Supporting Information

Hydrophilic and Electroneutral Nanoparticles to Overcome Mucus Trapping and Enhance Oral Delivery of Insulin

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S1. Determination of encapsulation efficiency of the insulin-loaded nanoparticles

Encapsulation efficiency (EE%), the mass ratio of encapsulated insulin inside nanoparticles, was determined during preparation processes and calculated based on the equation as below.

$$\mathrm{EE}\ (\%) = \frac{M_{encapsulated\ insulin}}{M_{loaded\ insulin}} \times 100\% = \frac{M_{loaded\ insulin} - M_{unencapsulated\ insulin}}{M_{loaded\ insulin}} \times 100\%$$

$$= \frac{\left(M_{dosage\ insulin} - M_{discarded\ insulin}\right) - M_{unencapsulated\ insulin}}{M_{dosage\ insulin} - M_{discarded\ insulin}} \times 100\%$$

$M_{encapsulated\ insulin}$, $M_{unencapsulated\ insulin}$ and $M_{loaded\ insulin}$ were the mass of encapsulated insulin inside nanoparticles, unencapsulated insulin outside nanoparticles and total insulin loaded by nanoparticles, respectively. $M_{encapsulated\ insulin}$ was the difference between $M_{loaded\ insulin}$ and $M_{unencapsulated\ insulin}$, which was calculated based on the assayed centrifuged supernatant of final insulin-loaded nanoparticles. In addition, $M_{dosage\ insulin}$ and $M_{discarded\ insulin}$ were the added dosage mass and discarded mass. $M_{dosage\ insulin}$ was calculated based on the originally added insulin solution. $M_{discarded\ insulin}$ was calculated based on the discarded solution in washing processes. Concentrations of samples were assayed *via* the HPLC method in the Section 2.6., and mass values were calculated based on the assayed concentrations and dilution ratios.

(1) For INS@MSN without washing processes, $M_{loaded\ insulin}$ was equal to $M_{dosage\ insulin}$. After centrifugation of INS@MSN, the supernatant was collected and assayed, and then $M_{unencapsulated\ insulin}$ was calculated. The originally added insulin solution for preparation processes was also assayed, and then $M_{dosage\ insulin}$, namely $M_{loaded\ insulin}$, was calculated. Finally, EE% was calculated based on the equation.

(2) For INS@MSN@PLA−PEG, the amount of loaded insulin was the difference between the dosage and discarded amount in washing processes. Discarded liquid samples for several times were

collected, diluted and mixed with dichloromethane. After stirring 30 min, the upper aqueous phase was diluted by trifluoroacetic acid solution (1%), and then concentrations were assayed [1]. $M_{discarded\ insulin}$ was the mass sum of discarded liquid samples. The originally added insulin solution for preparation processes was also assayed, and then $M_{dosage\ insulin}$ and further $M_{loaded\ insulin}$ were calculated. After centrifugation of INS@MSN@PLA−PEG, the supernatant was collected and treated as above. Then, $M_{unencapsulated\ insulin}$ was calculated based on the assayed concentrations, and, EE% was calculated based on the equation finally.

(3) For INS@MSN@PLA−PEG-CPP, discarded insulin was produced in washing processes of INS@MSN@PLA−PEG-MAL and INS@MSN@PLA−PEG-CPP. Similar with INS@MSN@PLA−PEG, discarded liquid samples were treated as above. $M_{discarded\ insulin}$ was the mass sum of discarded liquid samples. The originally added insulin solution for preparation processes was also assayed, and then $M_{dosage\ insulin}$ and further $M_{loaded\ insulin}$ were calculated. After centrifugation of INS@MSN@PLA−PEG-CPP, the supernatant was collected and treated as above. Then, $M_{unencapsulated\ insulin}$ was calculated based on the assayed concentrations, and EE% was calculated based on the equation finally.

Reference

1. Giovino, C.; Ayensu, I.; Tetteh, J.; Boateng, J. S. Development and characterisation of chitosan films impregnated with insulin loaded PEG-b-PLA nanoparticles (NPs): a potential approach for buccal delivery of macromolecules. *Int. J. Pharm.* **2012,** *428*, (1-2), 143−151.

