1 h at 37 °C, and imaged using confocal laser scanning microscopy (FV1000, Olympus).

Transcellular Investigation. E12 cells were seeded on a polycarbonate membrane and cultured for 18−19 days. The cell monolayers with TEER values higher than 500 $\Omega \cdot cm^2$ were used for the experiment. Prior to the experiment, the media in the apical and basolateral chambers were replaced with prewarmed Hanks balanced salt solution (HBSS) and the cells were equilibrated for 30 min at 37 °C. Then HBSS in apical chambers was replaced by 200 μL of fresh HBSS with test samples (F-insulin, 0.25 mg/mL). At certain time internals, an aliquot of basolateral medium (200 μL) was withdrawn. The F-insulin concentration was determined and the Papp value was calculated accordingly. To investigate the influence of mucus on insulin permeation, experiments were also performed with a procedure to remove mucus with NAC.

Intracellular Trafficking. To study the intracellular trafficking of test NCs/NPs, the experiments were performed with organelle trackers or specific inhibitors as listed in Supporting Information Table S2.[34,41] For colocalization analysis, E12 cells were first incubated with test samples (F-insulin, 0.25 mg/mL), for 1 h at 37 °C, and then the suspensions were replaced by fresh medium with different organelle trackers, including Lyso-Tracker prober (50 nM), ER-Tracker probe (500 nM), Mito-Tracker probe (200 nM) and Golgi-Tracker (150 μg/mL). Then the cells were imaged using confocal microscope (FV1000, Olympus). In the inhibition study, cells were first incubated with different test samples (F-insulin, 0.25 mg/mL) for 2 h at 37 °C, and then washed with PBS twice. Subsequently, the cells were incubated with medium containing specific inhibitors for another 2 h. The amount of intracellular NCs/NPs was then measured as described above. The amount of NCs/NPs exocytosed out of the cells during incubation period was calculated.

Hypoglycemic Effect and Pharmacokinetic. The hypoglycemic effect and pharmacokinetic of the NPs following oral administration were evaluated on diabetic rats. All the experiments were approved by the Institutional Animal Care and Use Committee of Sichuan University. For the disease induction, male Sprague−Dawley rats weighting 180−220 g were injected with streptozotocin (65 mg/kg) as previously described.[42] Blood glucose level was determined using a glucose meter (JPS-6, Yicheng Biotech. Co. Ltd. Beijing, China). The rat that exhibited fasting blood glucose level over 16.0 mM 1 week after treatment was considered to be diabetic. The rats were fasted overnight but allowed free access to water prior to the experiment. Rats ($n = 5$) were chosen per group such that the mean initial blood glucose levels were the same per group. Free insulin solution, NCs and NPs-1 were administrated at a dose of 75 IU/kg *via* gavage. Other groups of diabetic rats were subcutaneous injected (s.c.) with insulin solution at a dose of 5 IU/kg or oral administration with saline. Blood samples were collected from the tail veins prior to the administration and at different time intervals after the administration. Blood glucose level was determined using a glucose meter. Plasma insulin levels were quantified using porcine insulin ELISA kit (R&D System, Inc.), and the endogenic level of insulin (before administration) was subtracted from the tested value for each mouse. The area above the curve (AAC) of the blood glucose level and the area under the curve (AUC) of plasma insulin concentration curve were calculated. The pharmacological availability (PA %) and bioavailability (*F* %) relative to subcutaneous injection was analyzed as following equations:

$$PA(\%) = \frac{AAC_{oral} \times Dose_{s.c.}}{AAC_{s.c.} \times Dose_{oral}} \times 100$$

$$F(\%) = \frac{AUC_{oral} \times Dose_{s.c.}}{AUC_{s.c.} \times Dose_{oral}} \times 100$$

Statistics. Statistical analyses of the data were performed with SPSS program 16.0 by using two tail Student's *t* test. All experiments were performed in triplicate unless otherwise stated. Error bars used in this work are SD. A $p < 0.05$ is considered statistically significant ($^{\star}p < 0.05$).

