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pH-Responsive Nanoparticles Shelled with Chitosan for Oral Delivery of Insulin: From Mechanism to Therapeutic Applications

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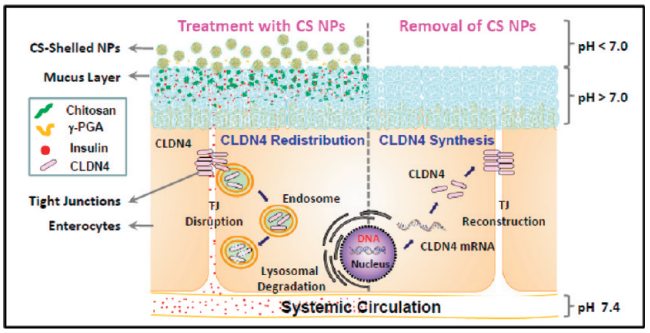

Despite advances in drug-delivery technologies, successful oral administration of protein drugs remains an elusive challenge. When protein drugs are administered orally, they can rapidly denature or degrade before they reach their targets. Such drugs also may not absorb adequately within the small intestine.

As a protein drug for treating diabetes, insulin is conventionally administered via subcutaneous (SC) injection, yet often fails to achieve the glucose homeostasis observed in nondiabetic subjects. Some of this difference may relate to insulin transport: normally, endogenously secreted insulin moves to the liver via portal circulation. When administered subcutaneously, insulin moves through the body via peripheral circulation, which can produce a peripheral hyperinsulinemia. In addition, because SC treatment requires multiple daily injections of insulin, patients often do not fully comply with treatment. Oral administration of exogenous insulin would deliver the drug directly into the liver through portal circulation, mimicking the physiological fate of endogenously secreted insulin. This characteristic may offer the needed hepatic activation, while avoiding hyperinsulinemia and its associated long-term complications.

This Account demonstrates the feasibility of using chitosan nanoparticles for oral insulin delivery. Nanoparticle (NP) delivery systems may provide an alternative means of orally administering protein drugs. In addition to protecting the drugs against a harmful gastric environment, the encapsulation of protein drugs in particulate carriers can avert enzymatic degradation, while controlling the drug release and enhancing their absorption in the small intestine. Our recent study described a pH-responsive NP system composed of chitosan (CS) and poly(γ-glutamic acid) for oral delivery of insulin. As a nontoxic, soft-tissue compatible, cationic polysaccharide, CS also adheres to the mucosal surface and transiently opens the tight junctions (TJs) between contiguous epithelial cells. Therefore, drugs made with CS NPs would have delivery advantages over traditional tablet or powder formulations. This Account focuses on the premise that these CS NPs can adhere to and infiltrate the mucus layer in the small intestine. Subsequently, the infiltrated CS NPs transiently open the TJs between epithelial cells. Because they are pH-sensitive, the nanoparticles become less stable and disintegrate, releasing the loaded insulin. The insulin then permeates through the opened paracellular pathway and moves into the systemic circulation.

Introduction

Although the oral route is considered to be the most convenient and comfortable means of administering drugs to patients, oral administration of protein drugs encounters many difficulties.[1−4] First, protein drugs are readily degraded by the low pH of gastric medium in the stomach. Second, various digestive enzymes in the stomach and small intestine may degrade protein drugs.[2,3] Finally, the

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intestinal epithelium is a major barrier to the absorption of hydrophilic macromolecules such as proteins, polysaccharides, and nucleic acids owing to their high molecular weight and hydrophilicity, which makes it impossible for them to diffuse across the cells through the lipid-bilayer cell membranes.[5−7] Therefore, enhancing the paracellular delivery of hydrophilic drugs has received considerable attention. Nevertheless, the delivery of hydrophilic macromolecules via the paracellular pathway is severely restricted by tight junctions (TJs) between neighboring epithelial cells.[8,9]

To overcome the above problems, we developed a nanoparticle (NP) carrier system self-assembled by hydrophilic chitosan (CS) and poly(γ-glutamic acid) (γ-PGA), as a platform technology, for oral delivery of hydrophilic macromolecules via the paracellular pathway.[10] The Food and Drug Administration (FDA) has approved CS for use in wound dressings[11] and for dietary applications in many countries.[12−14] CS is characterized by its ability to adhere to the mucosal surface and mediate reversible epithelial TJ opening.[13,14] These unique characteristics, together with an extremely safe toxicity profile, make CS an exciting and promising excipient in the pharmaceutical industry.[15−20] Preparation of NPs by the ionic gelation of CS with tripolyphosphate sodium (TPP) or poly(acrylic acid) (PAA) to improve the intestinal absorption of protein molecules was reported previously in the literature.[21,22] However, the stability of CS/TPP NPs was suboptimal, while CS/PAA NPs had large particle sizes with a broad size distribution.[21,22]

Over the past 6 years, our laboratory together with collaborators has actively explored the feasibility of using CS NPs to orally deliver insulin and other hydrophilic macromolecules for therapeutic applications.[23−30] This Account summarizes some of our major findings. The CS-mediated reversible epithelial TJ opening is discussed briefly, along with a description of the pH-responsive characteristics of CS NPs. The in vivo results then demonstrate that orally administered NPs with excessive CS on their surfaces can adhere to and infiltrate the mucosa of the intestinal tract, thereby prolonging their intestinal residence and subsequently mediating the opening of epithelial TJs. The functionality, efficacy, and biodistribution of the orally administered insulin-loaded NPs are also discussed. Finally, safety issues of this CS NP delivery system are addressed, along with other therapeutic applications of this promising platform technology.

