Supporting Information

Sustained-release of exendin 4 using injectable and ionic-nano-complex forming polymer hydrogel system for long-term treatment of type 2 diabetes mellitus

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Supporting figures

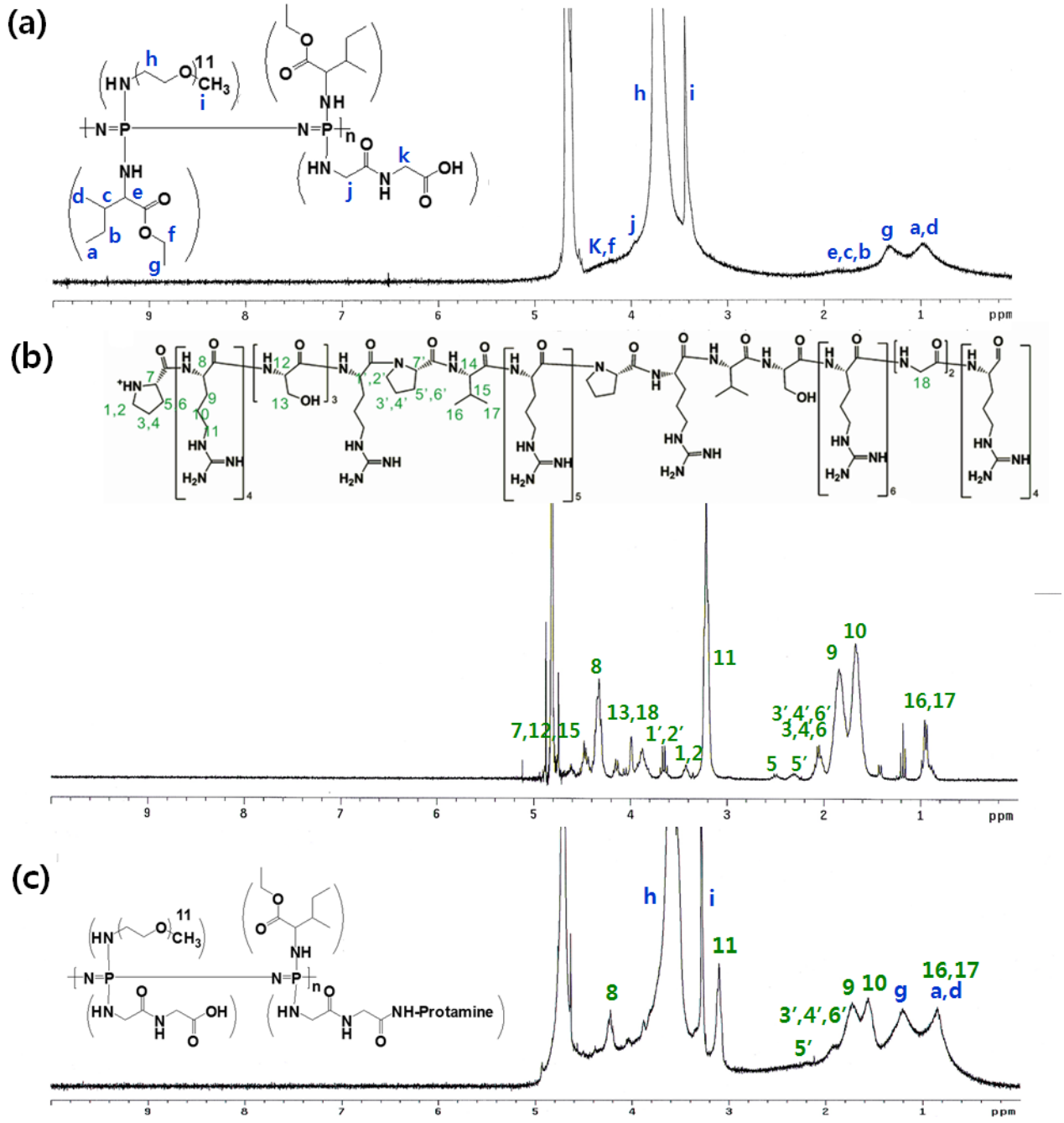
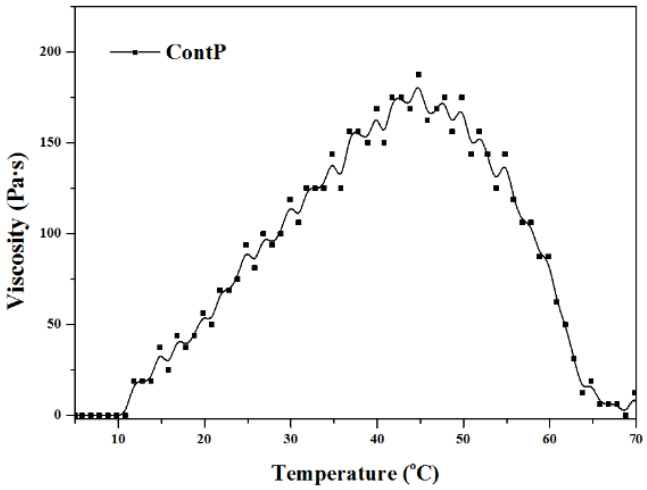
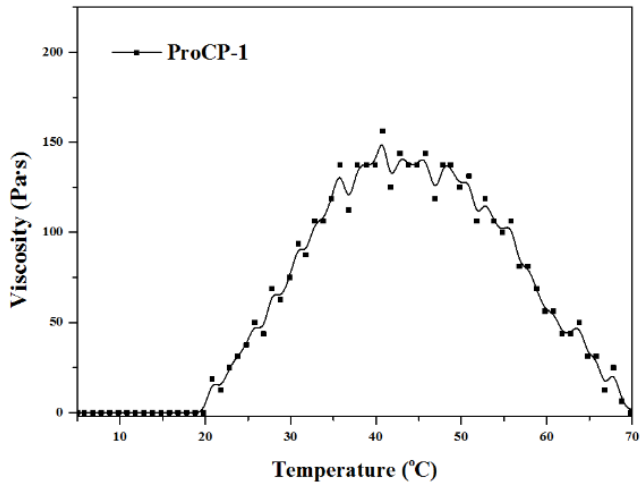


