ARTICLE

Thermo-sensitive Injectable Hydroxypropyl Chitin Hydrogel for The Sustained Salmon Calcitonin Release with Enhanced Osteogenesis and Hypocalcemic Effect

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Supporting Information

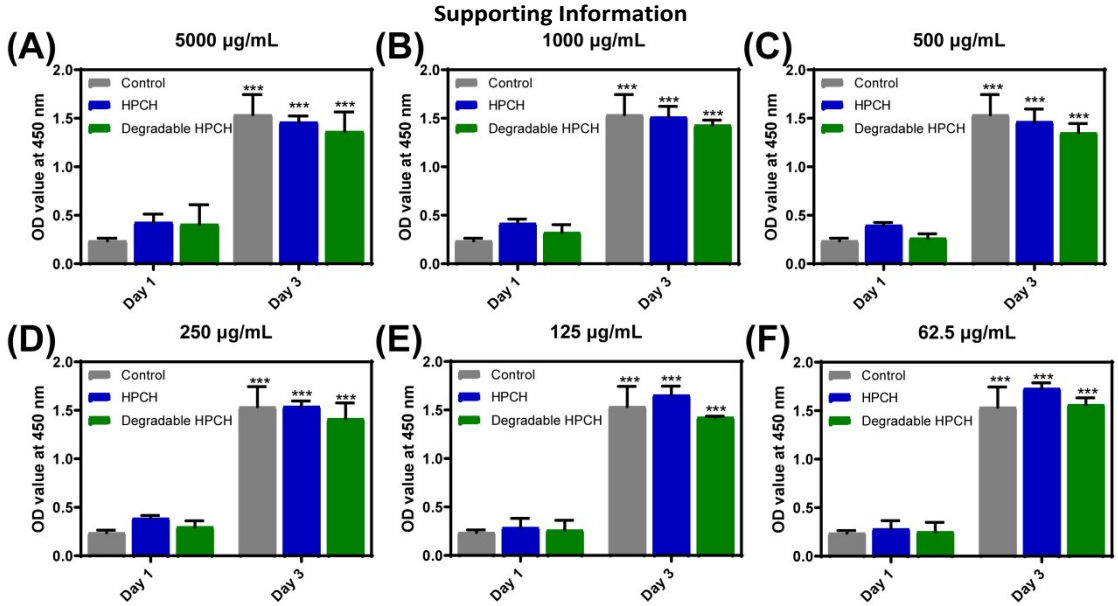

Figure S1. Proliferation of MC3T3-E1 cells co-cultured with HPCH and its degraded product at the concentration of (A) 5,000; (B) 1000; (C) 500; (D) 250; (E)125 and (F) 62.5 μg/mL. ****p*<0.001.

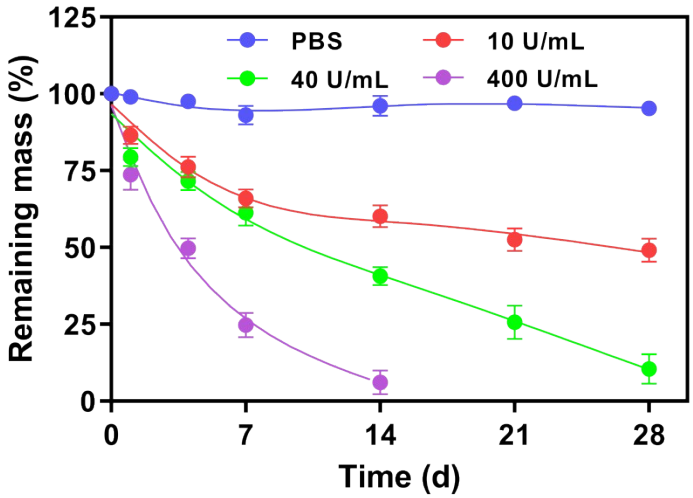

Figure S2. *In vitro* degradation of HA-HPCH in PBS and lysozyme solution (10, 40 and 400 U/mL, respectively).

