Supporting information

Crosslinked zwitterionic microcapsules to overcome gastrointestinal barriers for oral insulin delivery

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Materials

PVA (M_n = 1.6 kg/mol, OH degree: 85%), triethylamine (Et_3N, 99%), 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (I2959, 98%), span 80 (99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro (EDC), diethyl malonate (99%), *N*-hydroxysuccinimide (NHS), sulfo-cyanine 5-NH_2 (Cy5, 95%), fluorescein isothiocyanate (FITC, 95%), 3,6-dioxane-1,8-octanedithiol (98%), and glycerin (98%) were purchased from Energy Chemical (Shanghai, China) and used as received. ε-Caprolactone (ε-CL, 98%, Aldrich) was dried with calcium hydride and then distilled under reduced pressure. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), β-propiolactone (> 95%), *N,N*-dimethylaminopropyl acrylamide (99%), and streptozocin (STZ, 98%) were acquired from Macklin (Shanghai, China). Insulin (bovine pancreas, 27 U/mg) was purchased from Shyuanye (Shanghai, China). Mucin was obtained from Diamond (Shanghai, China). Betaine was acquired from Solarbio (Beijing, China). L-tryptophan and anti-occludin antibodies were provided by Sigma-Aldrich. The molecular probe of anti-rabbit IgG Fab2 Alexa Fluor 488 was acquired from Cell Signaling Technology (USA). Bovine insulin ELISA Kit was obtained from AiFang Biological (Changsha, China).

Caco-2 and HT-29 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with high glucose, 1% NEAA, 1% L-glutamine, 1% penicillin and streptomycin, and 10% fetal bovine serum at 37 °C with 5% CO_2.

Table S1. Encapsulation efficiency and loading capacity of microcapsules

Sample	Loading capacity (%)	Encapsulation efficiency (%)
CB-MCs@INS	42.3	98.7
MCs@INS	37.8	98.5

Table S2. Various pharmacokinetic parameters of tested formulations after administration in Type 1 diabetic mice.

Formulation	AUC (mIU·h/L)	AAC (%·h/L)	*A*%	*B%*
INS (s.c.) 5 U/kg	214.4 ± 39.52	200.8±39.52	100	100
INS (p.o.) 140 U/kg	10.04 ± 4.479	12.1±11.53	0.44 ± 1.96	0.18 ± 0.18
MCs@INS (p.o.) 140 U/kg	35.09 ± 6.419	38.92±10.74	0.692 ± 0.027	0.585 ± 0.074
CB-MCs@INS (p.o.) 140 U/kg	333.5 ± 20.65	402.9±25.13	14.66 ± 0.36	5.59 ± 0.363

