Supplementary Information

Optimization of GC/SA-PGGA double-layered nanogel's size

Blank SA-PGGA nanogel was prepared by gelation method using calcium dichloride. SA-PGGA was dissolved into deionized water, and then calcium dichloride solution (1.1 mg mL^{-1}) was treated with five different weight ratios of SA-PGGA to calcium dichloride. Each sample was vortexed for 5 min and then dialyzed against deionized water overnight (MWCO: 10000 g mol^{-1}). Blank GC/SA-PGGA double-layered nanogel was prepared by electrostatic interaction between SA-PGGA and GC. GC was treated with five different weight ratios of GC to SA-PGGA. Each sample was vortexed for 5 min and then dialyzed against deionized water (MWCO: 10000 g mol^{-1}). The particle size and zeta potential of all the samples were determined by DLS method and electrophoretic scattering method.

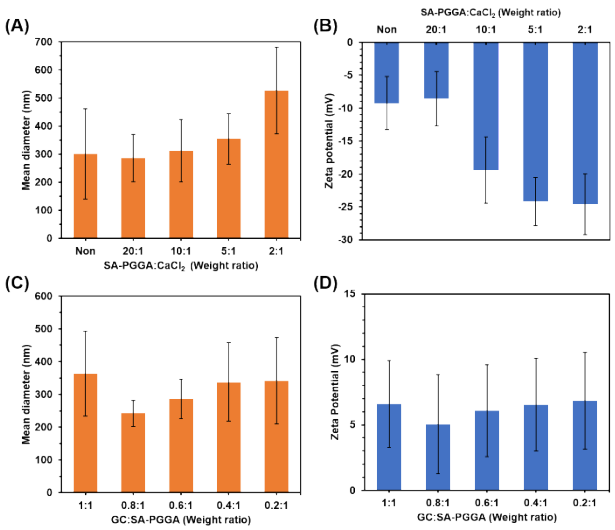

Figure S1 (a) Mean diameter of SA-PGGA nanogel with different weight ratio of SA-PGGA to CaCl2, (b) Zeta potential of SA-PGGA nanogel with different weight of SA-PGGA to CaCl2, (c) Mean diameter of GC/SA-PGGA double-layered nanogel with different weight ratio of GC to SA-PGGA, (d) Zeta potential of GC/SA-PGGA double-layered nanogel with different weight ratio of GC to SA-PGGA.

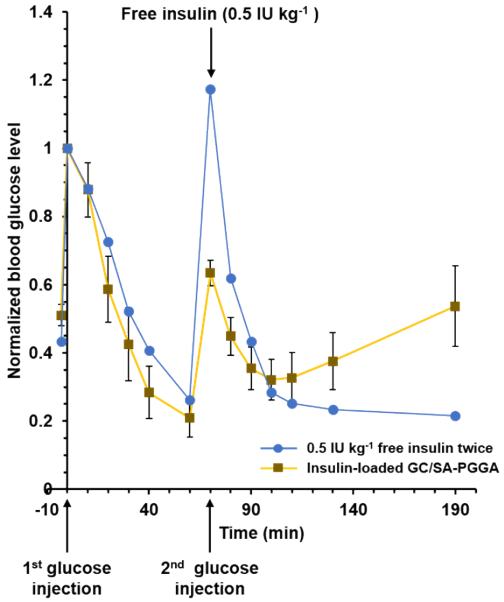

Figure S2 Normalized blood glucose levels in mice by retro-orbital injection of free insulin injected twice and insulin-loaded GC/SA-PGGA (n = 5). The error bars are expressed as standard error.

	Carbon Weight percent (%)	Nitrogen Weight percent (%)	Hydrogen Weight percent (%)
SA	29.941 ± 0.029	0.113 ± 0.016	4.607 ± 0.011
SA-PGGA	56.219 ± 0.251	7.094 ± 0.115	5.942 ± 0.031

Table S1 Elemental weight percent of SA and SA-PGGA by element analysis (n = 2). The results were presented with mean standard error.

	Mean diameter (nm)	Zeta potential (mV)	Loading efficiency (%)
SA-PGGA	343.5 ± 113.6 [a)]	-28.1 ± 4.7 [b)]	-
GC/SA-PGGA	767.9 ± 170.4	15 ± 3.37	71 ± 3.5[c)]

[a)] Determined by dynamic light scattering method. [b)] Determined by electrophoretic scattering method. [c)] Quantified by insulin standard curve using UV-Vis spectrometry.)

Table S2 Mean diameter, zeta potential and loading efficiency of insulin-loaded SA-PGGA and GC/SA-PGGA nanogel.

	190 min	250 min	260 min	270 min	280 min	290 min	300 min	320 min
Blank GC/SA-PGGA	0.9235 ± 0.0452	0.9061 ± 0.0359	0.9206 ± 0.0742	0.9349 ± 0.0392	0.9822 ± 0.0572	0.9469 ± 0.0541	0.9437 ± 0.0476	0.9793 ± 0.0692
Free insulin	0.5632 ± 0.0736	0.6213 ± 0.0766	1.0815 ± 0.0850	0.9145 ± 0.0890	0.8670 ± 0.0765	0.8633 ± 0.0727	0.8669 ± 0.0862	0.8562 ± 0.0859
Insulin-loaded GC/SA	0.6361 ± 0.0916	0.6005 ± 0.0559	1.1710 ± 0.1149	0.9371 ± 0.0974	0.9557 ± 0.1361	0.9221 ± 0.1187	0.9135 ± 0.1003	0.9269 ± 0.1031
Insulin-loaded GC/SA-PGGA	0.5370 ± 0.1318	0.5370 ± 0.1178	0.6235 ± 0.1290	1.2446 ± 0.2199	0.9094 ± 0.1022	0.8728 ± 0.0974	0.8561 ± 0.0963	0.8246 ± 0.0993

Table S3 Normalized blood glucose levels in mice by retro-orbital injection of blank GC/SA-PGGA, free insulin, insulin-loaded GC/SA and insulin-loaded GC/SA-PGGA from 250 to 320 min (n = 4). The results were presented with standard error.