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Characterizations of pHPMA derivatives, inhibitors with different functions used in the cellular exocytosis pathway study, spectra of monomers and polymer, insulin release profile, enzymatic degradation profiles of insulin with trypsin or α-chymotrypsin, cytotoxicity of NPs, TEER values of the cell monolayers over time with the incubation of tested samples, exocytosis of fluorescent-labeled NPs on E12 cells, blood glucose values and the videos of particle motion (avi). This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Harris, J. M.; Chess, R. B. Effect of Pegylation on Pharmaceuticals. *Nat. Rev. Drug Discovery* **2003**, *2*, 214–221.
2. Chin, R. L.; Martinez, R.; Garmel, G. Gas Gangrene from Subcutaneous Insulin Administration. *Am. J. Emerg. Med.* **1993**, *11*, 622–625.
3. Carino, G. P.; Mathiowitz, E. Oral Insulin Delivery. *Adv. Drug Delivery Rev.* **1999**, *35*, 249–257.
4. Porter, C. J.; Trevaskis, N. L.; Charman, W. N. Lipids and Lipid-Based Formulations: Optimizing the Oral Delivery of Lipophilic Drugs. *Nat. Rev. Drug Discovery* **2007**, *6*, 231–248.
5. Mo, R.; Jiang, T.; Di, J.; Tai, W.; Gu, Z. Emerging Micro- and Nanotechnology Based Synthetic Approaches for Insulin Delivery. *Chem. Soc. Rev.* **2014**, *43*, 3595–3629.
6. Jin, Y.; Song, Y.; Zhu, X.; Zhou, D.; Chen, C.; Zhang, Z.; Huang, Y. Goblet Cell-Targeting Nanoparticles for Oral Insulin Delivery and the Influence of Mucus on Insulin Transport. *Biomaterials* **2012**, *33*, 1573–1582.
7. Pridgen, E. M.; Alexis, F.; Kuo, T. T.; Levy-Nissenbaum, E.; Karnik, R.; Blumberg, R. S.; Langer, R.; Farokhzad, O. C. Transepithelial Transport of Fc-Targeted Nanoparticles by the Neonatal Fc Receptor for Oral Delivery. *Sci. Transl. Med.* **2013**, *5*, 213ra167.
8. Ensign, L. M.; Cone, R.; Hanes, J. Oral Drug Delivery with Polymeric Nanoparticles: The Gastrointestinal Mucus Barriers. *Adv. Drug Delivery Rev.* **2012**, *64*, 557–570.
9. Dhuria, S. V.; Hanson, L. R.; Frey, W. H., II. Intranasal Delivery to the Central Nervous System: Mechanisms and Experimental Considerations. *J. Pharm. Sci.* **2010**, *99*, 1654–1673.
10. Lai, S. K.; Wang, Y. Y.; Hanes, J. Mucus-Penetrating Nanoparticles for Drug and Gene Delivery to Mucosal Tissues. *Adv. Drug Delivery Rev.* **2009**, *61*, 158–171.
11. Wang, Y. Y.; Lai, S. K.; Suk, J. S.; Pace, A.; Cone, R.; Hanes, J. Addressing the PEG Mucoadhesivity Paradox to Engineer Nanoparticles That "Slip" through the Human Mucus Barrier. *Angew. Chem., Int. Ed.* **2008**, *47*, 9726–9729.
12. Yang, M.; Lai, S. K.; Wang, Y. Y.; Zhong, W.; Happe, C.; Zhang, M.; Fu, J.; Hanes, J. Biodegradable Nanoparticles Composed Entirely of Safe Materials That Rapidly Penetrate Human Mucus. *Angew. Chem., Int. Ed.* **2011**, *50*, 2597–2600.
13. Xu, Q.; Boylan, N. J.; Cai, S.; Miao, B.; Patel, H.; Hanes, J. Scalable Method to Produce Biodegradable Nanoparticles That Rapidly Penetrate Human Mucus. *J. Controlled Release* **2013**, *170*, 279–286.
14. Yang, X. Z.; Du, J. Z.; Dou, S.; Mao, C. Q.; Long, H. Y.; Wang, J. Sheddable Ternary Nanoparticles for Tumor Acidity-Targeted siRNA Delivery. *ACS Nano* **2012**, *6*, 771–781.
15. Oh, E.; Delehanty, J. B.; Sapsford, K. E.; Susumu, K.; Goswami, R.; Blanco-Canosa, J. B.; Dawson, P. E.; Granek, J.; Shoff, M.; Zhang, Q.; et al. Cellular Uptake and Fate of PEGylated Gold Nanoparticles Is Dependent on Both Cell-Penetration Peptides and Particle Size. *ACS Nano* **2011**, *5*, 6434–6448.
16. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives. *Angew. Chem., Int. Ed.* **2010**, *49*, 6288–6308.
17. Khafagy el, S.; Morishita, M. Oral Biodrug Delivery Using Cell-Penetrating Peptide. *Adv. Drug Delivery Rev.* **2012**, *64*, 531–539.