Figure S1. ^{1}H NMR spectra of synthesized protamine conjugated poly(organophosphazene). (a) Pre-ProCP, (b) protamine sulfate, and (C) ProCP.

(a)



(b)



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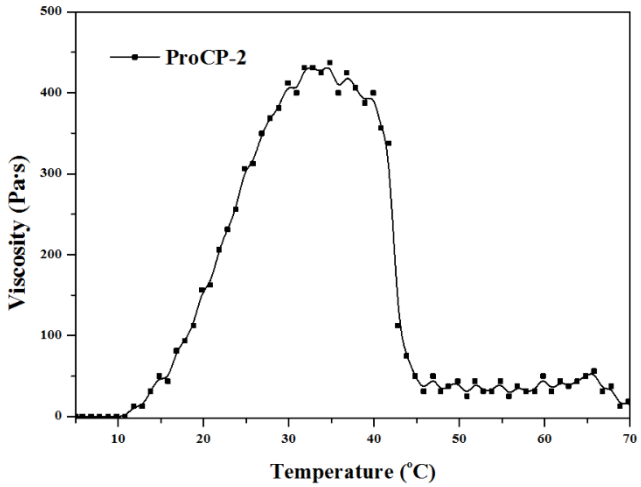


Figure S2. Temperature-dependent viscosity changes of ContP (a), ProCP-1 (b), and ProCP-2 (c) solutions (15 wt% of ProCP in phosphate buffered solution).

Table S1. Characteristics of ProCP with different amount of protamine group.