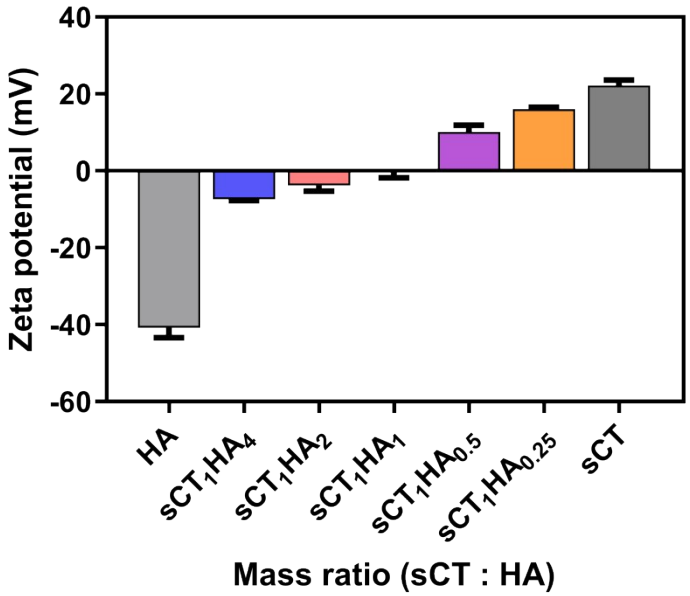

Figure S3. Zeta potential of HA, sCT, and sCT-HA complex fabricated at different mass ratio.

