex} = 493\) nm, \(\lambda_{em} = 525\) nm) or tetramethylrhodamine-5-(and-6)-isothiocyanate (TRITC, \(\lambda_{ex} = 543\) nm, \(\lambda_{em} = 571\) nm) for confocal laser scanning microscopy or in vivo imaging. For FITC labeling, 20 mg PEI-vitE<sub>6</sub> (or PEI25) was dissolved in 4 mL DMSO and stirred at room temperature. A solution of FITC in DMSO (5 mg/mL, 125 \(\mu\)L) was added dropwise into the polymer solution and stirred at 30°C for 4 h. The obtained products were purified by dialysis (MWCO 2000 Da) against PBS for 24 h. The dialysate was diluted to proper concentration for use (500 ng/\(\mu\)L). The procedure for TRITC labeling was the same as described above.

**Determination of buffering capacity.** The buffering capacity of PEI-vitE<sub>n</sub> conjugates and control PEIs (1.8 kDa and 25 kDa) was determined using acid-base titration assays with pH values of solutions ranging from 2.0 to 11.0 according to a previous report\(^2\) with minor modification. Briefly, a certain amount of modified and unmodified PEIs (0.2 mmol of nitrogen atoms) was dissolved in 10 mL NaCl solution (150 mM). The PEI solutions were adjusted to a starting pH of 2.0 with 0.1 M HCl, and were then titrated with 0.1 M NaOH. The pH values of above solutions were monitored using a pH meter (Mettler Toledo FE20). For comparison, 150 mM NaCl was also titrated in the same way. The buffering capacity of these PEI conjugates and control PEIs were calculated according to the following equation: Buffer Capacity (%) = 100 \times (\Delta V_{NaOH} \times 0.1 \text{ M})/N\text{ mol}, wherein \(\Delta V_{NaOH}\) is the volume of 0.1 M NaOH solution that was required to adjust the pH value of PEI solutions from 5.1 to 7.4, and N mol is the total moles of amine groups that could be protonated in the tested PEIs (0.2 mmol).

**References**

**Figure S1.** Buffering capacity of PEI1.8, PEI25 and PEI-vitEₙ by acid-base titration. 150 mM NaCl was used as a control.

**Figure S2.** DNA protection by PEI-vitE₆ against DNase or serum. Lane 1: naked pDNA; Lane 2: PEI-vitE₆/pDNA (N/P = 2); Lane 3: pDNA with DNase I or 10% serum for 2 h; Lane 4: PEI-vitE₆/pDNA with DNase I or 10% serum for 2 h; Lane 5: PEI-vitE₆/pDNA treated with heparin; Lane 6: PEI-vitE₆/pDNA with DNase or 10% serum for 2 h followed by incubation at 65°C for 10 min to deactivate DNase and then treated with heparin.
Figure S3. AFM images of PEI-vitE/pDNA complex at N/P ratio of 20. a) 3D topography image; b) 2D image; c) height image of the cross-section indicated by the white line in (b).

Figure S4. A) HEK-293A Cell viability at the presence of vitamin E at various concentrations for 48 h. B) HEK-293A Cell viability at the presence of PEI25, PEI1.8, and PEI-vitEₙ (n=2, 4, 6) polymers at various concentrations for 48 h, respectively. Data were expressed as mean ± S.D.
Figure S5. A) GFP gene expression imaged by High Content Screening System, scale bar is 200 μm. Gene transfection experiments of PEI-vitE₆ at different N/P ratios were conducted, with Lipo 2000 (2 μL) used as a control. B) GFP gene expression efficiency was evaluated by the number of GFP positive cells measured by flow cytometry.
Figure S6. Uptake mechanism study of PEI-vitE₆ by flow cytometry. Entrance inhibition of FITC-labelled PEI-vitE₆/pDNA complexes by four different endocytosis inhibitors as well as low temperature were investigated. PC stands for positive control without addition of inhibitors and incubated at 37°C. Methyl-β-cyclodextrin (M-β-CD, 16 μM), genistein (150 nM), amiloride (60 μM), chlorpromazine (CPZ, 15 μg/mL) were chosen as inhibitors. The inhibition ratios were normalized by positive control.

Figure S7. Competition experiments of vitE over FITC-labelled PEI-vitE₆/pDNA complexes (A) and non FITC-labelled PEI25/pDNA complexes (B). HEK-293A cells were pre-incubated with vitE (100 μM, 200 μM, 400 μM). Cells without vitE pre-incubation were used as positive control (PC) and the inhibition ratio was normalized over positive control.
Figure S8. Body weight change of mice injected PEI25 or PEI-vitE₆ for 40 days with PBS injection as control. The mice injected with PEI25 all died in 24 h post injection.

![Body weight change graph](image)

Figure S9. Distribution of TRITC-PEI-vitE₆/pDNA in different organs over time. a) Control with no complexes injected; b) 0.5 h after complexes injected; c) 1 h after complexes injected; d) 2 h after complexes injected; e) 4 h after complexes injected.

![Distribution images](image)
Figure S10. Fluorescence images for liver histology slices of mice injected with naked pDNA, PEI/pDNA, and PEI-vitE₆/pDNA, respectively.
$^{1}H$ & $^{13}C$ NMR spectra of intermediates
Mass Spectrometry of intermediates