Polymer	Structure [a]	T_{ass} (°C)[b]	T_{max} (°C)[c]	$V_{37°C}$ (Pa.s)[d]	V_{max} (Pa.s)[e]
ProCP-2	$[NP(IleOEt)_{60.5}(GlyGlyOH)_{9.0}$**(protamine)$_{2.5}$**$(AMPEG)_{28.0}]_n$	11.8	34.8	425	437.5
ProCP-2'	$[NP(IleOEt)_{60.5}(GlyGlyOH)_{9.5}$**(protamine)$_{2.0}$**$(AMPEG)_{28.0}]_n$	7.8	29.8	825	1000
ProCP-2''	$[NP(IleOEt)_{60.5}(GlyGlyOH)_{10.0}$**(protamine)$_{1.5}$**$(AMPEG)_{28.0}]_n$	5	28.8	1175	1406.5

[a] The substituted ratios were determined by ^{1}H-NMR.
[b] The association temperature at which the viscosity start to increase. Viscosity was measured at 15 wt% of polymer concentration in PBS (pH 7.4).
[c] The temperature at which viscosity reaches the maximum value.
[d] Viscosities at 37 °C.
[e] Maximum viscosity.

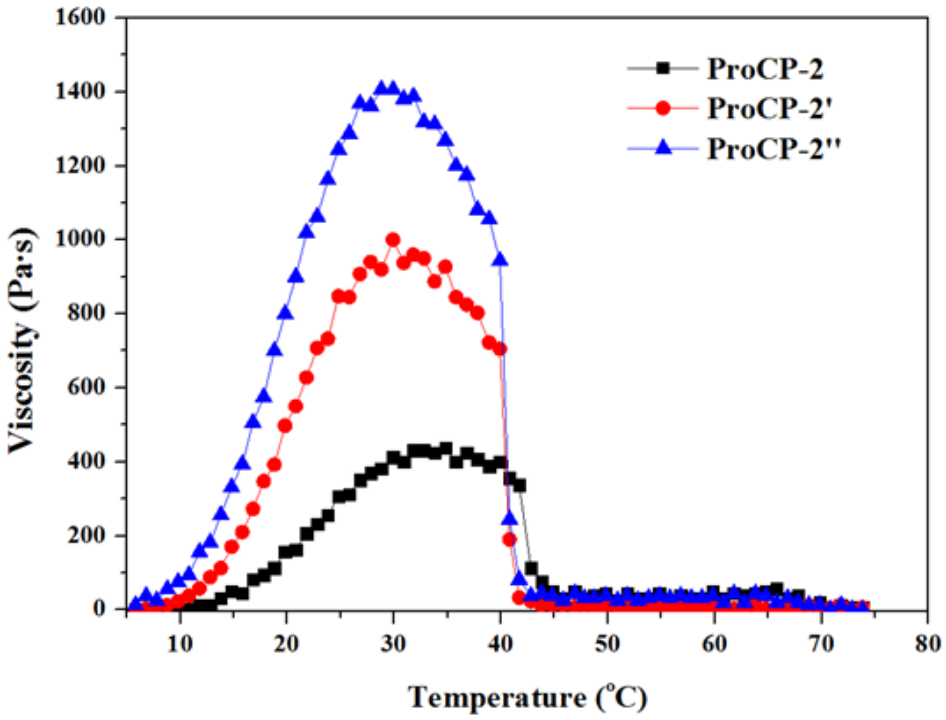


Figure S3. Temperature-dependent viscosity changes of ProCP-2, ProCP-2', and ProCP-2'' solutions (15 wt% of ProCP in phosphate buffered solution).