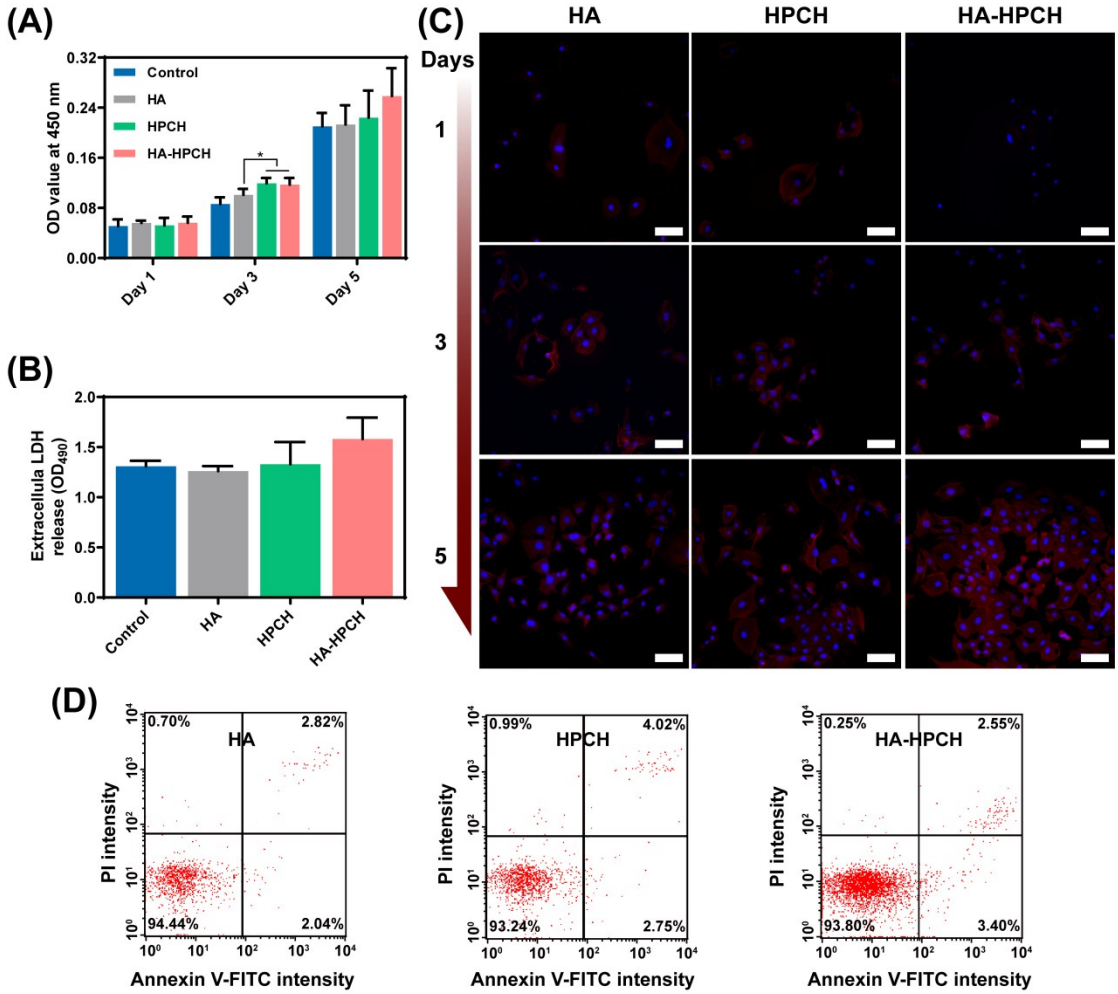

Figure S4. **Biocompatibility of sCT carriers.** (A) Proliferation and (B) LDH release, (C) morphology (scale bar: 100 μm) and (D) apoptosis of MC3T3-E1 cells co-cultured with α-MEM complete medium containing HA, HPCH and HA-HPCH. **p*<0.05.

