Supporting Information for

N-Trimethyl Chitosan Chloride-Coated PLGA Nanoparticles Overcoming Multiple Barriers to Oral Insulin Absorption

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Table S1: Mean Size, Polydispersity Index (PDI), ζ-Potential, Encapsulation Efficiency (EE) and Drug-Loading Capacity (DLC) of Fluorescein- or ^{125}I-Labeled NPs (*n*=3).

NPs	size (nm)	PDI	ζ-potential (mV)	EE (%)	DLC (%)
RB-Ins PLGA NPs	**137.1±5.3**	**0.179±0.077**	**-12.0±4.2**	**48.6±3.8**	**8.3±0.7**
RB-Ins TMC-PLGA NPs	**232.7±8.4**	**0.231±0.021**	**42.5±6.8**	**47.4±5.1**	**7.5±0.2**
^{125}I-Ins PLGA NPs	**128.5±5.9**	**0.111±0.076**	**-11.9±3.2**	**48.5±3.1**	**8.2±0.2**
^{125}I-Ins TMC-PLGA NPs	**252.2±7.3**	**0.210±0.089**	**44.6±7.1**	**47.3±4.7**	**7.6±0.7**
RB-Ins FPR648-PLGA NPs	**127.8±5.5**	**0.121±0.012**	**-12.6±3.7**	**48.6±4.0**	**8.3±0.5**
RB-Ins TMC-FPR648-PLGA NPs	**262.5±6.9**	**0.283±0.058**	**41.3±4.9**	**45.5±3.4**	**7.4±0.5**

Insulin was labeled with fluorescein Rhodamine B (RB-Ins) or radioactive isotope ^{125}Iodine (^{125}I-Ins) for *in vitro* or *in vivo* evaluation. Fluorescein FPR648 labeled PLGA (FPR648-PLGA) was used in nanoparticles preparation for *in vitro* visualization. The RB-Ins loaded NPs (RB-Ins TMC-PLGA NPs, RB-Ins PLGA NPs) or ^{125}I-Ins loaded NPs (^{125}I-Ins TMC-PLGA NPs, ^{125}I-Ins PLGA NPs) were prepared using the same method as insulin loaded NPs. The RB-Ins loaded FPR648-labeled NPs (RB-Ins TMC-FPR648-PLGA NPs, RB-Ins FPR648-PLGA NPs) were prepared using FPR648-PLGA instead of PLGA by the same method.

The fluorescein or radioisotope labeling of insulin or PLGA had no significant effect on the size, ζ-potential, EE or DLC of the nanoparticles (Table S1). Therefore, fluorescein- or radioisotope-labeled nanoparticles could be used for *in vitro* visualization or *in vivo* tracking.

Table S2: The BGL of Diabetic Rats in Different Groups. (*n*=6)

BGL (mmol/L)	PBS	Ins	Ins PLGA NPs	Ins TMC-PLGA NPs	Ins TMC-PLGA NPs	Ins TMC-PLGA NPs	Ins
		50 IU/kg	20 IU/kg	20 IU/kg	10 IU/kg	40 IU/kg	2 IU/kg
time (h)	ig.	ig.	ig.	ig.	Ig.	Ig.	sc.
0	21.1±3.2	22.4±3.9	20.4±1.9	20.4±1.9	20.6±2.2	22.3±3.6	19.8±2.9
0.5	24.9±4.2	24.7±3.6	20.1±1.8	20.1±1.8	20.9±3.5	23.6±3.1	12.6±3.6
1	23.5±4.4	23.7±3.5	21.1±2	21.1±2	21.7±3.6	23.2±3	8.5±2.5
1.5	23.7±4.3	23.2±3.3	22±2	22±2	20±4.2	21.4±2.8	6.9±2.8
2	24.1±4.1	22.7±3.2	21.5±2.3	21.5±2.3	18.8±3.7	19.8±2.6	5.5±2.3
3	23.5±5	21.9±3.1	20.3±1.9	20.3±1.9	18.4±3.9	18.5±2.4	9.3±2.4
4	23.7±4.1	22.8±3.3	19±2	19±2	17±3.7	16.7±2.2	11.4±2.7
5	22.6±3.7	22.9±3.4	19±2.1	19±2.1	15.7±3.8	15.6±2	15.2±2.8
6	22.4±3.6	24±3.4	18.4±2.7	18.4±2.7	15.2±3.2	14.5±1.9	19.4±3.3
7	23.4±4.1	23.9±3.3	18±2.1	18±2.1	14.3±2.6	13.4±1.7	19.5±3.6
8	21.8±3.4	24.3±3.4	17.9±1.6[a]	17.9±1.6	13.6±2.3	13.1±1.7	20.1±3.5
10	22.8±3.9	23.7±3.3	17.5±1.7[a]	17.5±1.7[b]	12.7±2.3	12.6±1.7	21.4±5
12	22.7±4.3	23.5±3.3	17.1±2[a]	17.1±2[b]	12.4±1.9	12.2±1.7	21.2±4.8

[a] $P<0.05$, compared with Ins solution 50 IU/kg ig.; [b] $P<0.05$, compared with Ins PLGA NPs 20 IU/kg ig.; [c] $P<0.05$, compared with Ins TMC-PLGA NPs 20 IU/kg ig..

Because of the difference of basal BGL of each diabetic rats, the hypoglycemia effect was measured by calculating the percentage of BGL to the initial level individually.