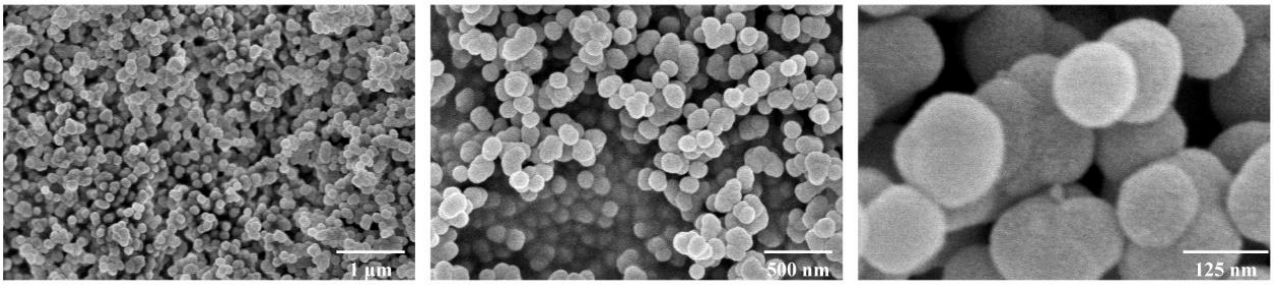

Figure S1. SEM images of MSN.

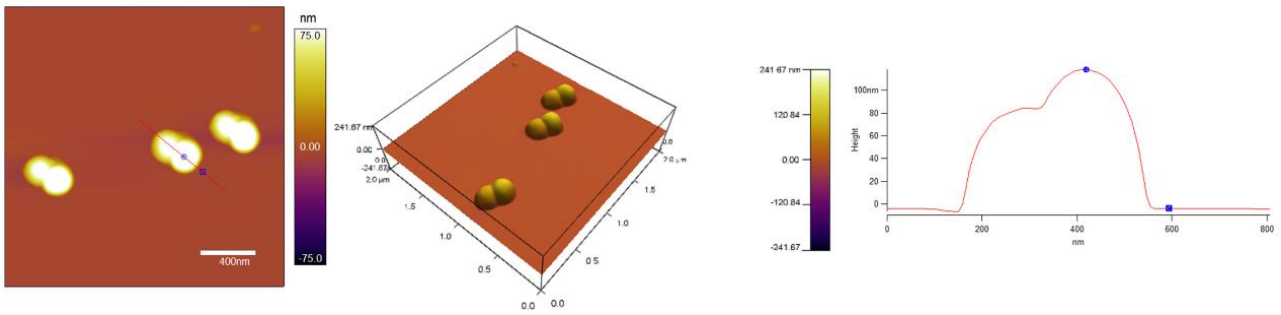

Figure S2. AFM images of MSN.

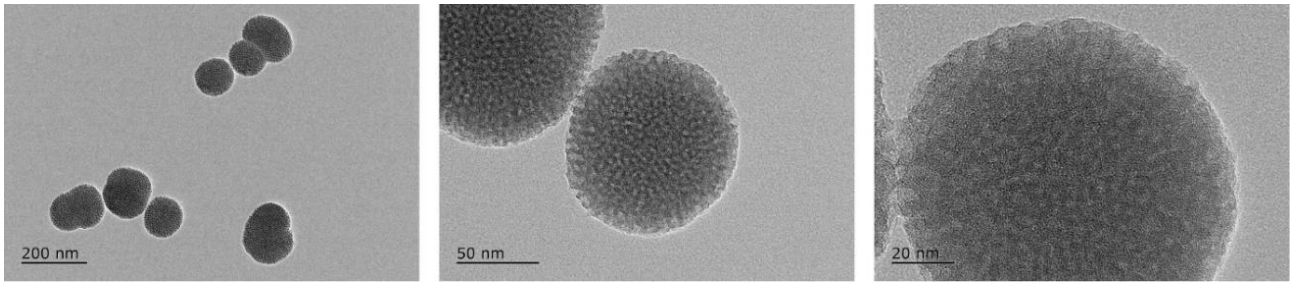

Figure S3. TEM images of MSN.