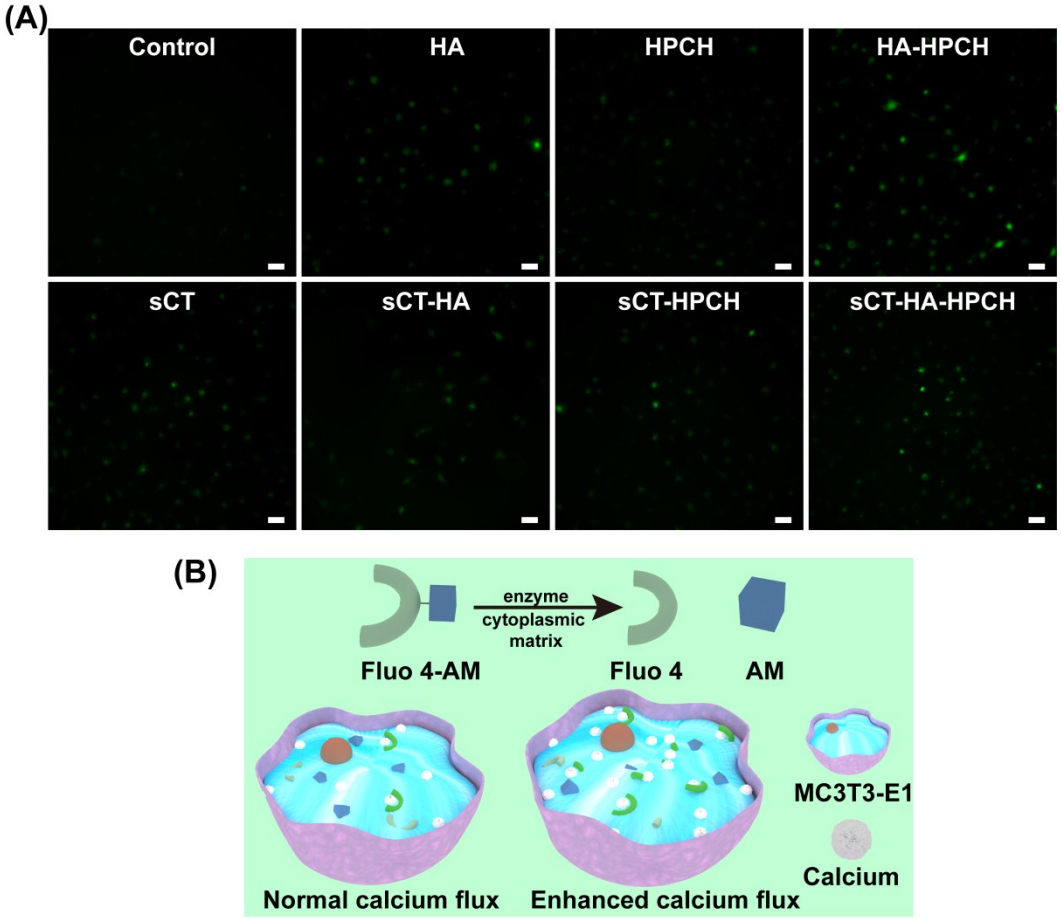

Figure S5. Calcium flux of MC3T3-E1 cells co-cultured with different materials. (A) Representative images (scale bar: 100 μm), and (B) the mechanism of enhanced calcium flux.

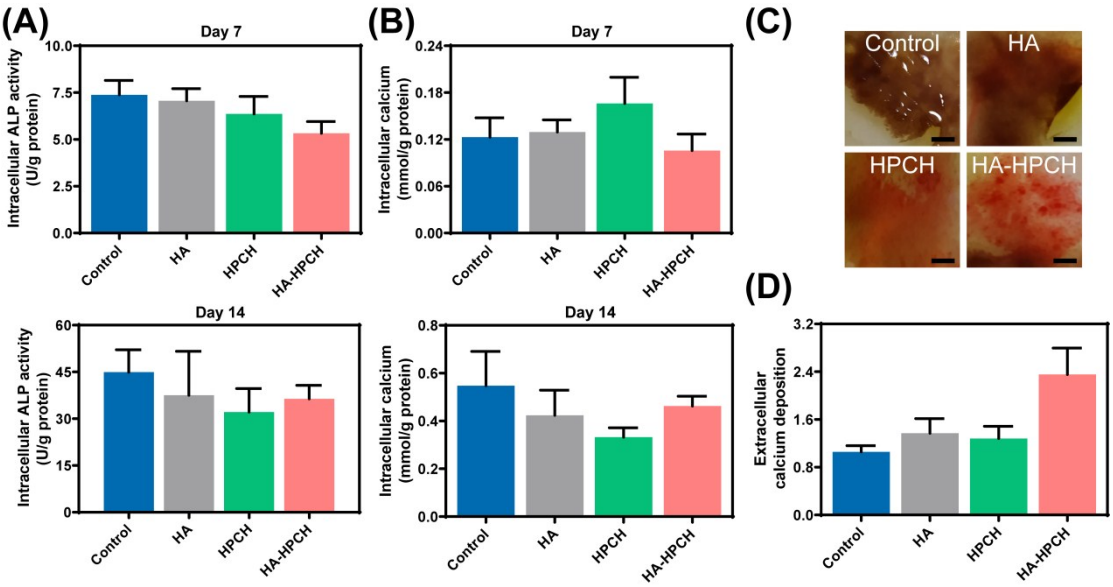

Figure S6. **Osteogenic differentiation of MC3T3-E1 cells co-cultured with different sCT carriers.** Intracellular ALP activity at (A) day 7 and day 14; (B) calcium concentration at Day 7 and Day 14; (C) images of extracellular calcium deposition (scale bar: 1 mm) and (D) quantitative analysis of mineralized nodules.

