Electronic Supplementary Material (ESI) for Nanoscale.
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Supplementary Information

Scalable production of core-shell nanoparticles by flash nanocomplexation to enhance mucosal transport for oral delivery of insulin

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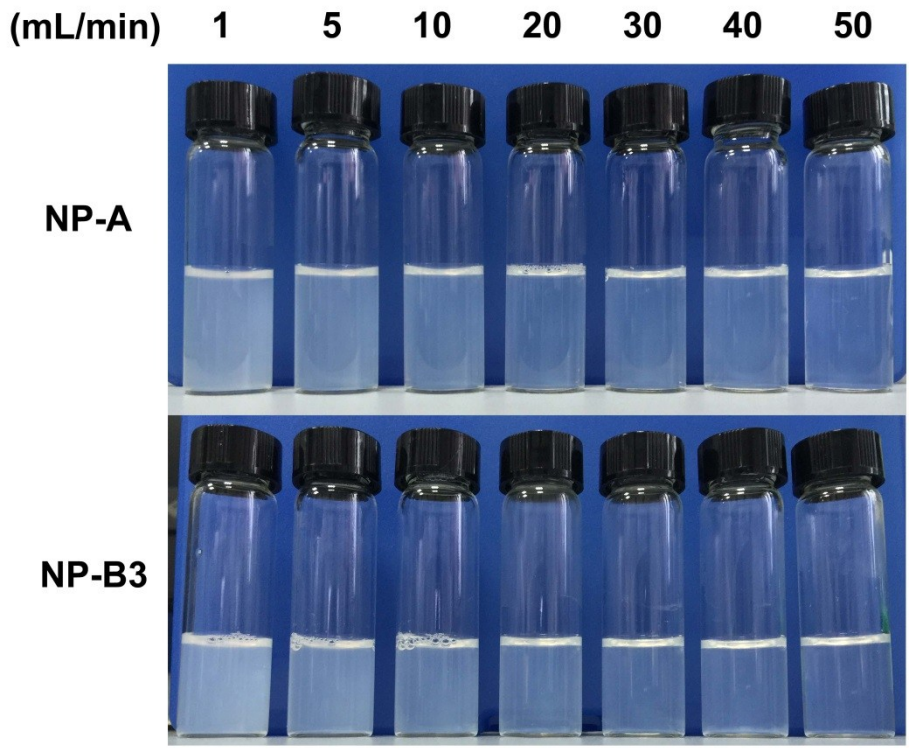

Fig. S1. The optical photos of samples (NP-A, NP-B3) fabricated under different flow rates (1 - 50 mL/min).

Table S1. Physicochemical properties of freshly prepared and reconstituted

NP-B3 nanoparticles upon lyophilization

Parameter	Freshly Prepared	Reconstituted
Z-average diameter	104.5 ± 3.2 nm	102.4 ± 4.1 nm
Zeta potential	-20.1 ± 0.5 mV	-19.2 ± 0.7 mV
Polydispersity index	0.07 ± 0.01	0.08 ± 0.02
Encapsulation efficiency	97.1 ± 1.7%	95.8 ± 2.4%
Loading content	66.6 ± 0.5%	66.3 ± 0.6%
pH	6.3	6.3
Rehydration time	~	~10 sec

Note: Data are shown as means ± SD (n = 6). The cryoprotectant applied in lyophilization is 2% sorbitol (w/v).

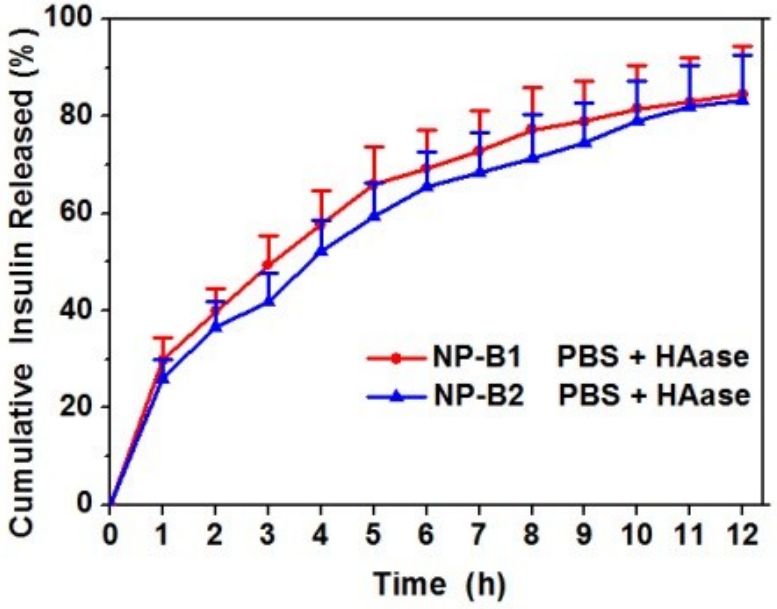

Fig. S2. *In vitro* release profiles of insulin from NP-B1 and NP-B2 in PBS (pH 7.4) with hyaluronidase (0.01 mg/mL). Data are shown as means ± SD (n = 4).

