Supporting Information

PEGylated Oligoproline-Derived Nanocarrier for Dual Stimuli-Responsive Gene Delivery

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**Table S1.** GPC and \(^1\)H NMR based molecular weights of control polymer mPEG\(_{113}\)-b-PDMAEMA\(_{50}\)-b-P(DMAEMA\(_{16}\)-co-BMA\(_{42}\)-co-PAA\(_{17}\)) (PDDBP) and precursor polymers prepared by RAFT polymerization.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Polymer</th>
<th>(M_n,\text{NMR}^a)</th>
<th>(M_n,\text{GPC}^b)</th>
<th>PDI(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>mPEG(_{113})-ECT</td>
<td>5262</td>
<td>5300</td>
<td>1.05</td>
</tr>
<tr>
<td>2.</td>
<td>mPEG(<em>{113})-b-PDMAEMA(</em>{50})-ECT</td>
<td>13112</td>
<td>14800</td>
<td>1.55</td>
</tr>
<tr>
<td>3.</td>
<td>mPEG(<em>{113})-b-PDMAEMA(</em>{50})-b-P(DMAEMA(<em>{16})-co-BMA(</em>{42})-co-PAA(_{17}))</td>
<td>23528</td>
<td>25300</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\(^a\)Molecular weight (\(M_n,\text{NMR}\)) of polymers calculate by \(^1\)H NMR, \(^b\)Number average molecular weight (\(M_n\)) of polymers by GPC, \(^c\)Polydispersity index (PDI) of polymers.

**Scheme S1.** Stepwise synthesis of non-cleavable control polymer mPEG\(_{113}\)-b-PDMAEMA\(_{50}\)-b-P(DMAEMA\(_{16}\)-co-BMA\(_{42}\)-co-PAA\(_{17}\)) (PDDBP).
**Fig. S1.** LC-MS spectra of CP$_5$K peptide (A) Total ion current (TIC) spectrum of CP$_5$K peptide, (B-D) Mass spectrum of peptide ion at 0.080 (B), 0.551 (C) and 0.56 min (D) retention time indicate characteristic mass peak of peptide at m/z 776.2.
Fig. S2. $^1$H NMR spectra. (A) mPEG$_{113}$-MAL, (B) mPEG$_{113}$-$b$-CP$_5$K-NH$_2$, disappearance of maleimide peak at 6.7 ppm and appearance of peptide peaks indicate conjugation of peptide to mPEG-MAL. (C) mPEG$_{113}$-$b$-CP$_5$K-ECT, appearance of peak characteristic to ECT macro CTA indicate ECT conjugation to mPEG$_{113}$-$b$-CP$_5$K-NH$_2$. (D) mPEG$_{113}$-$b$-CP$_5$K-$b$-PDMAEMA$_{42}$-ECT. (E) mPEG$_{113}$-$b$-CP$_5$K-$b$-PDMAEMA$_{42}$-$b$(DMAEMA$_{22}$-co-BMA$_{40}$-co-PAA$_{24}$)(PPDDBP) in CDCl$_3$. 
Fig. S3. $^1$H NMR spectra of (A) mPEG$_{113}$-ECT, (B) mPEG$_{113}$-b-PDMAEMA$_{50}$-ECT, and (C) mPEG$_{113}$-b-PDMAEMA$_{50}$-b-P(DMAEMA$_{16}$-co-BMA$_{42}$-co-PAA$_{17}$)(PDDBP) in CDCl$_3$. 
Fig. S4. GPC chromatogram of mPEG\textsubscript{113}-ECT, mPEG\textsubscript{113}-b-PDMAEMA\textsubscript{50}-ECT, and mPEG\textsubscript{113}-b-PDMAEMA\textsubscript{50}-b-P(DMAEMA\textsubscript{16}-co- BMA\textsubscript{42}-co- PAA\textsubscript{17}).
Fig. S5. (A) The PPDBBP and control PDDBP copolymer assembles into stable micelles (0.2 mg/mL) in DPBS at pH 7.4 with an average diameter of 37 and 46 nm, respectively as confirmed by DLS based size measurement. (B) ROS-mediated dePEGylation of PPDBBP polymer micelles (open square) through cleavage of CP5K peptide linkers after SIN-1 treatment (4 mM) for 20h. An increase in zeta potential from 1.54 to 8.45 mV after SIN-1 treatment indicates dePEGylation mediated exposure of positively charged amine groups of PDMAEMA block to aqueous medium. The control polymer without CP5K peptide (open triangle) under similar conditions doesn’t show any significant change in zeta potential indicating ROS induces dePEGylation of PPDBBP.

Fig. S6. DLS based measurement of critical micelle concentration (CMC) of PPDBBP micelles. The polymer micelles show destabilization at 0.01 mg/mL.
**Figure S7.** PPDDBP polymer micelles (1 mg/mL) in DPBS (pH 7.4) before and after SIN-1 (4 mM) treatment for 24h. The size of polymer micelles remain unchanged after SIN-1 mediated dePEGylation, indicating that the stability of polymer micelles can be maintained by hydrophilic PDMAEMA corona and hydrophobic P(DMAEMA-co-BMA-co-PAA) core.

**Fig. S8.** The formation of PPDDBP/pDNA polyplexes at different N/P ratio was confirmed by gel electrophoresis assay. The absence of free pDNA indicates effective polyplex formation between PPDDBP and pDNA for all N/P ratio tested. Abbreviations: L = Ladder (1 kb, EZ Load 1 kb Molecular Ruler), P = pDNA. The numbers indicate the corresponding N/P ratios.
Fig. S9. The serum stability of pDNA loaded into PPDDBP polyplexes at different time points (0.25, 0.5, 1, 2, 3 and 6h) in 50% serum +/- sodium dodecyl sulfate (SDS, 1% w/v) was determined by agarose gel electrophoresis. After incubation, pDNA was exposed outside the PPDDBP polyplexes due to structural disruption by addition of SDS. In the presence of serum and SDS, the band for released pDNA showed up in the same position as the one of free pDNA in PBS for all the time points, suggesting effective protection by PPDDPBD from nucleases in serum considering no band from unprotected pDNA in 50 % serum (P+S). In the presence of serum without SDS, the absence of free pDNA in all the lanes at various N/P ratio indicates no pDNA exposure due to stable encapsulation of pDNA by polyplexes. Abbreviations: L = Ladder (1 kb, EZ Load 1 kb Molecular Ruler), P = pDNA, P+S = pDNA+serum, S = serum, SDS = sodium dodecyl sulfate.
Fig. S10. (A) pH dependent hemolysis of erythrocytes with PPDBP polymer micelles and (B) PPDBP/pDNA polyplex (N/P10) at 1, 5 and 40 μg/mL polymer concentration. Triton X-100 (1% (v/v))-treated condition was set as a positive control. Data are expressed as mean ± SD (n = 3).