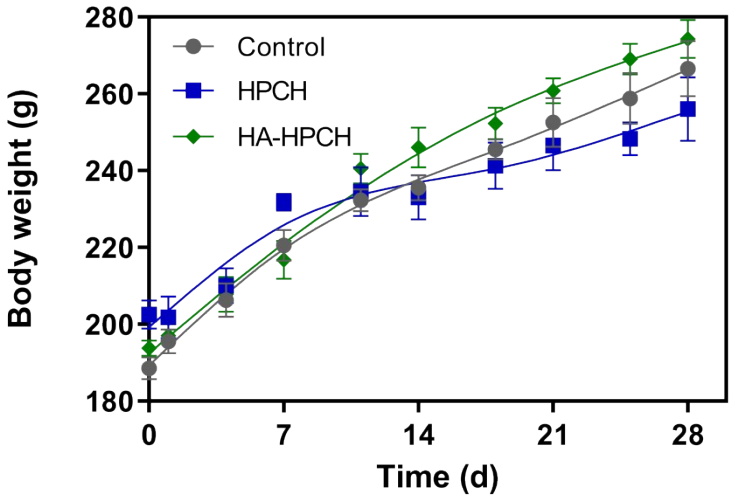

Figure S7. Body weight of SD rats subcutaneously injected by control (saline), HPCH and HA-HPCH at different time points.

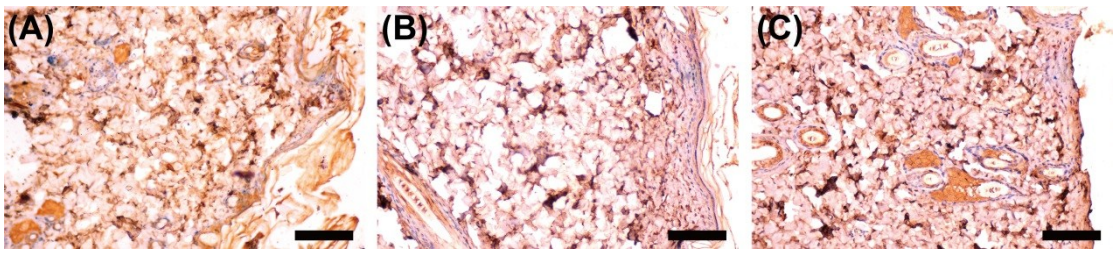

Figure S8. IgG staining of skins after subcutaneous injection of (A) Control (saline); (B) HPCH solution (2 wt%) and (C) HA-HPCH (2 wt% of HPCH) on Day 28. (scale bar: 200 μm)