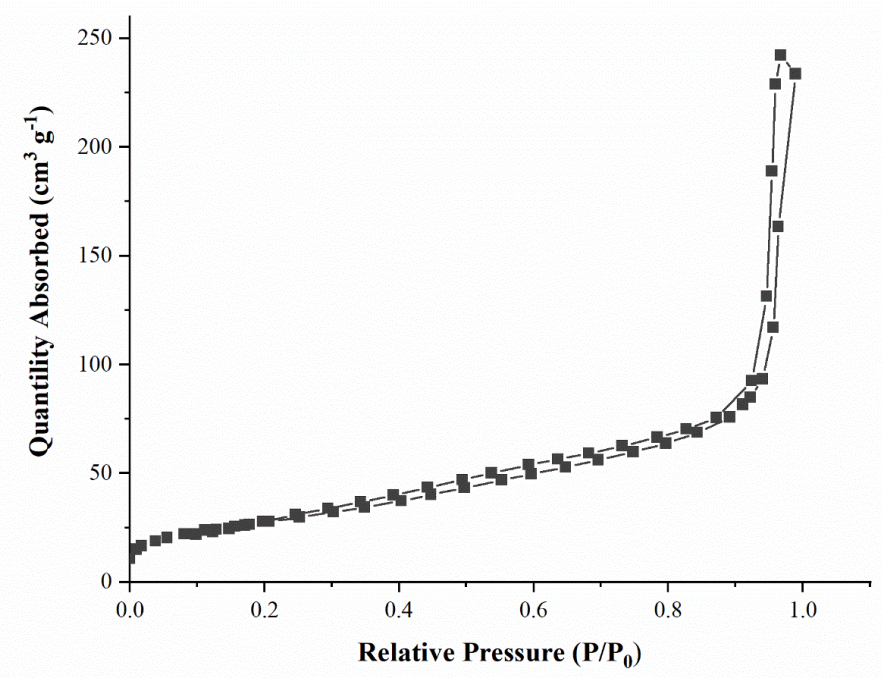

Figure S4. N_2 adsorption−desorption isotherms of MSN.

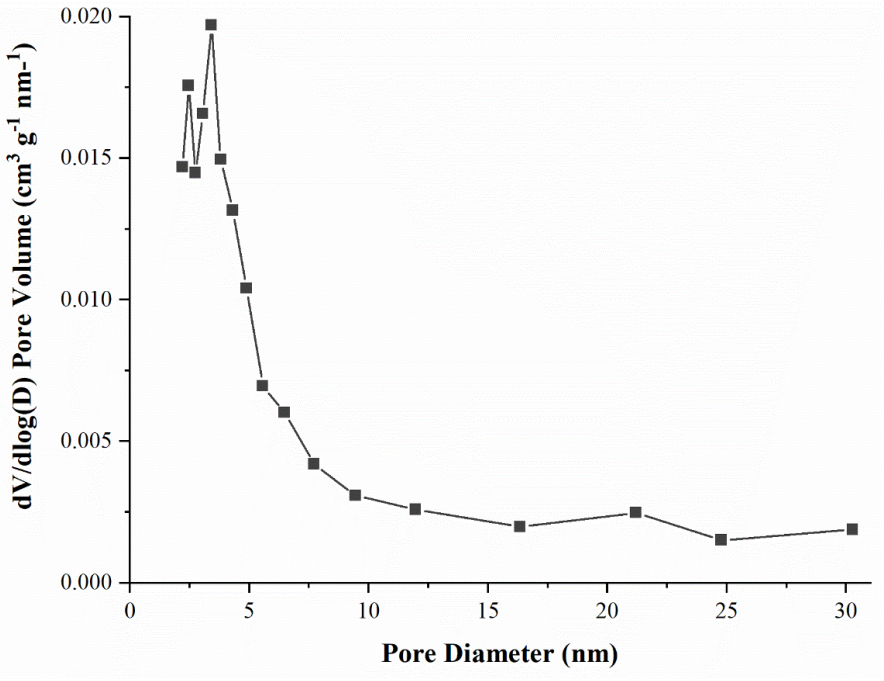

Figure S5. Pore diameter distribution of MSN.

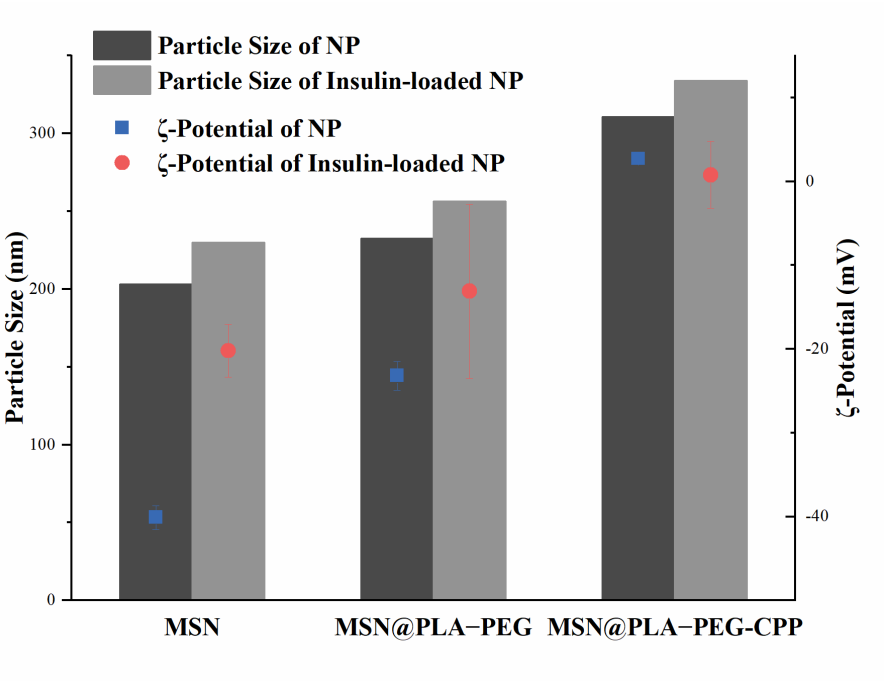

Figure S6. Particle size and ζ-potential of nanoparticles with or without insulin loading.