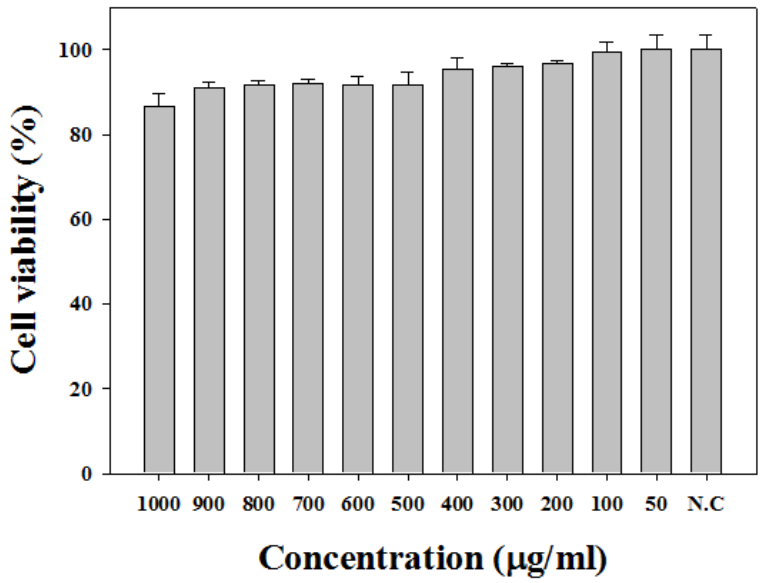


Figure S4. Cytotoxicity test of ProCP-2 on NIH3T3 cell. The cell viability were confirmed after 24 hours (n=6).

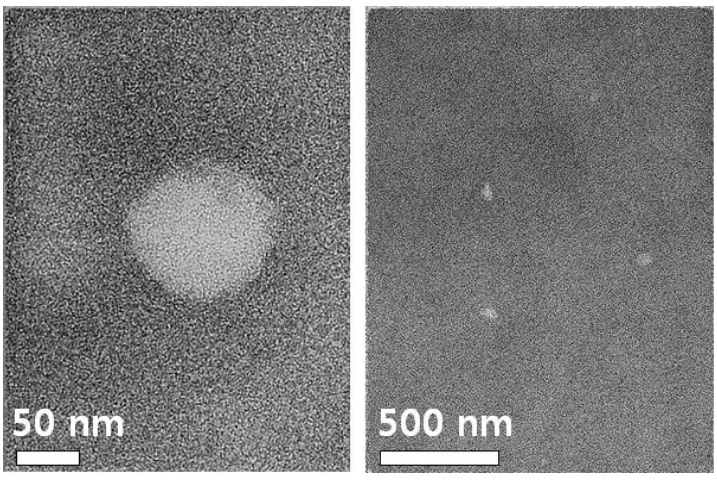
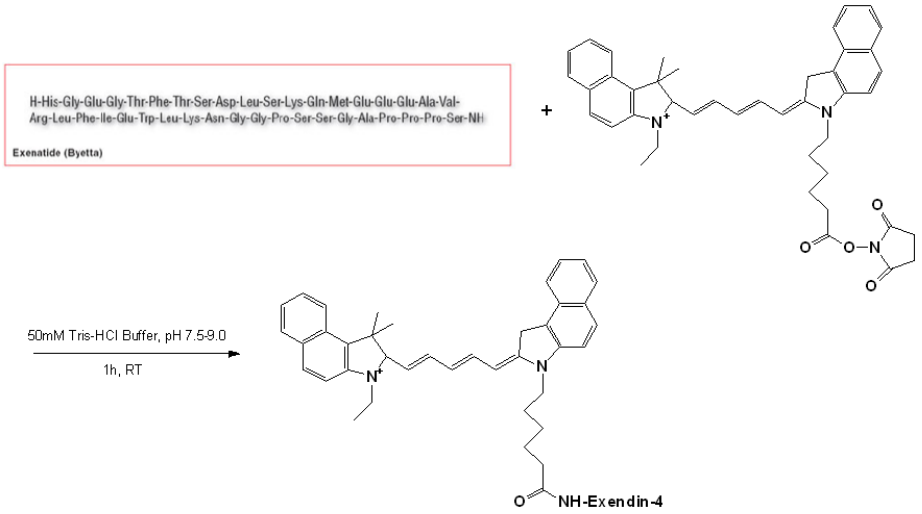


Figure S5. Transmission electron microscopy (TEM) images of the ProCP-2 nano-particles (left) and Ex-4/ProCP-2 nano-complexes (right).