Mechanism

The reversible opening of TJs was observed at an ultrastructural level by using transmission electron microscopy (TEM) to investigate the interaction between CS and Caco-2 cell monolayers, a widely used in vitro model for intestinal permeability studies. As shown in our TEM micrographs, following treatment with CS, distinct ultrastructural changes in TJ morphology, including dilation of the intercellular spaces, were observed; the intercellular spaces were recovered after removal of CS (Figure 1A). The above results demonstrate that CS can transiently and reversibly open the TJs between Caco-2 cells, thus increasing their paracellular permeability.[31]

The CS-mediated expression of claudin-4 (CLDN4), a transmembrane protein responsible for the maintenance of TJ integrity,[32] was then evaluated. Following treatment with CS, CLDN4 staining at cell−cell contact sites became discontinuous, indicating the loss of TJ integrity (insets in Figure 1A). Conversely, after removal of CS, CLDN4 expression at TJs increased significantly. Western blot analysis results confirmed this observation. Following treatment with CS, the levels of CLDN4 were significantly reduced at the cell membrane and in the cytosol. In contrast, after removal of CS, the expression of CLDN4 protein was significantly elevated (Figure 1B). The above results indicate that treatment with CS leads to redistribution of CLDN4 protein from the cell membrane to the cytosol, resulting in the disruption of TJs, i.e., an indication of TJ opening. After removal of CS, CLDN4 must be synthesized to ensure a complete recovery of TJs (figure in Conspectus).[31]

Preparation and Characterization of CS NPs

The pK_a values of CS and γ-PGA are approximately 6.5 and 2.9, respectively.[23−25] Drug-loaded NPs were obtained upon addition of an aqueous γ-PGA blended with protein drugs such as insulin into an aqueous CS under magnetic stirring.[25−27] Electrostatic interaction between the two polyelectrolytes (negatively charged γ-PGA and positively charged CS) in an aqueous environment (pH 6.0) instantaneously induced the formation of hydrophobic segments, resulting in highly neutralized complexes that segregated into colloidal NPs.[24] Zinc (Zn^{2+}) is generally added during the biosynthesis and storage of insulin; the histidine and glutamic acid residues in insulin may complex with Zn^{2+} to increase its stability.[33] γ-PGA is unique in that it is composed of naturally occurring L-glutamic acid monomers linked together through amide bonds.[34] Therefore, insulin may conjugate with γ-PGA via Zn^{2+}, subsequently increasing its loading efficiency and content in the prepared CS NPs.[24−26]