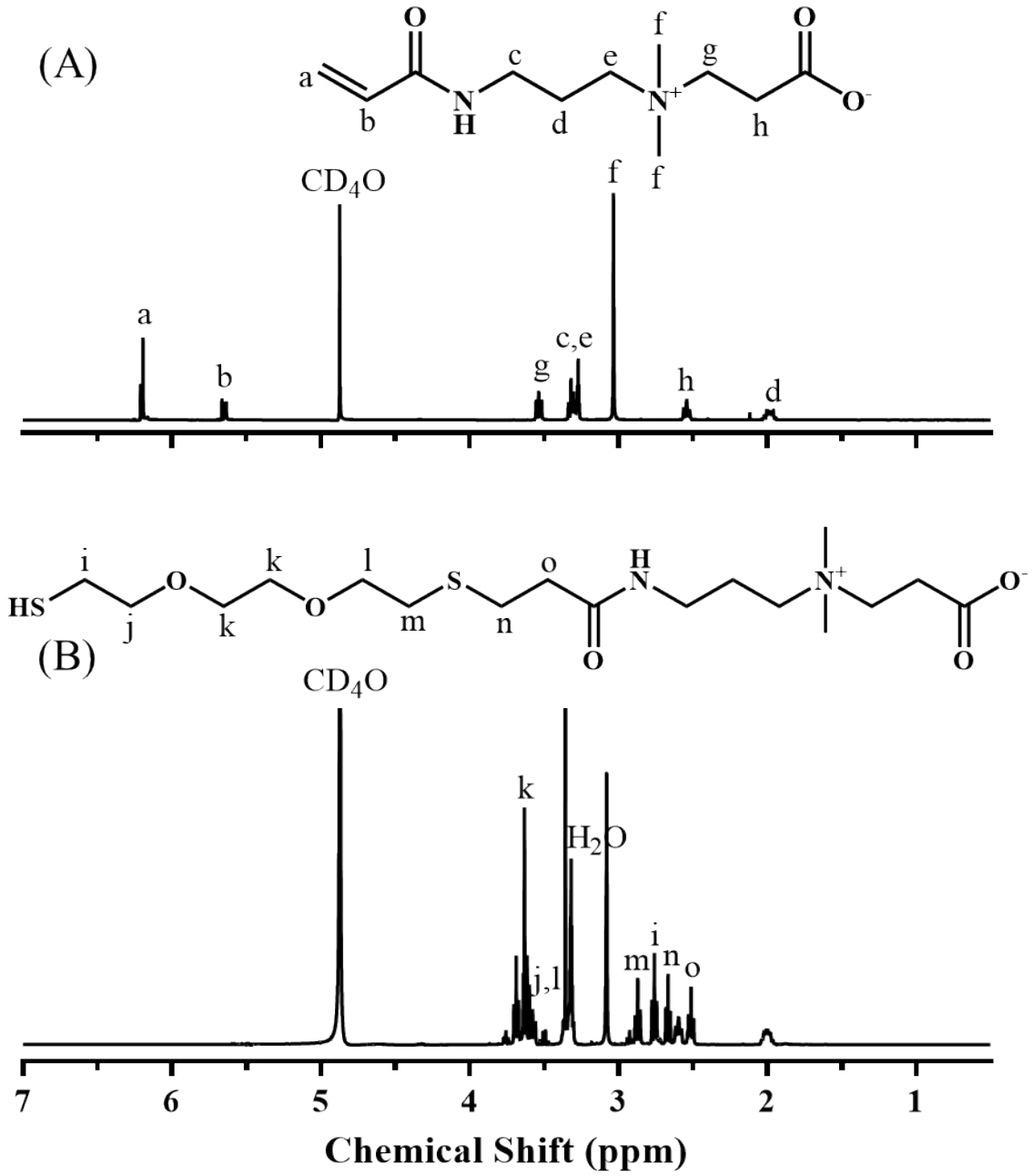

Fig. S1 ^{1}H NMR of (A) CB (400 MHz, CD_4O), and (B) TCB (400 MHz, CD_4O)