(a)



(b)

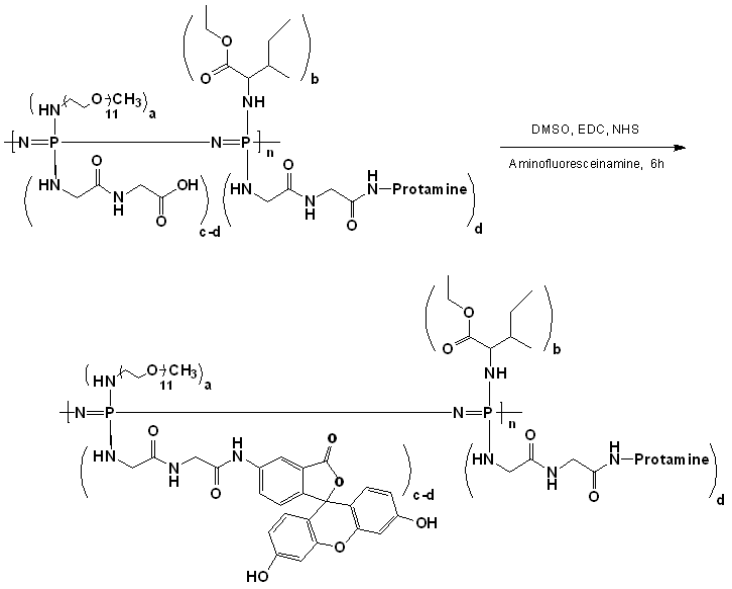


Figure S6. Cy5.5-conjugated Ex-4 (a) and aminofluorescein-conjugated ProCP (AF-ProCP) (b) for *in vivo* retention study.

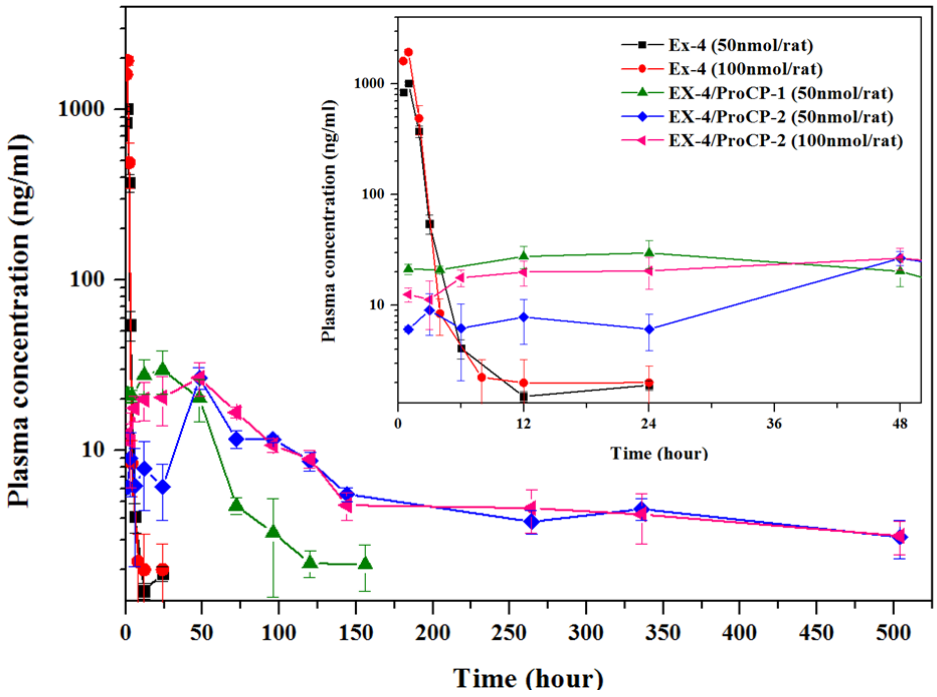


Figure S7. Pharmacokinetic study of exendin 4 (Ex-4) in Sprague-Dawley (SD) rats. Plasma concentration of Ex-4 in SD rats following subcutaneous injections of Ex-4 solution (50 nmol) (■), Ex-4 solution (100 nmol) (●), Ex-4/protamine-conjugated polymer 1 (ProCP-1) nano-complexes (50 nmol) (▲), Ex-4/ProCP-2 nano-complexes (50 nmol) (♦), and Ex-4/ProCP-2 nano-complexes (100 nmol) (◀). Error bars represent standard deviation (n = 4).