A. Characteristics of CS NPs. The mean particle size and surface charge of the prepared NPs can be controlled by the

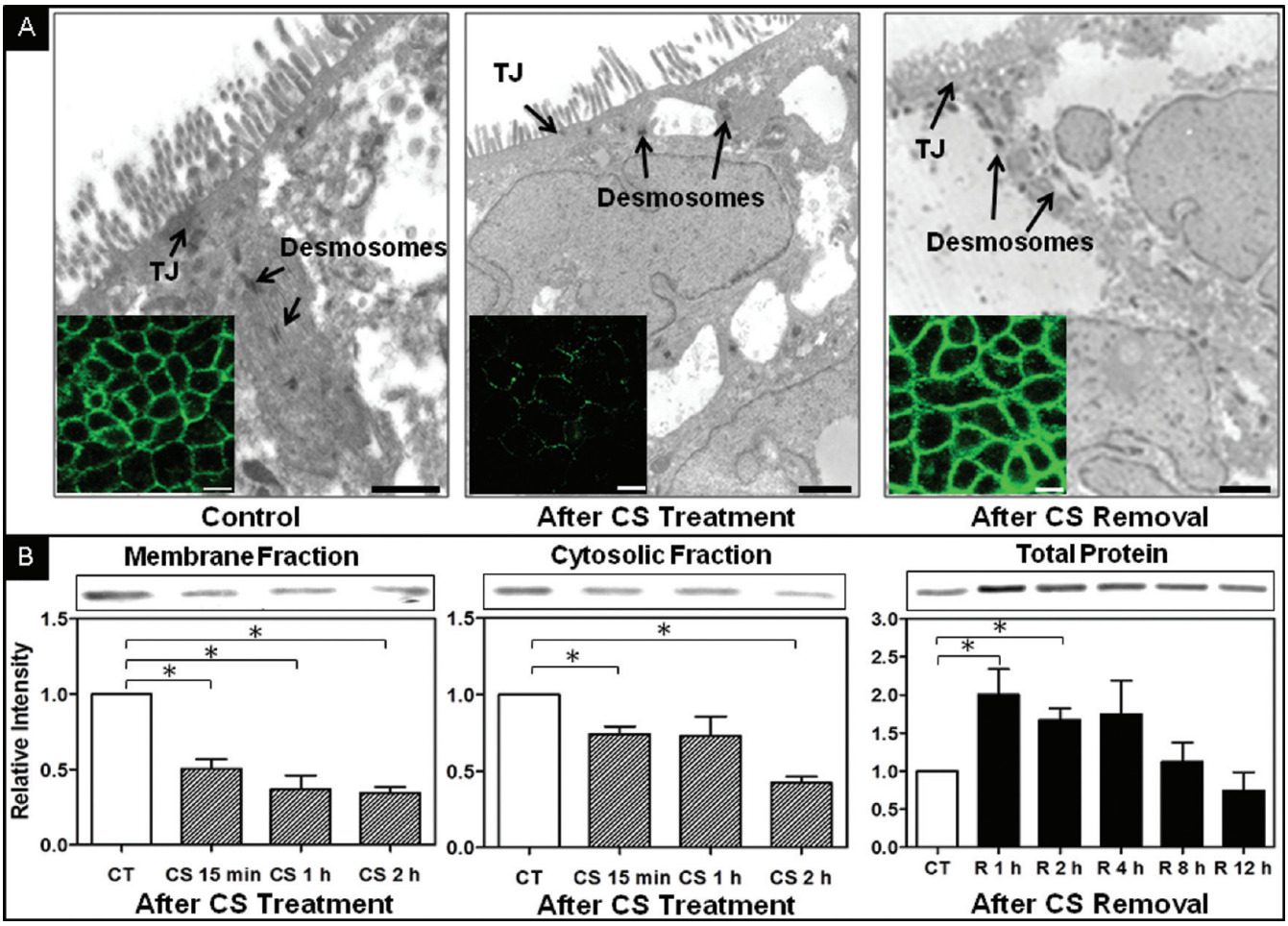

FIGURE 1. Chitosan (CS)-mediated reversible opening of intercellular tight junctions (TJs). (A) TEM micrographs of the polarized Caco-2 cells before CS treatment (Control), after CS treatment and following CS removal (scale bars, 0.5 μm); insets showing the redistribution of claudin-4 (CLDN4, a TJ protein) after CS treatment and following CS removal, studied by using confocal microscopy (scale bars, 15 μm); (B) Expression of CLDN4 at the cell membrane and in the cytosol after CS treatment and total CLDN4 following CS removal, obtained from Western blot analysis ($n = 3$). (*) Statistical significance at a level of $P < 0.05$. Adapted with permission from ref 31. Copyright 2011 Elsevier.

relative concentrations of CS to γ-PGA used. The NPs used in the study had a positive surface charge, with excessive CS shelled on their surfaces.[23−25] With an excessive CS, the NPs prepared in deionized water had a diameter of approximately 250 nm and a zeta potential of 25 mV; in addition, their insulin loading efficiency and content were 75% and 15%, respectively.[26] The morphology of CS NPs was spherical in shape. Figure 2A displays the small-angle X-ray scattering (SAXS) profiles in a log−log plot of suspensions of CS NPs. The observed low-q scattering patterns indicate that CS NPs had a compact structure with a smooth surface. The characteristic correlation distance between the insulin molecules distributed within the individual CS NP, as calculated from the high-q peak via $d = 2/q_m$, was approximately 4.8 nm.[30]

B. pH-Sensitivity of CS NPs and Paracellular Transport of Insulin. The prepared CS NPs were responsive to their surrounding pH environment. In the pH environment below 7.0 (in the lumen of the duodenum and jejunum), CS NPs remained intact, while destabilizing and disintegrating at pH > 7.0 (in the mucus layer and intercellular spaces, Figure 2B).[28] This observation is caused by the deprotonation of CS in the neutral/basic pH environment, and therefore, the neutrally charged CS was not able to complex with the negatively charged γ-PGA. In this study, the ability of CS NPs in enhancing the paracellular transport of insulin was evaluated in Caco-2 cell monolayers cultured in a trans-well insert. The donor compartment was maintained at pH < 7.0, and the receiver compartment at pH 7.4. Next, the transport of insulin through the opened TJs was directly observed by preparing fluorescent NPs through the use of cyanine 3-labeled insulin (Cy3-insulin), cyanine 5-labeled CS (Cy5-CS), and fluorescein-amine-labeled γ-PGA (FA-γ-PGA). According to Figure 2C,