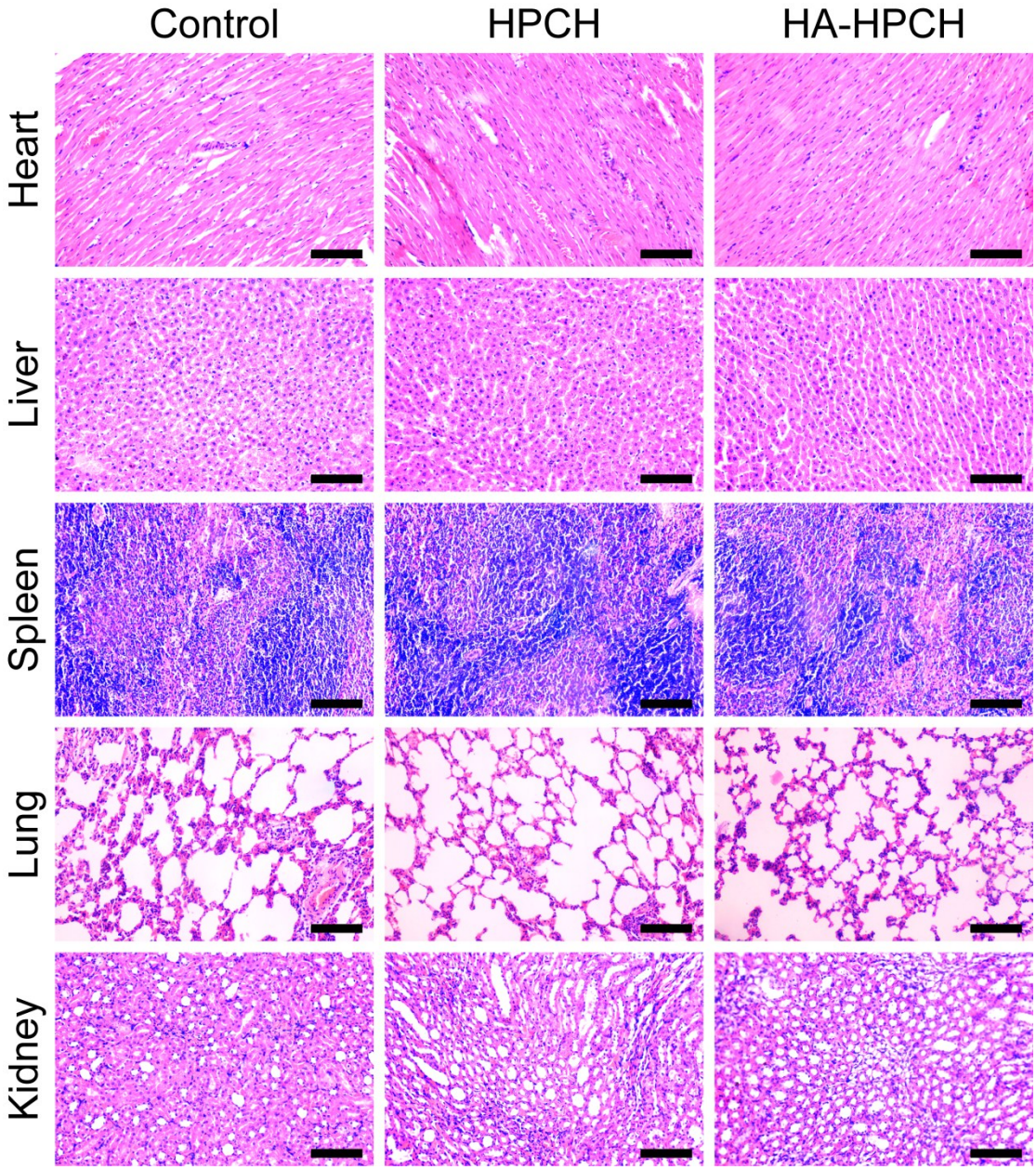

Figure S9. H&E staining of major organs (heart, liver, spleen, lung and kidneys) after subcutaneous injection of saline (Control), HPCH solution (2 wt%) and HA-HPCH (2 wt% of HPCH) on Day 28. (scale bar: 200 μm)

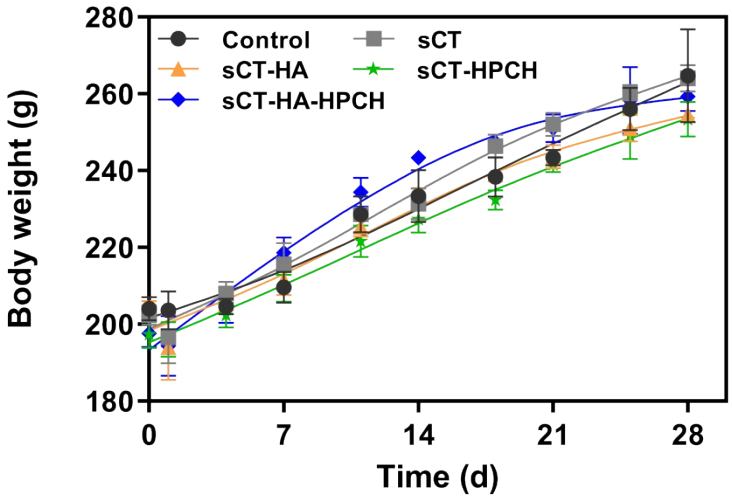

Figure S10. Body weight of SD rats subcutaneously injected by Control (saline), sCT, sCT-HA, sCT-HPCH and sCT-HA-HPCH on day 28.