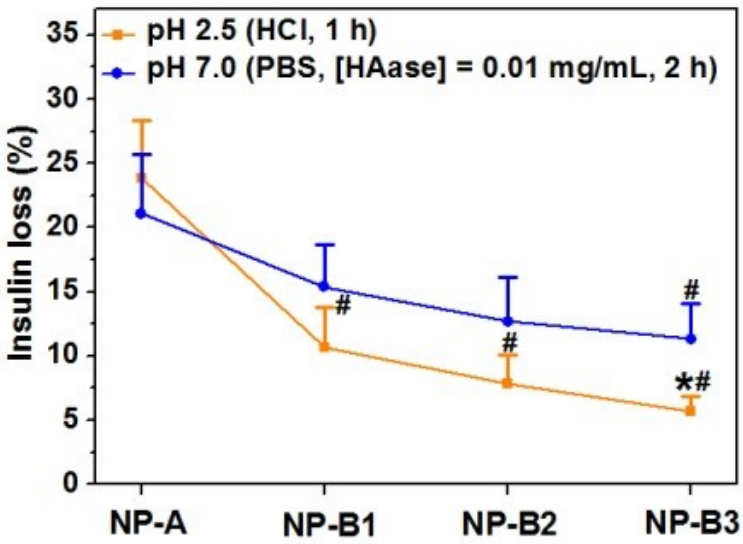

Fig. S3. Effect of medium pH on insulin loss from tested NPs *in vitro* at pH 2.5 and 7.0, simulating the pH environments in the fasting stomach and mucus layer, respectively. Data are shown as means ± SD (n = 6).

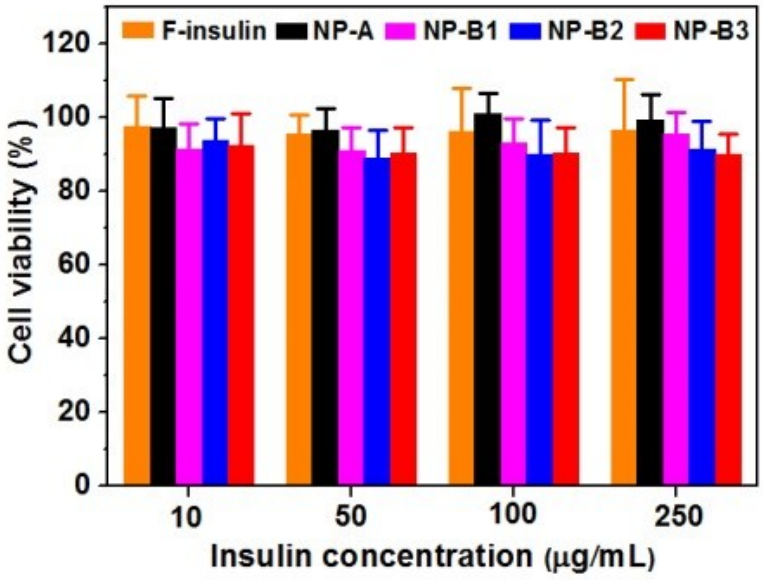

Fig. S4. Cytotoxicity of all tested NPs on HT29-MTX cells. Data are shown as means ± SD (n = 6).