Table S2. Pharmacokinetic parameters of **Figure S7**.

	Ex-4 Sol. (50nmol/rat)	**Ex-4 Sol. (100nmol/rat)**	**Ex-4/ProCP-1 (50nmol/rat)**	**Ex-4/ProCP-2 (50nmol/rat)**	**Ex-4/ProCP-2 (100nmol/rat)**
AUC	1572.1	2635.7	1676.0	3172.8	3724.0
$T^{1/2}$ (hour)	1.2	2.7	28.8	108.7	174.5
T_{max} (hour)	1.0	1.0	24.0	48.0	48.0
C_{max} (ng/mL)	1012.3	1942.1	29.7	26.7	26.7

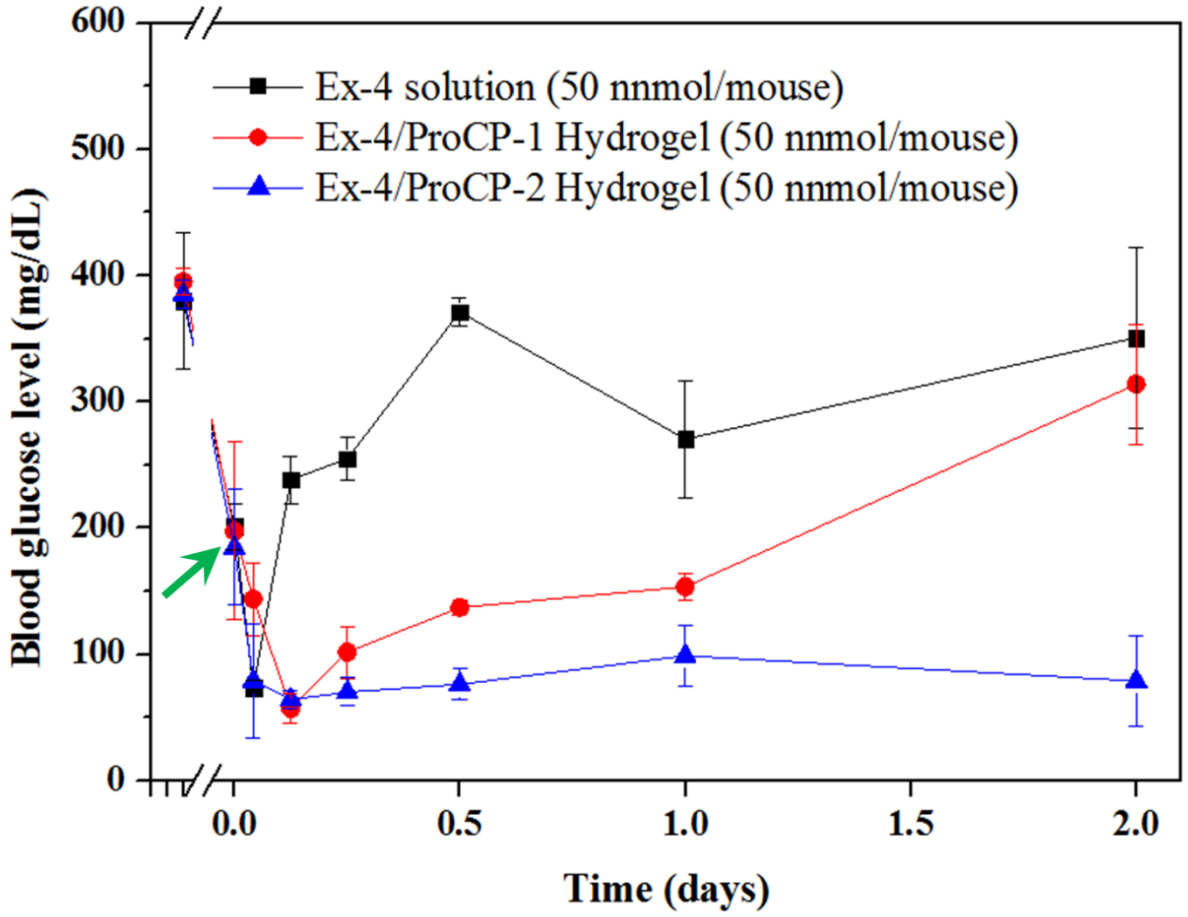


Figure S8. Pharmacodynamic studies of exendin 4 (Ex-4) release after one-time subcutaneous (SC) injection of the Ex-4/protamine-conjugated polymer (ProCP) nano-complex system in diabetic (*db/db*) mice. This magnified graph shows the early time points in Figure 6 (A). The green arrow indicates the injection point.

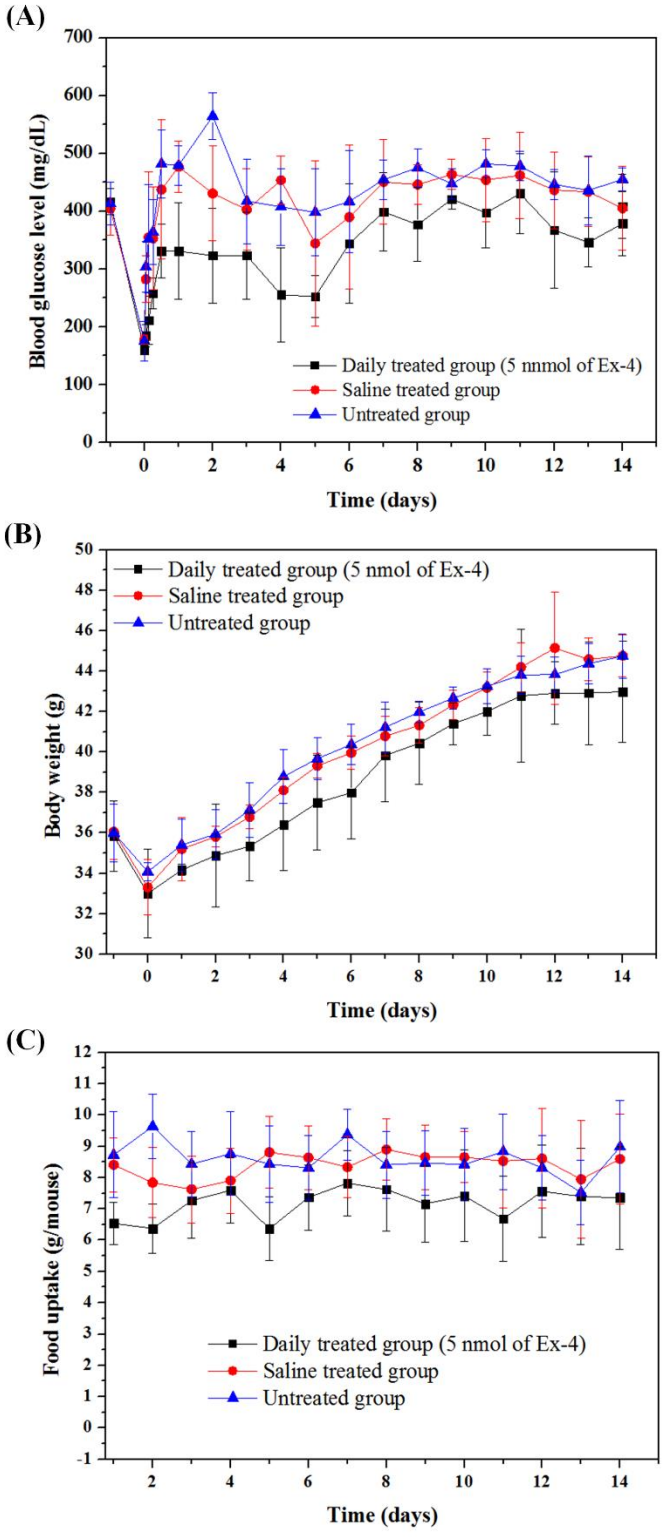


Figure S9. Pharmacodynamic studies of control groups (daily Ex-4 injection, daily saline injection, and untreated groups). (A) Blood glucose level, (B) body weight, (C) and food uptake were monitored for 14 days (n = 5).