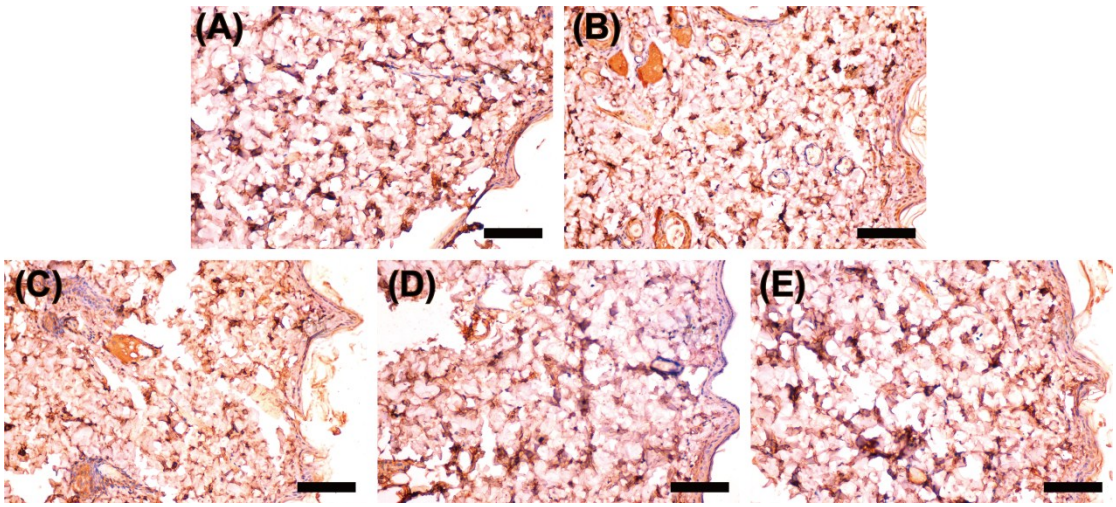

Figure S11. IgG staining of skins after subcutaneous administration of (A) Control (saline); (B) sCT; (C) sCT-HA, (D) sCT-HPCH and (E) sCT-HA-HPCH on Day 28 (scale bar: 200 μm).