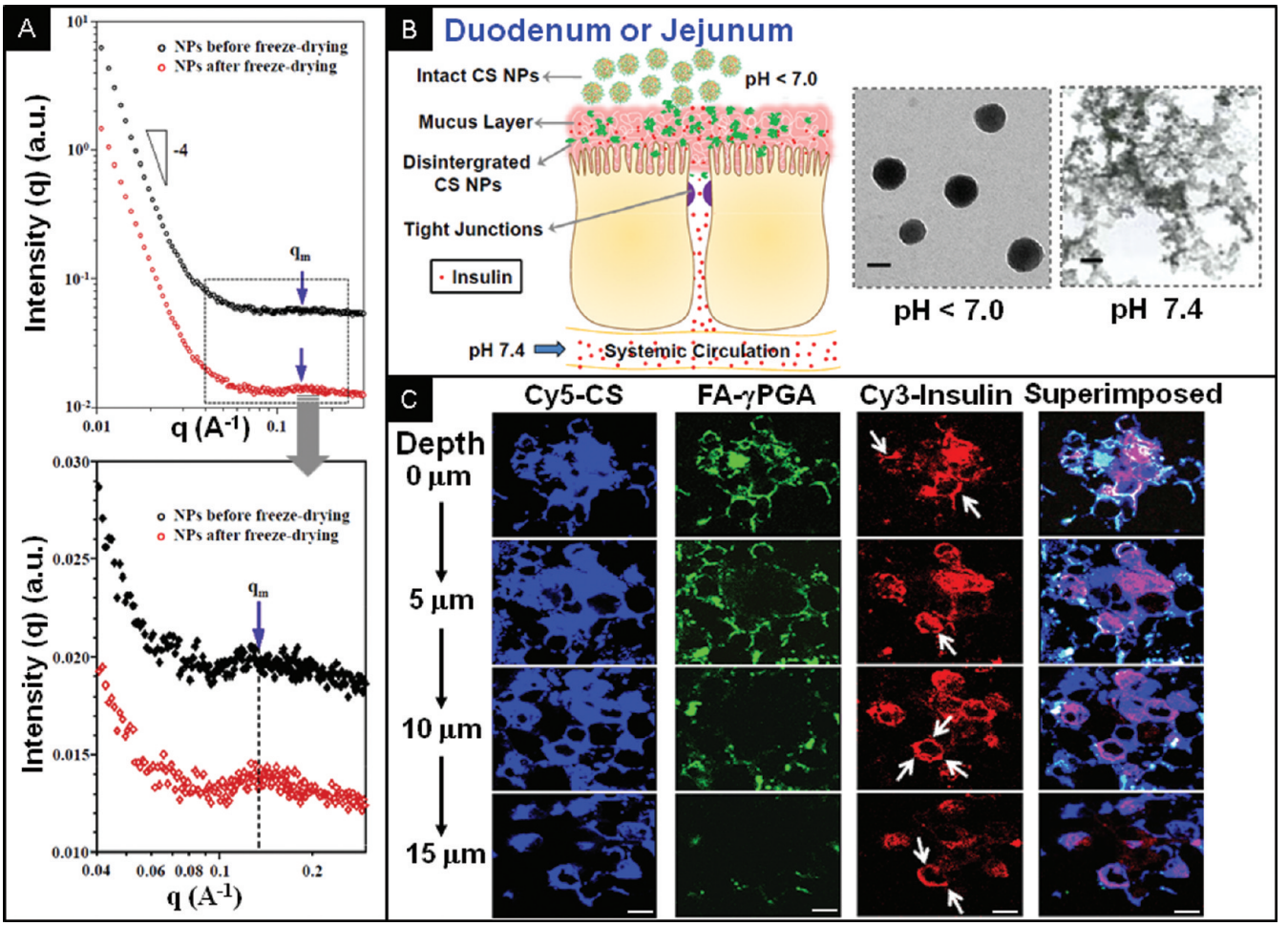

FIGURE 2. (A) Small angle X-ray scattering (SAXS) profiles in a log−log plot of suspensions of CS NPs before and after freeze-drying. (B) CS NPs were responsive to their surrounding pH environment; schematic illustrations showing the presumed mechanism of the paracellular transport of insulin released from disintegrated CS NPs and their TEM micrographs at distinct pH environments (scale bars, 100 μm). (C) Fluorescence images of Caco-2 cell monolayers incubated with fluorescence-labeled CS NPs for 120 min at pH 6.6 in the donor compartment and pH 7.4 in the receiver compartment (scale bars, 15 μm). CS NPs, chitosan nanoparticles; Cy5-CS, cyanine 5-labeled chitosan; FA-γ-PGA, fluoresceinamine-labeled γ-PGA; Cy3-insulin, cyanine 3-labeled insulin. Modified with permission from refs 28 and 30. Copyright 2009 and 2010 Elsevier.

following treatment with CS NPs, the insulin released from the disintegrated NPs could transport across the opened paracellular pathway (as indicated by white arrows).[26,28]

C. Pharmacological Activity of Insulin. Pharmacological activity of the insulin loaded in CS NPs was evaluated in a rat model. Following subcutaneous (SC) injection of the insulin-loaded NPs, a similar hypoglycemic response to that of the free-form insulin solution, yet with a 2 h time lag, was observed, implying that the activity of the insulin released from CS NPs was still intact.[28] This finding is attributed to the fact that the insulin-loaded NPs were prepared under mild aqueous-based conditions at room temperature. Organic solvents or elevated temperatures may cause denaturation or degradation of protein molecules.

