Overcoming the Diffusion Barrier of Mucus and Absorption Barrier of Epithelium by Self-Assembled Nanoparticles for Oral Delivery of Insulin

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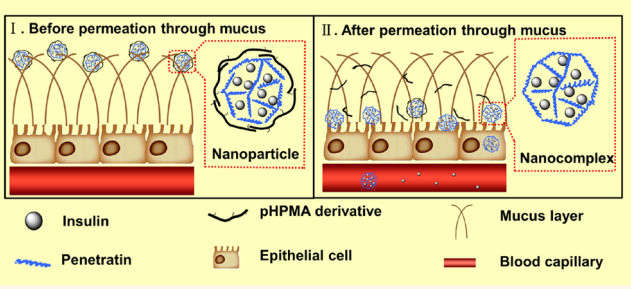
ABSTRACT Nanoparticles (NPs) have demonstrated great potential for the oral delivery of protein drugs that have very limited oral bioavailability. Orally administered NPs could be absorbed by the epithelial tissue only if they successfully permeate through the mucus that covers the epithelium. However, efficient epithelial absorption and mucus permeation require very different surface properties of a nanocarrier. We herein report self-assembled NPs for efficient oral delivery of insulin by facilitating both of these two processes. The NPs possess a nanocomplex core composed of insulin and cell penetrating peptide (CPP), and a dissociable hydrophilic coating of *N*-(2-hydroxypropyl) methacrylamide copolymer (pHPMA) derivatives. After systematic screening using mucus-secreting epithelial cells, NPs exhibit excellent permeation in mucus due to the "mucus-inert" pHPMA coating, as well as high epithelial absorption mediated by CPP. The investigation of NP behavior shows that the pHPMA molecules gradually dissociate from the NP surface as it permeates through mucus, and the CPP-rich core is revealed in time for subsequent transepithelial transport through the secretory endoplasmic reticulum/Golgi pathway and endocytic recycling pathway. The NPs exhibit 20-fold higher absorption than free insulin on mucus-secreting epithelium cells, and orally administered NPs generate a prominent hypoglycemic response and an increase of the serum insulin concentration in diabetic rats. Our study provides the evidence of using pHPMA as dissociable "mucus-inert" agent to enhance mucus permeation of NPs, and validates a strategy to overcome the multiple absorption barriers using NP platform with dissociable hydrophilic coating and drug-loaded CPP-rich core.



KEYWORDS: oral delivery · insulin · nanoparticles · mucus · epithelium · cell penetrating peptide

Protein therapeutics are increasingly being applied for the treatment of various diseases in clinic due to their high therapeutic efficacy and excellent selectivity.[1] Despite the frequent dosing that is required for patients with many diseases, especially chronic ones, the administration of these drugs are largely limited to parenteral routes and, thus, causes problems including low patient compliance and safety issues.[2] The oral bioavailability of these biomolecules is very limited (<1%) due to their inherent low permeability across the epithelium and the rapid indigestive degradation.[3] The advance of nanotechnology and nanomedicine in the past decade has opened a new perspective for oral delivery of biomolecules. Numerous nanocarriers have been reported for the oral delivery application, such as liposomes, nanogels, and polymeric nanoparticles (NPs).[4,5] Improved bioavailability was achieved with these nanocarriers utilizing different absorption strategies, such as paracellular permeation through opened tight junctions and transcytosis mediated by certain receptors.[6,7]

Different from the NPs developed for parenteral application, the behavior of orally administered NPs is greatly influenced by their interaction with the mucus that covers and protects the underlying epithelium.[8] Intestinal mucus is a layer of slippery secretion in constant and fast renewal and, thus, can rapidly trap and remove foreign particles, especially those

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