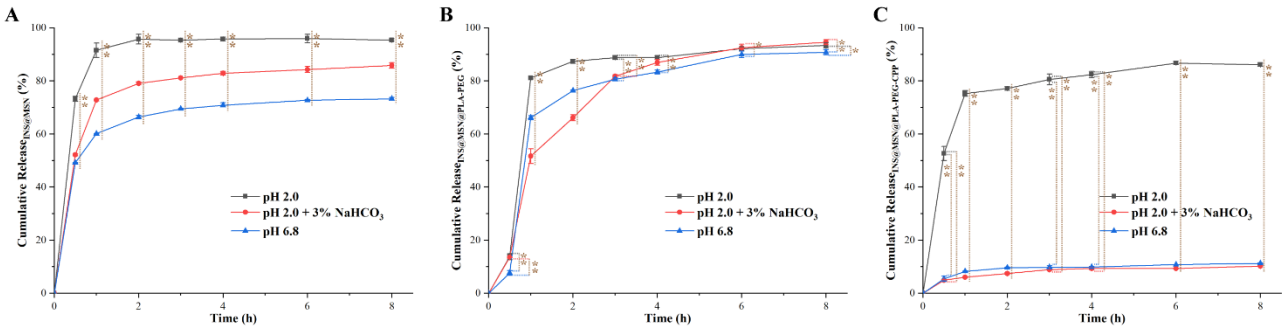

Figure S7. Cumulative release behaviors of insulin from (A) INS@MSN, (B) INS@MSN@PLA−PEG, and (C) INS@PLA−PEG-CPP in medium at pH 2.0 (black), pH 2.0 + 3% $NaHCO_3$ (red), or pH 6.8 (blue). (*$p < 0.05$ and **$p < 0.01$)

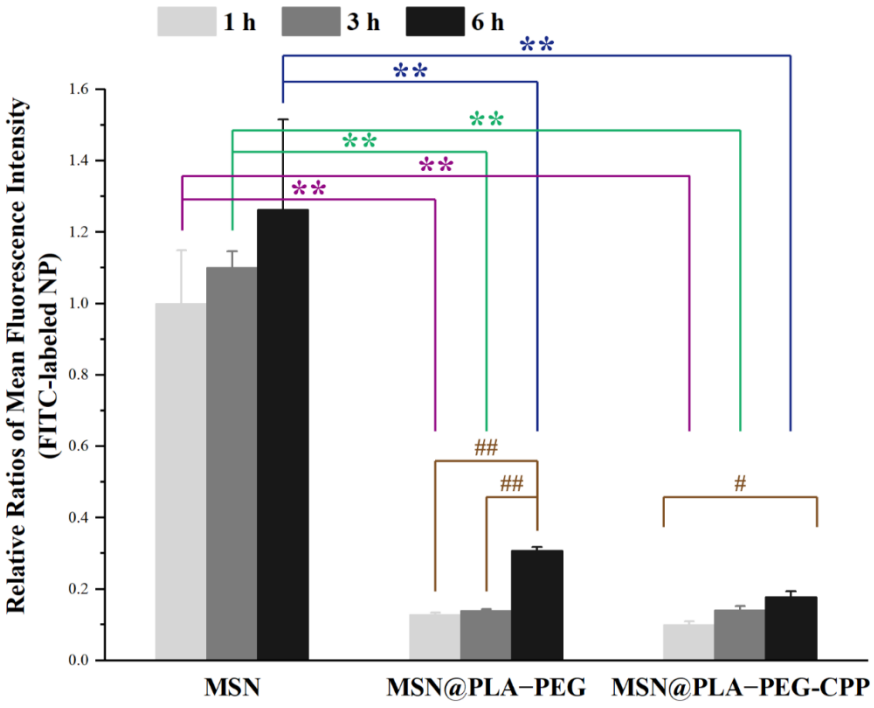

Figure S8. Cellular uptake of relative ratios of mean fluorescence intensity and of nonmucous Caco-2 cells incubated with FITC-labeled nanoparticles for different cultured time, compared with that of MSN at 1 h. ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{**}p < 0.01$).