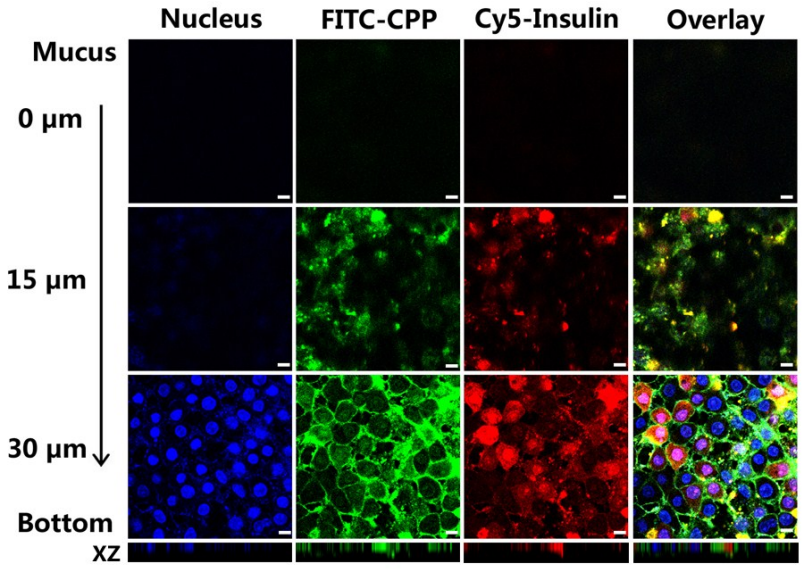

Fig. S5. Insulin transport across mucus layer covered on HT29-MTX cell monolayer visualized by confocal microscopy imaging. NP-B2 (HA coating: 35 kDa) was prepared from Cy5-labeled insulin and FITC-labeled CPP and incubated with HT29-MTX monolayer for 3 h before imaging. Cell nuclei were stained with DAPI (shown in blue). Scale bars: 10 μm.

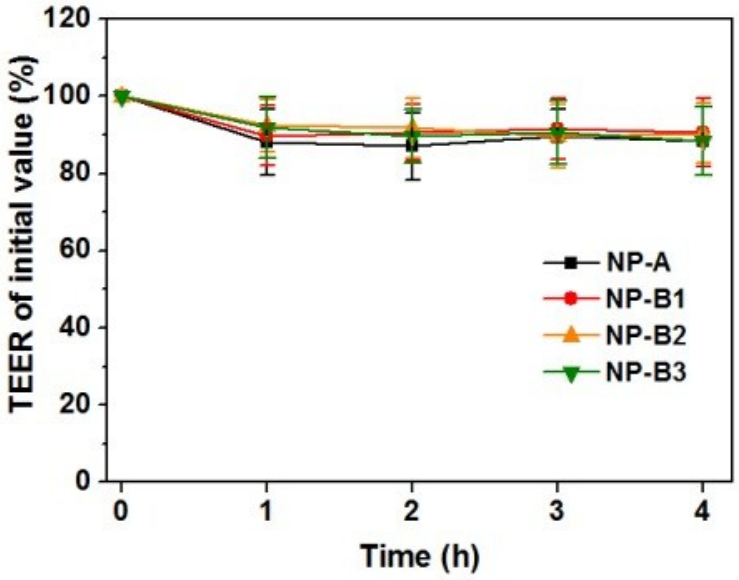

Fig. S6. Effect of tested NPs on TEER values of Caco-2 cell monolayer. The TEER value was presented as the percentage of the initial value before this test. Data are shown as means ± SD (n = 6).

