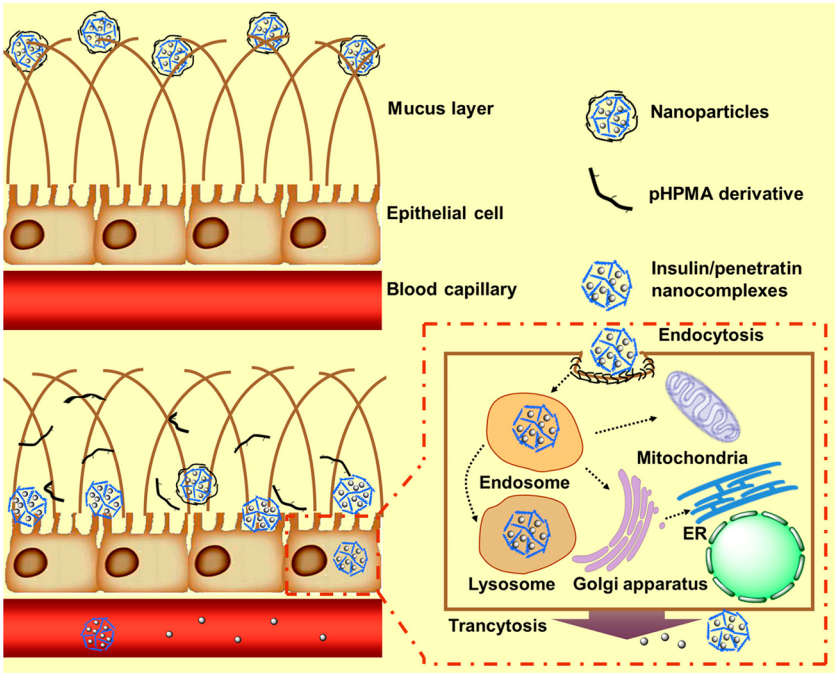

Figure 6. Schematic illustration of the process of the NP permeation across the mucus layer and the intracellular transport of NP in the epithelial cells.

(Figure 4A,C,D), which both involved secretory endoplasmic reticulum/Golgi pathway and endocytic recycling pathway, the improved transepithelial transport of NPs-1 may mainly be attributed to the better mucus permeation ability and timely revealing of the CPP-rich core for cell interaction. Moreover, the unchanged TEER value of cell monolayers throughout the treatment suggested that the translocation of drug happened through a transcellular pathway without compromising the integrity of the epithelium (Supporting Information Figure S10). Thus, this NP platform for transepithelial transport can avoid the potential safety issues associated with manipulating the permeability of epithelium.[7] Furthermore, in diabetic rats, NPs-1 was able to generate an excellent hypoglycemic response and increase the serum insulin level after oral administration. The relative bioavailability of NPs-1 was 2.08-fold higher than that of NCs.

In summary, we rationally developed a distinctive self-assembled NP platform for effective oral delivery of insulin. With systematic *in vitro* screening using mucus-secreting cells, NPs were demonstrated to possess the ability to overcome both the mucus barrier and epithelial barrier. We provide the first demonstration of enhanced mucus permeation of NP using pHPMA derivatives as a coating agent. However, an important fact that might need to be taken into account was that the mucus on the cell model might renew in a much slower rate than it happened in the gastrointestinal track. This might account for the higher difference between NP-1 and NC in the *in vivo* study compared with the *in vitro* results, and also suggested that further screening of the NP formulation using animal model might generate even better efficiency of delivery. In addition, this is the first example of applying dissociable hydrophilic coating and a cationic CPP-rich nanocomplex core to overcome both diffusion barrier of mucus and absorption barrier of epithelium, and our study might underscore the importance of overcoming the multiple barriers in a multistep strategy.

EXPERIMENTAL SECTION

Materials. Penetratin peptide was chemically synthesized by Kaijie Biopharmaceuticals Co., Ltd. (Sichuan, China). Porcine insulin (28.3 IU/mg) was purchased from Wanbang Bio-Chemical Co., Ltd. (Jiangsu, China). Fluorescein isothiocyanate (FITC), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and nocodazole were all purchased from Sigma-Aldrich (St. Louis, MO). Lyso-Tracker Red and Mito-Tracker Red were purchased from Invitrogen (Carlsbad, CA). ER-Tracker Red, Golgi-Tracker Red, Brefeldin A, Monensin and LY294002 were acquired from Beyotime (Haimen, Jiangsu, China). Tetraethyl rhodamine isothiocyanate (TRITC) was purchased from FanboBiochemicals (Beijing, China). All chemical reagents utilized in study were analytic grade.

Synthesis and Characterization of pHPMA Derivatives. The monomers of *N*-(2-hydroxypropyl) methacrylamide (HPMA) and *N*-methacryloyl-glycylglycine (MA-GG-OH) were synthesized according to previous reports.[36,37] Then three kinds of HPMA copolymer derivatives containing different amounts of MA-GG-OH monomers were synthesized by radical solution polymerization in absolute methanol (AIBN, 2 wt %; monomer concentration 12.5 wt %; molar ratio HPMA/MA-GG-OH were 95:5, 90:10, and 80:20, respectively). The copolymerization was performed in sealed ampules under nitrogen at 50 °C for 24 h. The pHPMA was isolated from polymerization mixture by precipitation into diethyl ether; then, dialyzed and lyophilized. The ^{1}H NMR spectra and mass spectrograms of monomers and