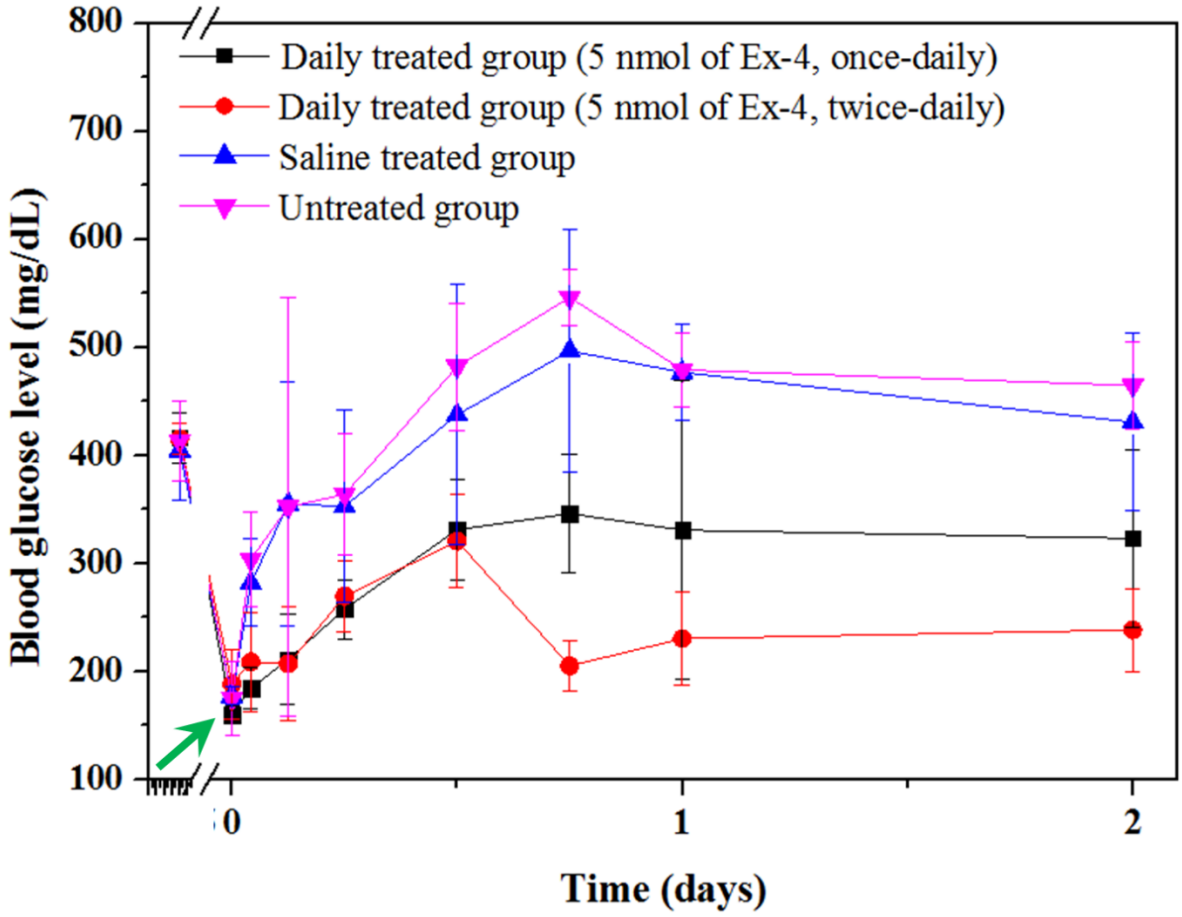


Figure S10. Blood glucose levels of control groups (once-daily Ex-4 injection, twice-daily injection, daily saline injection, and untreated groups) (n = 5). The green arrow shows the first injection point.