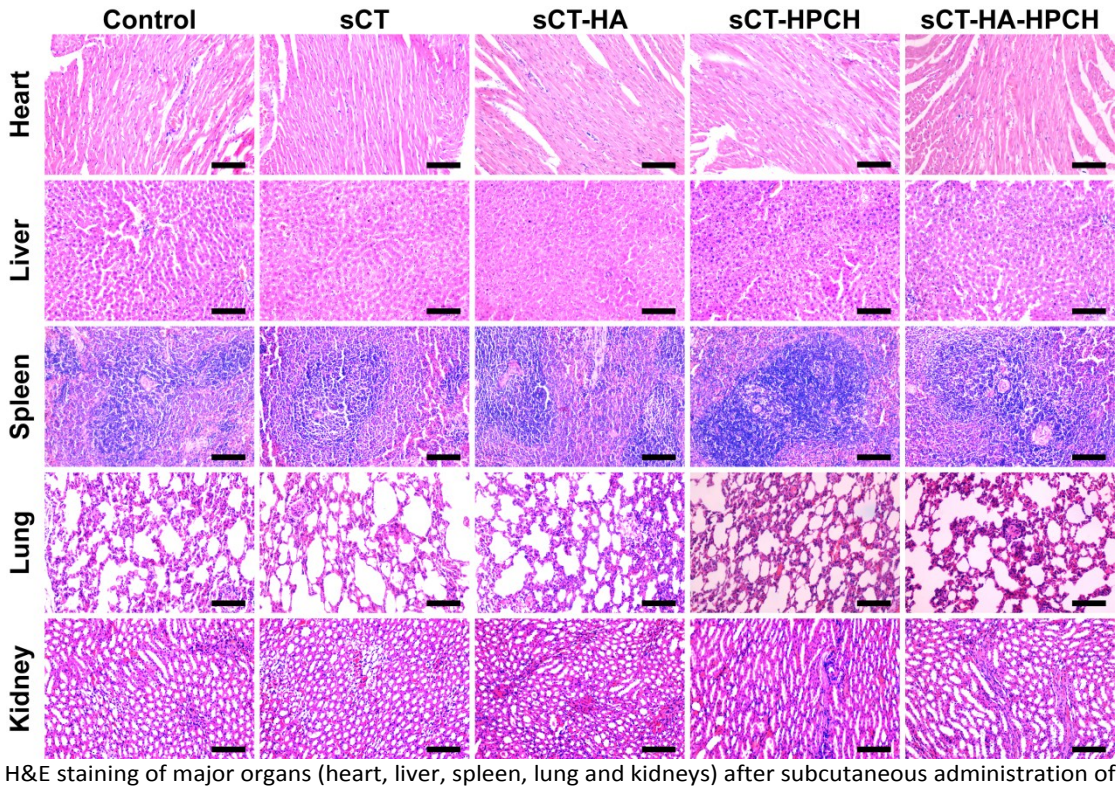

Figure S12. H&E staining of major organs (heart, liver, spleen, lung and kidneys) after subcutaneous administration of Control (saline), sCT, sCT-HA, sCT-HPCH, sCT-HA-HPCH on Day 28. (scale bar: 100 μm)

Table S1. The kinetics of *in vitro* sCT release.

Model	sCT [a]	sCT-HA	sCT-HPCH	sCT-HA-HPCH
Zero-order	R = 0.778 t + 0.278 (R^2 = 0.733)	R = 0.034 t + 0.055 (R^2 = 0.981)	R = 0.030 t + 0.043 (R^2 = 0.976)	R = 0.025 t + 0.022 (R^2 = 0.968)
First-order	Ln (1-R) = -2.304 t - 0.231 (R^2 = 0.970)	Ln (1-R) = -0.089 t + 0.071 (R^2 = 0.886)	Ln (1-R) = -0.075 t + 0.081 (R^2 = 0.725)	Ln (1-R) = -0.042 t - 0.022 (R^2 = 0.850)
Higuchi	R = 0.944 $t^{1/2}$ + 0.070 (R^2 = 0.948)	R = 0.171 $t^{1/2}$ - 0.058 (R^2 = 0.940)	R = 0.146 $t^{1/2}$ - 0.049 (R^2 = 0.896)	R = 0.120 $t^{1/2}$ - 0.052 (R^2 = 0.870)
Weibull	Lnln [1/(1-R)] = 0.676 ln t +1.161 (R^2 = 0.979)	Lnln [1/(1-R)] = 0.610 ln t – 1.875 (R^2 = 0.876)	Lnln [1/(1-R)] = 0.548 ln t – 1.995 (R^2 = 0.941)	Lnln [1/(1-R)] = 0.531 ln t -2.368 (R^2 = 0.846)
Ritger-peppas	Ln R = 0.468 ln t + 4.669 (R^2 = 0.941)	Ln R = 0.498 ln t + 2.563 (R^2 = 0.929)	Ln R = 0.457 ln t + 2.474 (R^2 = 0.910)	Ln R = 0.471 ln t + 2.145 (R^2 = 0.883)

[a] The equation was fitted within 1 day.
R: Cumulative release.

Table S2. The pharmcokinetic parameters of *in vivo* sCT release.

Group	Cmax (pg/mL)	Tmax (min)	Relative Bioavailability （%）	Cmin	Tmin (h)	Relative pharmacological availability (%)
sCT	64.67 ± 9.13	60	-	70.73 ± 0.68	12	-
sCT-HA	97.56 ± 5.32	60	95.43 ± 5.79	66.11 ± 2.52	24	156.77 ± 10.56
sCT-HPCH	84.38 ± 10.07	60	129.44 ± 26.23	75.32 ± 6.26	12	162.49 ± 17.51
sCT-HA-HPCH	91.43 ± 8.96	60	178.64 ± 17.00	65.36 ± 2.34	24	236.12 ± 18.31