In Vivo Functionality of CS NPs

Functionality of CS NPs was explored in vivo by using a rat model. CS NPs labeled with quantum dot (QD, to provide the electron density) or fluorescence suspending in deionized water were orally administered via an oral feeding needle. After 3 h, rats were sacrificed and their small intestines were retrieved and then examined under TEM or confocal laser scanning microscopy (CLSM).

A. Mucosal Adhesion, Infiltration, and TJ Opening. The orally administered CS NPs could adhere to and infiltrate the mucus layer and approach the epithelial surface (Figure 3A).[35] The mucoadhesive feature of CS is attributed to an interaction between the positively charged CS and negatively charged sialic acid groups in mucin.[36] Additionally, we found that the mucoadhesive properties of CS NPs

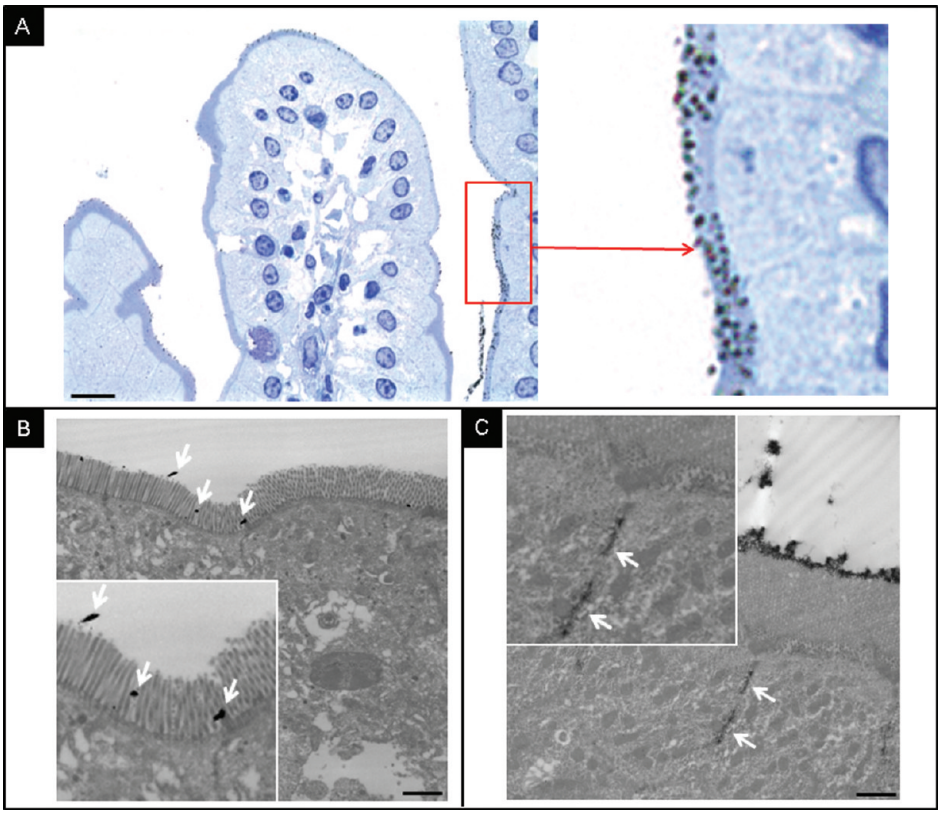

FIGURE 3. (A) Photomicrographs (scale bar, 10 μm) and (B) TEM micrographs (scale bars, 2 μm) of silver-enhanced intestinal sections showing the mucoadhesion and infiltration of quantum-dot-labeled CS NPs (black dots) and (C) permeation of lanthanum through the opened paracellular space (indicated by white arrows) in mice treated with CS NPs. Areas defined by rectangles are shown at a higher magnification in the insets. CS NPs: chitosan nanoparticles. Adapted with permission from ref 35. Copyright 2011 Elsevier.

were affected by their pH environment exposed; significantly more CS NPs adhered to the luminal surface in the duodenum (pH 6.0−6.6) than in the jejunum (pH ~ 7.0) and ileum (pH ~ 7.4).[28] A gradually increasing environmental pH along the intestinal tract decreased the positive surface charge of CS NPs (owing to the deprotonation of CS), ultimately reducing its mucoadhesive capability.

