Electronic Supplementary Information (ESI)

for

Dual functionalized amino poly(glycerol methacrylate) with guanidine and Schiff-base linked imidazole for enhanced gene transfection and minimized cytotoxicity

Pan Guo, a Wen-Xing Gu, a Qixian Chen, b Hongguang Lu, a Xiongqi Han, a Wei Li, a

Hui Gao* a

a School of Chemistry and Chemical Engineering, Tianjin Key Laboratory of Organic Solar Cells and Photochemical Conversion, Tianjin University of Technology, Tianjin 300384, P. R. China, E-mail: hgao@tjut.edu.cn

b Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States
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1. **Synthesis and characterization of polymers.**

**Synthesis of EP.** PGMA was synthesized by atom transfer radical polymerization (ATRP), followed by further modification with ethanediamine according to our previous reports.\(^1\) Briefly, ethanediamine was added in 50-fold excess to GMA unit. The reaction mixture was stirred for 24 h, purified by dialysis (Spectra/Por RC, cut off 7000 Da) against deionized water for 2 days, and then freeze-dried to yield EP (Yield: 78%).

**Synthesis of GEP.** The primary amines on EP were then guanidinylated. The reaction was conducted in aqueous solution with 1.5 molar equivalent amounts of HPC and DIPEA to primary amines of EP for 24 h at room temperature. Then, the modified polymer was further purified by dialysis (Spectra/Por RC, cut off 7000 Da) against deionized water for 2 days before freeze-dried. The final product, guanidine modification of EP (GEP), was obtained as a white solid (Yield: 80%).

**Synthesis of IGEP.** GEP (0.2 g, 5 mmol) was dissolved in 3 mL of deionized water, and an aqueous solution of 4-imidazolecarboxaldehyde (0.1125 g, 4 mmol) was slowly added at dropwise with a time span over 20 min. The mixture was stirred at room temperature for 24 h. The obtained solution was condensed and precipitated in excessive amount of anhydrous acetone to obtain crude product. The products were then filtered, rinsed thrice with anhydrous acetone. The purified IGEP was collected after vacuum-dried.

**Characterization of polymers.** The \(^1\)H NMR spectra of the polymers were acquired on a 400 MHz Bruker Avance-400 spectrometer (400 MHz, Bruker, Freemont, CA) using D\(_2\)O as solvents. FTIR spectra of polymers were recorded on a Bio-Rad 6000 (Thermo Electron, USA) spectrometer using KBr pellets. Each sample was ground along with 50 mg of KBr to afford a fine powder and compressed into a pellet. Molecular weights and polydispersity of polymers were assessed by gel permeation
chromatography (GPC) equipped with a refractive index detector, using THF as a mobile phase at a flow rate of 1.0 mL min\(^{-1}\) at 35 °C. The GPC instrument was calibrated with a series of monodisperse polyethylene glycol compounds. All sample solutions were filtered through a 0.45 µm filter before analysis. The molar content of amino groups per gram of polymer was measured with an elemental analysis instrument (Elementar Vario EL, GER).

2. **Particle size, zeta potential and morphology measurement of DNA complexes.**

The size and zeta potential of complexes were determined in three independent experiments using a Zetasizer Nano ZS90 instrument (Malvern Instruments, Southborough, MA) at 25 °C. Three independent polymer/pDNA complexes for each polymer were prepared. The morphology of polymer/pDNA complexes was observed using a scanning electron microscope (SEM) on a JSM-6700F type field emission scanning electron microscope (JEOL, South Korea). SEM samples were prepared by depositing solutions of complexes (EP/pDNA, GEP/pDNA, IGEP/pDNA, N/P = 5.0) on a glass slide. After evaporation of water, the samples were coated with a thin gold layer.

3. **Agarose gel electrophoresis.**

The agarose gel retardation assay was performed to assess the mobility of polymer/pDNA complexes under electric field. Routinely, diverse formulations of polymer/pDNA complexes were prepared with N/P ratios ranging from 0.5 to 6.0. Then, complexes with several complexation ratios were loaded onto a 0.8% agarose gel containing ethidium bromide (EB), and a constant voltage (80 V) was applied to the complex-loaded gel in 0.5×TBE buffer for 60 min. DNA retardation was analyzed using a UV illuminator (Gel Documentation Systems, Bio-Rad, Hercules, CA).

4. **EB displacement assay.**

To quantitatively estimate the binding potency of polymer to pDNA, the EB displacement assay was conducted. Basically, 4 µL of EB (1 mg mL\(^{-1}\)) solution was mixed with pDNA (25 µg) to a final volume of 1.2 mL and incubated at room temperature for 15 min to prepare EB/DNA solution. Polymer was then added to the
mixture at varying N/P ratios, and the mixture was further incubated at room temperature for 15 min. Fluorescence spectra was recorded with three readings at an excitation wavelength of 485 nm and an emission wavelength of 595 nm using a Hitachi F-4500 fluorescence spectrophotometer (Hitachi Scientific Instruments, Finchampstead, UK). A pure EB solution and the EB/DNA solution without any polymer were used as negative and positive controls, respectively. The relative fluorescence was calculated as follows:

\[
\text{Relative fluorescence (\%)} = \frac{\text{fluorescence (polymers + EB + DNA)} - \text{fluorescence (EB)}}{\text{fluorescence (EB + DNA)} - \text{fluorescence (EB)}} \times 100
\]

5. Determination of Buffering Capacity.

The buffering capacity of cationic polymers in the pH range 2-10 was determined by acid-base titration. Polymers were dissolved into 10 mL of saline (0.9% NaCl solution) with a 10 mM amino group concentration. 0.1 M NaOH solution was used to adjust the pH to 10. Then, the solutions were titrated with a 0.1 M HCl solution with continuous volume increments. The pH of all the solutions was measured using a pH meter (Sartorius PB-10).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>AC(^a) (%)</th>
<th>Number of amino(^a)</th>
<th>Number of guanidine(^a)</th>
<th>Number of imidazole(^b)</th>
<th>Mn(^c) (Da)</th>
<th>PDI(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGMA</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>12000</td>
<td>1.32</td>
</tr>
<tr>
<td>EP</td>
<td>81.6</td>
<td>68.8</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>GEP</td>
<td>81.6</td>
<td>68.8</td>
<td>25.5</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>IGEP</td>
<td>81.6</td>
<td>68.8</td>
<td>25.5</td>
<td>12.7</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

AC: amination conversion; \(^a\)Determined by elemental analysis; \(^b\)Determined by \(^1\)H NMR; \(^c\)Determined by GPC.
Fig. S1 $^1$H NMR spectra of (A) EP, (B) GEP and (C) IGEP in D$_2$O.

Fig. S2 FTIR spectra of EP (black), GEP (red) and IGEP (blue).
Fig. S3 (A) DLS measurements and (B) the zeta-potentials of EP/pDNA, GEP/pDNA, IGEP/pDNA and PEI/pDNA complexes at varying N/P ratios.

Fig. S4 SEM images of polymer/DNA complexes at N/P =5.0. EP/pDNA (left) and GEP/pDNA (right).
Fig. S5 Titration profile of GEP and IGEP with HCl.

Reference