Table S3: Percentage of BGL to the Initial Level in Diabetic Rats of Different Groups. (*n*=6)

percentage of initial BGL (%)	PBS	Ins	Ins PLGA NPs	Ins TMC-PLGA NPs	Ins TMC-PLGA NPs	Ins TMC-PLGA NPs	Ins
time (h)	ig.	50 IU/kg ig.	20 IU/kg ig.	20 IU/kg ig.	10 IU/kg Ig.	40 IU/kg Ig.	2 IU/kg sc.
0	100	100	100	100	100	100±0	100
0.5	118±4.6	110.7±7	98.5±5.9	101±6.5	109.7±5.7	107±13.8	63±9.2
1	111.4±8.2	106.3±6.5	103.2±12	105±6.4	109±11.7	104.8±9.1	42.4±6.2
1.5	112.3±7.3	104±8.3	107.6±6.4	96.7±10	100.4±7.1	96.8±10.2	33.9±9
2	114.2±5.1	101.8±10.1	105.2±14.8	91±8.2	99.5±8.3	89.5±9.6	26.9±7.6
3	111±12.6	98.4±8.1	99.5±6.3	89±9.3	97.5±8.4	83.6±8.2	46.5±5.1
4	112.5±5.5	102.4±7.6	93.2±13.1	82±14	96.8±4.1	75.6±9.4	57.2±5.4
5	106.9±3.1	102.5±5.7	93.3±9.3	75.8±10.5	94.8±5.8	70.5±7.1	76.8±3.1
6	106.1±3	107.7±8.9	90.4±12.9	73.3±7.9	91.6±5.9	65.8±9.2	98.1±2.4
7	110.8±5.9	107.5±13.7	88.1±9.3	69±5.5[b]	90.2±8.7	60.8±7.2	98.3±4
8	103.3±1.7	109.6±14	87.8±5.2	66±4[bb]	89.7±9.1	59.4±6.6	101.5±2.8
10	108±5	106.3±10.5	85.7±6.8[a]	69.4±4.4[b]	86.6±9.3[c]	57.1±8.2	107.4±9.6
12	107.6±8.5	105.6±9.3	84±9.1[a]	63.1±3.1[b]	82.5±10.4[c]	55.4±8.3	106.2±8.5

[a] $P<0.05$, compared with Ins solution 50 IU/kg ig.; [b] $P<0.05$, compared with Ins PLGA NPs 20 IU/kg ig.; [bb] $P<0.01$, compared with Ins PLGA NPs 20 IU/kg ig.; [c] $P<0.05$, compared with Ins TMC-PLGA NPs 20 IU/kg ig..

Because it is difficult to increase further the insulin-encapsulation efficiency of the TMC-PLGA NPs, the concentration of the NPs in the suspension administered to diabetic rats had to be increased to reach higher dose of insulin. In particular, when the dose of insulin increase from 20 IU/kg to 40 IU/kg for orally delivered Ins TMC-PLGA NPs, the concentration of the NPs was doubled in the suspension administered. As indicated by the results of nanoparticles' stability in SIF or SGF, the ζ-potential of TMC-PLGA NPs might decrease in the GI tract, inducing aggregations of nanoparticles. The hypoglycemia effect of orally delivered Ins TMC-PLGA NPs failed to increase when the dose of insulin increased to 40 IU/kg, probably due to the aggregation of nanoparticles in the GI tract.

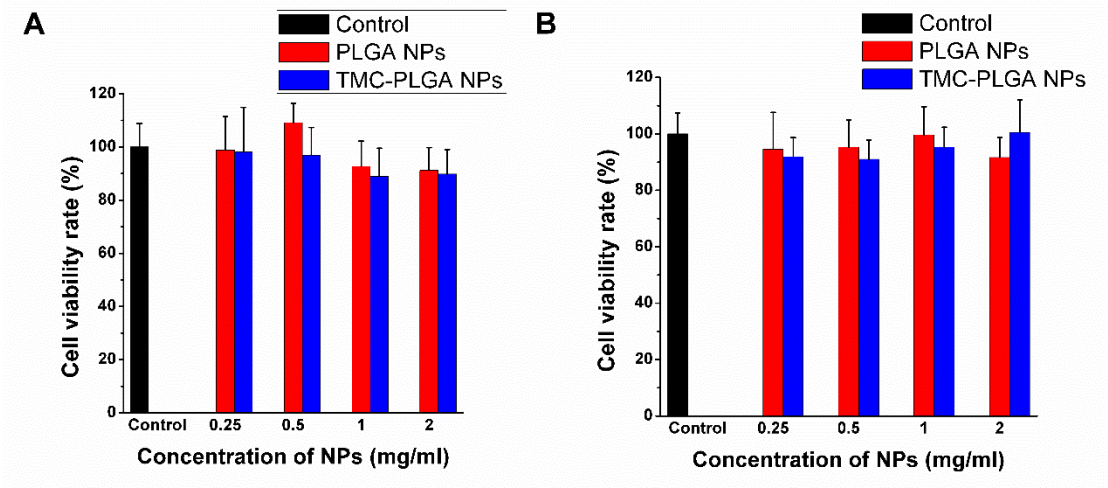

Figure S1: Effects of PLGA NPs and TMC-PLGA NPs on the viability of Caco-2 cells (A) and HT29-MTX cells (B) (*n*=6). The cells were incubated with PLGA NPs or TMC-PLGA NPs for 2 h at 37°C before the MTT assay.

Both TMC-PLGA NPs and PLGA NPs exhibited no significant impact on the viability of HT29-MTX or Caco-2 cells in the MTT assay, indicating that the TMC-PLGA NPs and PLGA NPs had no cytotoxic effects at or below a concentration of 2 mg/mL.