Similar findings were observed ultrastructurally in our TEM micrographs. The CS NPs labeled with QD could infiltrate the mucus layer and approach the microvilli on epithelial cells (Figure 3B).[35] However, whether the epithelial TJs were opened could not be determined, owing to the lack of electron density in their intercellular spaces. Therefore, following treatment with CS NPs, the same tissue samples were incubated with aqueous lanthanum (an electron-dense metal ion), which has been widely used to stain cells for TEM examination.[37] According to Figure 3C, lanthanum could infiltrate the intercellular spaces, suggesting that CS NPs were able to open the epithelial TJs and increase their paracellular permeability.

B. Intestinal Absorption. Following oral treatment with fluorescence-labeled CS NPs, the fluorescent images at distinct portions of the retrieved small intestine were obtained by using CLSM. According to our results, the intensity of fluorescence signals observed in the duodenum were stronger along the luminal surface (the *XY* plane) and appeared at a deeper level (the *XZ* plane) than that in the jejunum and ileum (Figure 4). This finding suggests that the intestinal absorption occurred mainly in the duodenum.[28]

C. Safety Issues. The GI tract is normally exposed to a number of chemical and bacterial toxins.[38] Fortunately, chemical toxins are not normally found in the intestine, unless ingested accidentally. In contrast, endotoxins (lipopolysaccharide, LPS, the negatively charged components from the cell wall of Gram-negative bacteria) are the major bacterial toxins in the GI tract. If delivered to the systemic circulation, LPS triggers a systemic inflammatory response that can progress to endotoxic shock and occasionally death.[39]

Whether CS can also enhance the absorption of endotoxins present in the small intestine is widely speculated.

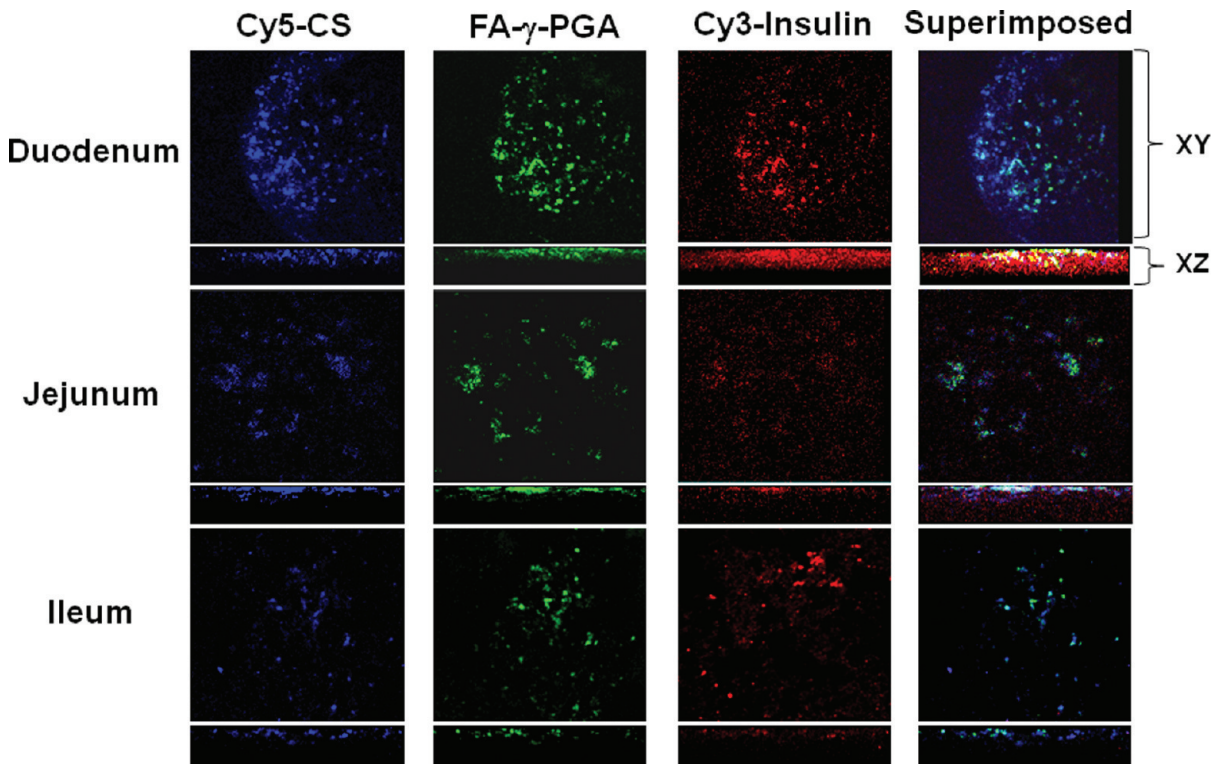

FIGURE 4. Fluorescence images of distinct intestinal segments of rats retrieved at 3 h after oral treatment with fluorescence-labeled CS NPs. *XY* plane, the plane parallel to the luminal surface of the intestine; *XZ* plane, the plane perpendicular to the intestinal surface. CS NPs, chitosan nanoparticles; Cy5-CS, cyanine 5-labeled chitosan; FA-γ-PGA, fluoresceinamine-labeled γ-PGA; Cy3-insulin, cyanine 3-labeled insulin. Adapted with permission from ref 28. Copyright 2009 Elsevier.