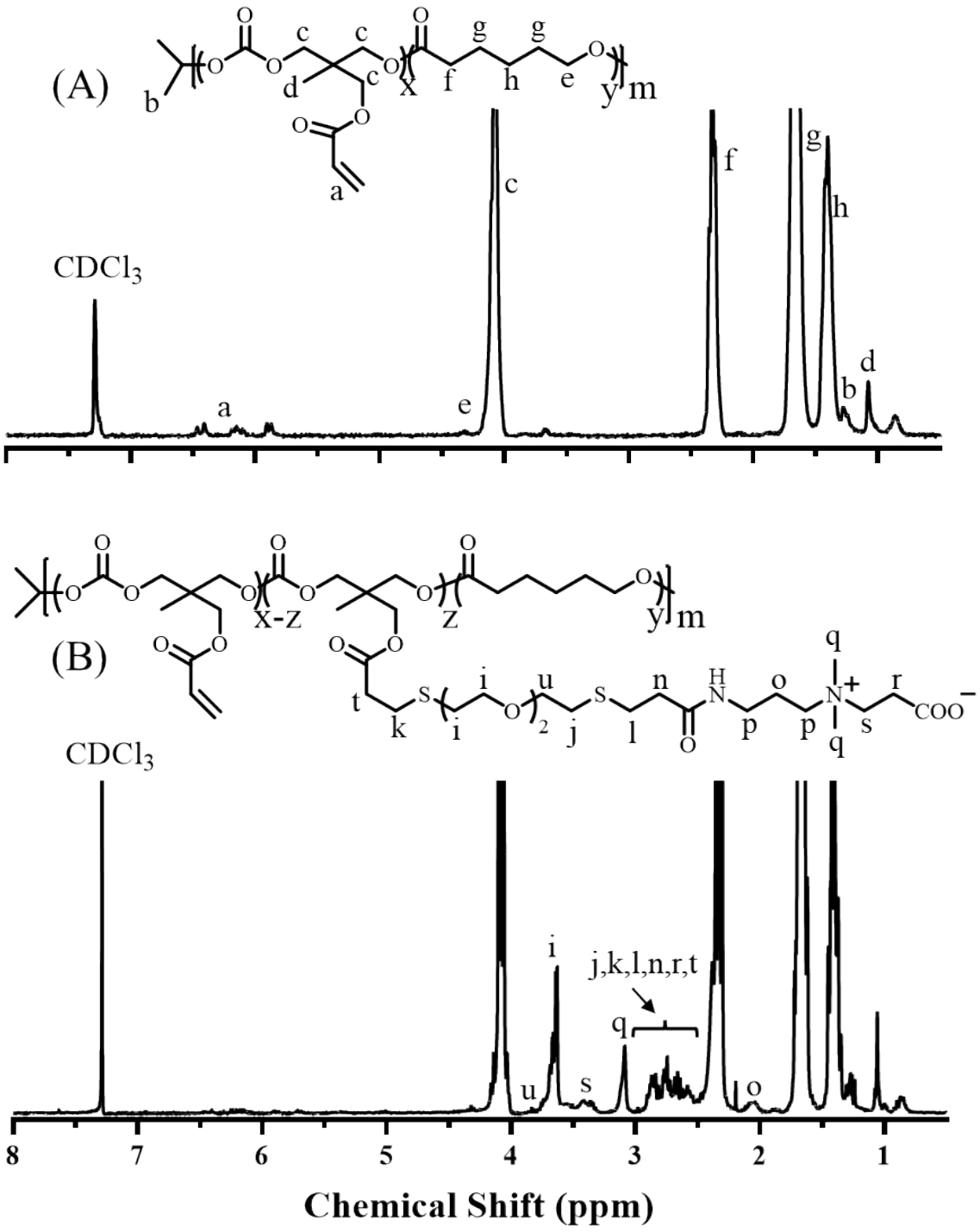

Fig. S2 (A) ^{1}H NMR of P(AC-CL) (400 MHz, $CDCl_3$) (B) ^{1}H NMR of P(AC_{CB}-AC-CL) (400 MHz, $CDCl_3$)

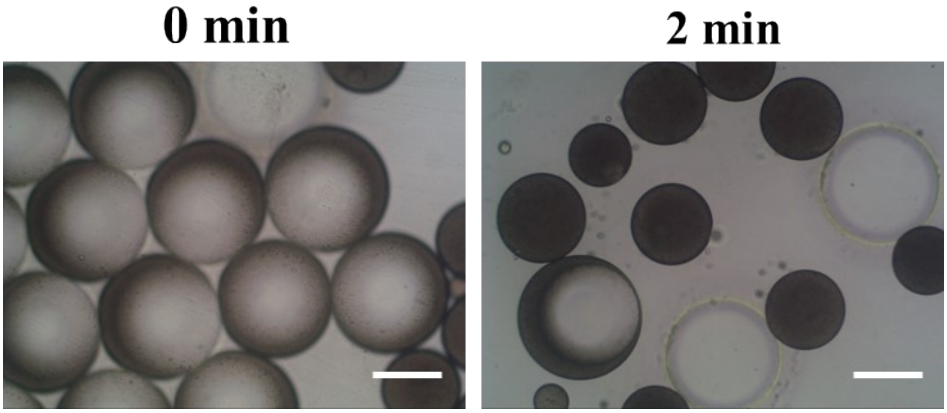

Fig. S3 The optical image of microcapsules degradation without UV-crosslinking ruptured after 2 min, scale bar: 100 μm.

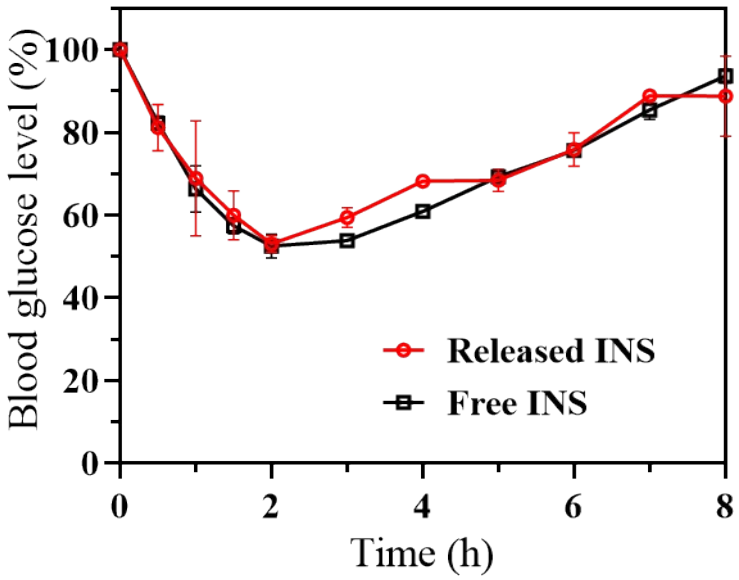

Fig. S4 Blood glucose response of normal mice after subcutaneous injection of equivalent doses of free insulin or insulin released from microcapsules (5 U/kg).