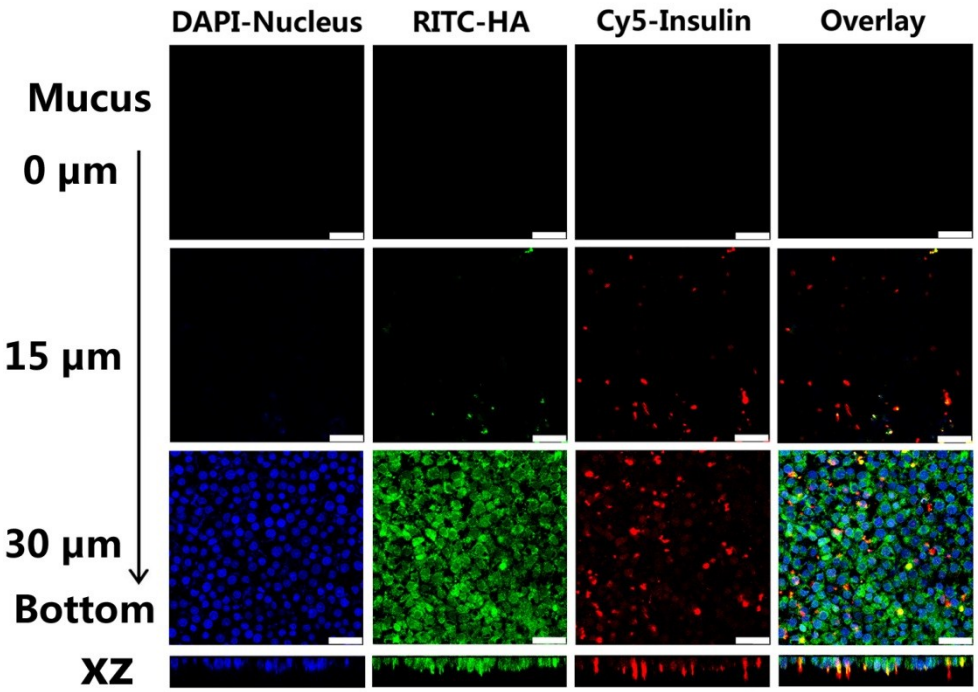

Fig. S7. CLSM images of the distribution of HA on mucus layer of HT29-MTX cell monolayer from apical to bottom side. NP-B2 (HA coating: 35 kDa) was prepared from Cy5-labeled insulin and RITC-labeled HA and incubated with HT29-MTX monolayer for 2 h before imaging. Blue: DAPI-cell nuclei; green: RITC-HA; red: Cy5-Insulin; yellow: co-localization of Cy5-insulin and RITC-HA. Scale bars: 50 μm. CLSM images on XZ axis represent the vertical distribution of HA and insulin on mucus layer of HT29-MTX cell monolayer.

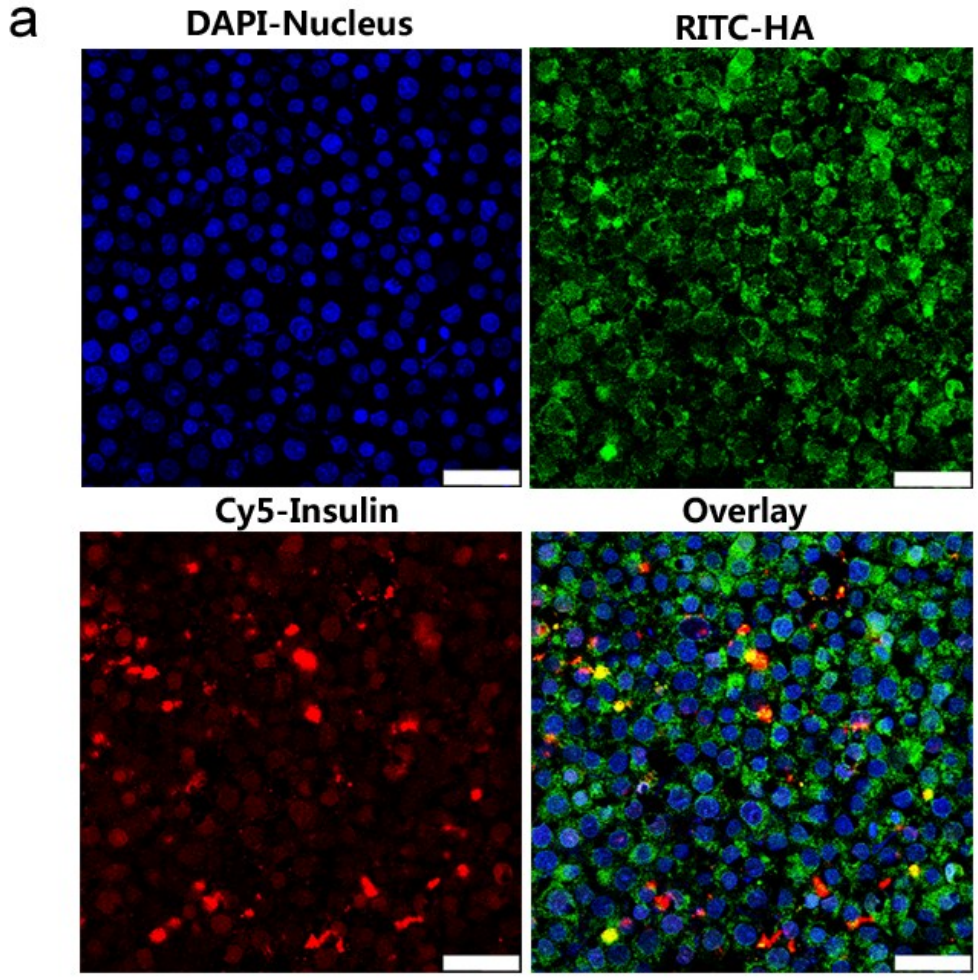
a
DAPI-Nucleus
RITC-HA
Cy5-Insulin
Overlay

Fig. S8. The enlarged CLSM images showing the distribution of HA and insulin in NP-B nanoparticles through the mucus layer of HT29-MTX cell monolayer at depth of 30 μm. Scale bars: 50 μm. (a) NP-B1 nanoparticle (HA coating: 4.7 kDa); (b) NP-B2 nanoparticle (HA coating: 35 kDa); (c) NP-B3 nanoparticle (HA coating: 190 kDa).