To address this concern, this study investigated how CS NPs affect the absorption of LPS. Their biodistribution was followed by single-photon emission computed tomography (SPECT)/computed tomography (CT), in which ^{99m}Tc-pertechnetate was used to label LPS (^{99m}Tc-LPS) and ^{123}iodine was used to label insulin (^{123}I-insulin). The ^{99m}Tc-LPS was then ingested 1 h before administering the ^{123}I-insulin-loaded NPs to replicate the physiological conditions. As shown in Figure 5A, the ^{99m}Tc-LPS remained mainly in the GI tract, while the ^{123}I-insulin could enter the systemic circulation and was observed in the urinary bladder. The above results suggest that the absorption enhancement by CS was specific for the loaded insulin only.[35]

An attempt was made to determine a specific reason for this observation by using the fluorescein isothiocyanate-labeled LPS (FITC-LPS) and Cy3-insulin-loaded NPs in order to repeat the study, examining by CLSM. As shown in Figure 5B, the Cy3-insulin could infiltrate the mucus layer and was observed underneath the epithelium (indicated by white arrows). Conversely, the FITC-LPS was restricted primarily outside the mucus layer (shown by blue arrows) and could not enter the systemic circulation.[35] This is probably owing to the charge repulsion between the anionic LPS and the negatively charged mucus layer.

Our previous investigation undertook an in vivo toxicity study to determine whether oral administration of CS NPs was safe.[28] Mice were treated with a daily dose of CS NPs for 14 days. The experimental group and the untreated control group did not significantly differ in clinical signs and body weight. The measured hematological and biochemical parameters for both studied groups were within the normal ranges. Moreover, no pathological changes were observed in the histological sections of the liver and kidney. CS has been reported to be effective as a hypolipidemic agent;[12] however, the dose of CS required to produce such an activity is much higher than that used in the formulation reported here.

In Vivo Efficacy of CS NPs

The efficacy of CS NPs for oral insulin delivery in a diabetic rat model was evaluated next. Diabetic rats were created by injecting streptozotocin (STZ) intraperitoneally; STZ has been used to induce diabetes in experimental animals, owing to its ability to cause selective destruction of pancreatic β-cells.[40] In this study, five test groups were