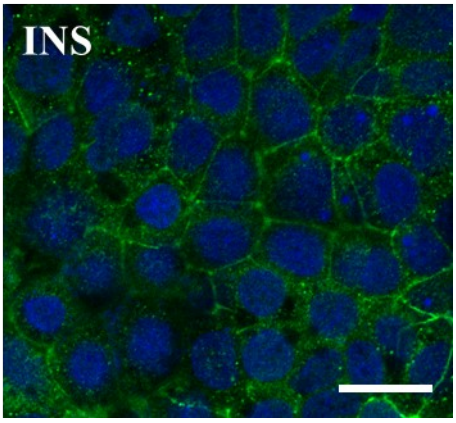

Fig. S5 Tight junctions were observed at different time points after being treated with INS (Scale bar: 20 μm).

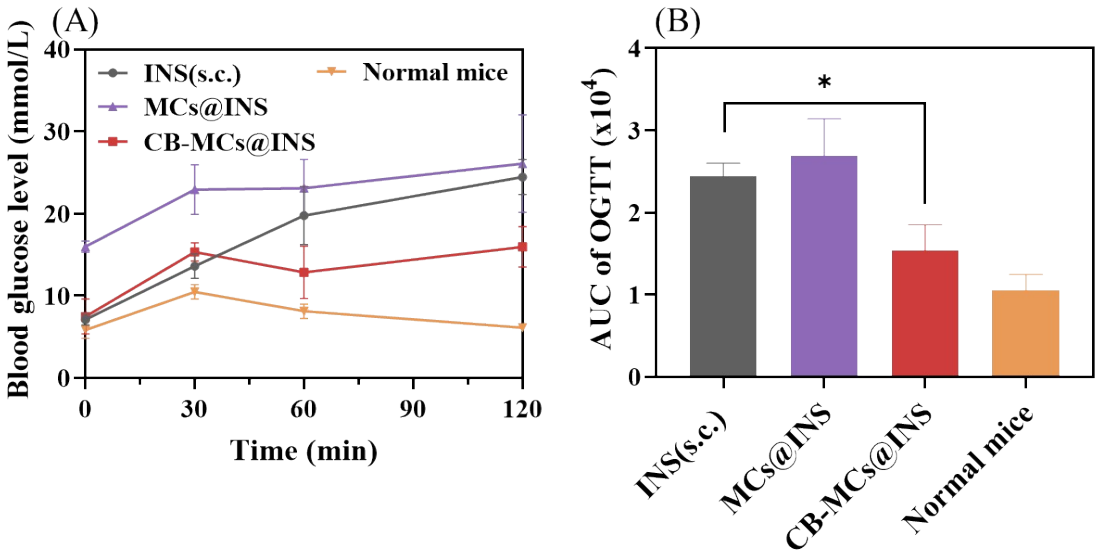

Fig. S6 (A) Glucose tolerance tests of diabetic mice at 1.5 h post-administration of CB-MCs@INS, MCs@INS, or subcutaneously injected with insulin; normal mice served as control. (B) Responsiveness was calculated based on the area under the curve in 120 min.

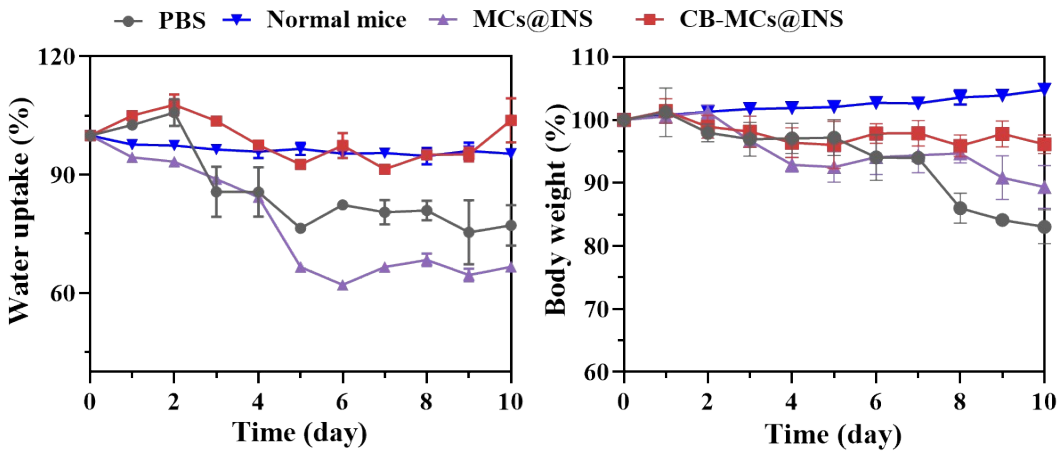

Fig. S7 Daily changes with water uptake and body weight in diabetic mice by orally administered different formulations for 10 consecutive days