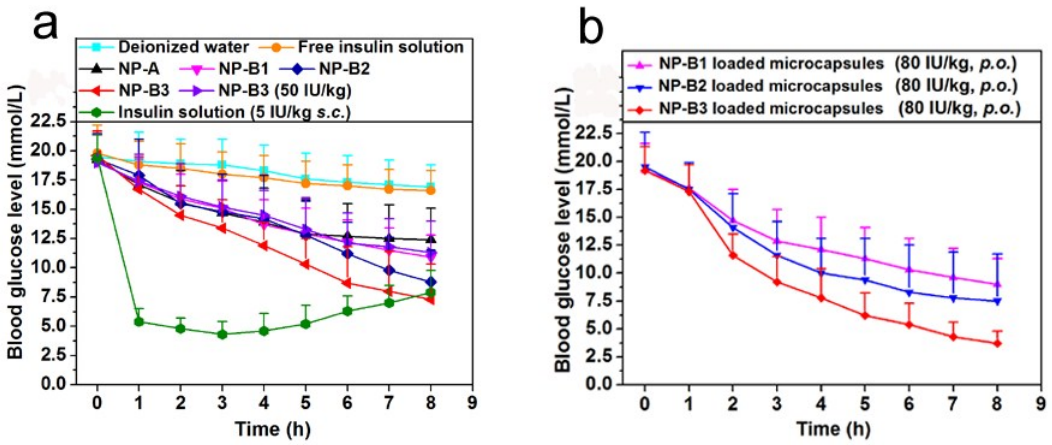

Fig. S9. Efficacy of insulin-loaded nanoparticles. (a) Variation of blood glucose levels of fasting diabetic rats following oral administration of deionized water (as a background control), free insulin (as a negative control) and different insulin formulations (all at insulin dose of 80 IU/kg, except an additional group of NP-B3 at 50 IU/kg as shown in the figure), or following *s.c.* injection free insulin solution at a dose of 5 IU/kg (as a positive control). Data are shown as means ± SD (n = 8). (b) Variation of blood glucose levels of diabetic rats after orally administering MC-1/2/3 (i.e. HPMCP-coated NP-B1/2/3 nanoparticles) at an insulin dose of 80 IU/kg. Data are shown as means ± SD (n = 6).

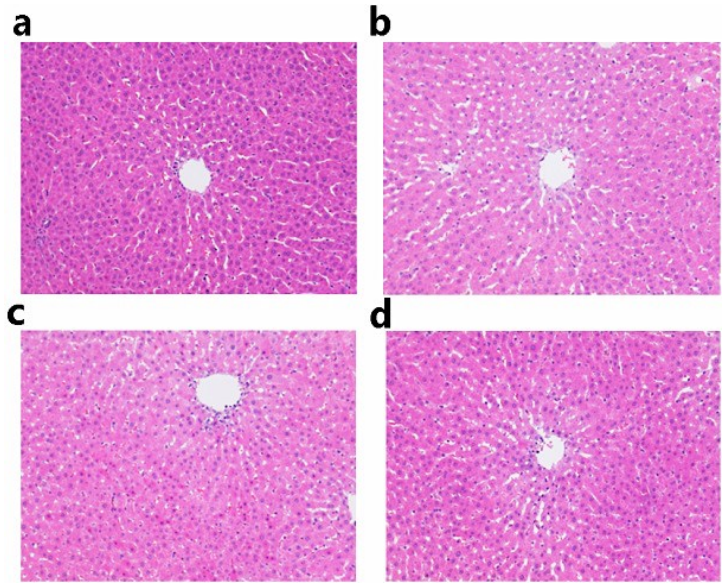

Fig. S10. Photomicrographs of the liver sections processed by hematoxylin-eosin (HE) staining. (a) Normal rats (non-diabetic) receiving PBS; (b) Diabetic rats receiving PBS; (c) Diabetic rats receiving NP-B3 nanoparticles; (d) Diabetic rats receiving MC3 suspension (loaded with NP-B3 nanoparticles).