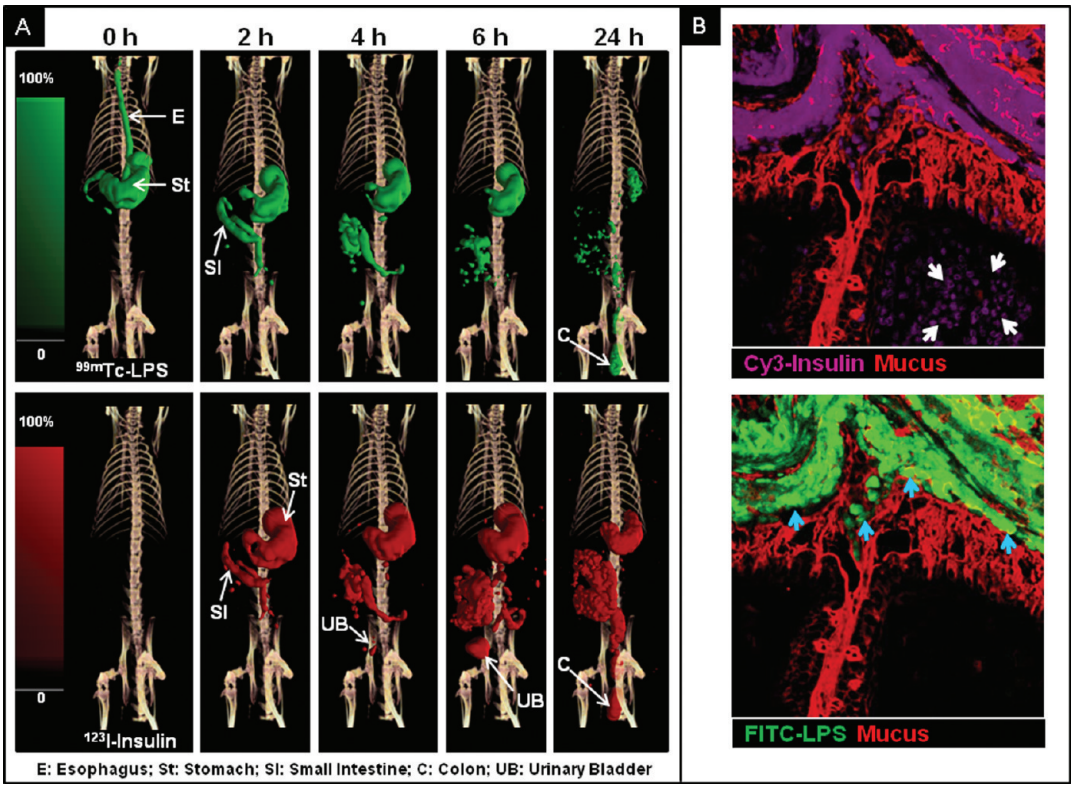

FIGURE 5. (A) Biodistribution of the ^{99m}Tc-LPS and ^{123}I-insulin in rats orally treated with the ^{99m}Tc-LPS followed by ^{123}I-insulin-loaded CS NPs. (B) Confocal images showing the intestinal villi retrieved from rats fed with FITC-LPS (green) followed by the administration of Cy3-insulin-loaded CS NPs. The white arrows indicate the absorbed insulin (purple) underneath the epithelium, while the FITC-LPS (indicated by blue arrows) mainly restricted outside the mucus layer (red). CS NPs, chitosan nanoparticles; ^{99m}Tc-LPS, ^{99m}Tc-pertechnetate-labeled lipopolysaccharide; ^{123}I-insulin, ^{123}iodine-labeled insulin; FITC-LPS, fluorescein isothiocyanate-labeled lipopolysaccharide; Cy3-insulin, cyanine 3-labeled insulin. Adapted with permission from ref 35. Copyright 2011 Elsevier.

investigated: the deionized water group (empty control), free-form insulin solution group, empty NP group, insulin-loaded NP group via oral administration, and free-form insulin solution group via SC injection.

A. Pharmacodynamic (PD)/Pharmacokinetic (PK) Profiles. As expected, for the SC injection group, the blood glucose level was reduced sharply within 2 h and then increased over time (Figure 6A).[28−30] For the insulin-loaded NP group via oral administration, the blood glucose level was reduced significantly within 4 h and maintained at approximately the same level for at least another 6 h. Conversely, the other three test groups did not exhibit a significant hypoglycemic action. The above results clearly demonstrate the effectiveness of our insulin-loaded NPs in reducing blood glucose levels in diabetic rats. Figure 6B displays the plasma insulin levels of diabetic rats treated with different formulations of insulin. Compared with the group treated with the free-form insulin solution subcutaneously, oral administration of the insulin-loaded NPs exhibited a slower absorption of insulin and reached the maximum concentration within 4−5 h, and thus might be suited for basal insulin therapy.[29] The relative bioavailability compared to SC injection was about 15%.[28] These results suggest that oral administration of insulin using our CS NPs may reduce the risk of hyperinsulinemia which has been commonly observed in patients receiving insulin subcutaneously.[29]

B. Biodistribution. Biodistribution of the isotope-labeled NPs, in which CS was labeled by ^{99m}Tc and insulin by ^{123}I, was examined by using a dual isotope dynamic SPECT/CT scanner. According to our results, CS mainly propagated from the stomach, through the small intestine, and then into the large intestine; meanwhile, insulin was clearly observed in the kidneys and in the urinary bladder.[29]

C. Capsule Formulation. The aforementioned study was performed with CS NPs suspended in deionized water. The SPECT/CT images revealed a significant portion of CS NPs, labeled by ^{99m}Tc, remaining in the stomach over an extended period after oral administration. Additionally, a certain amount of the loaded insulin might release from CS NPs,

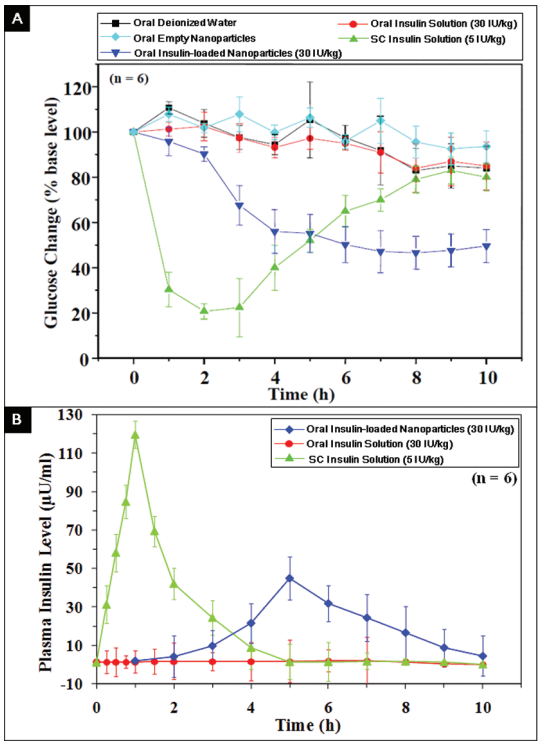

FIGURE 6. (A) Blood glucose level versus time profiles and (B) plasma insulin level versus time profiles of diabetic rats, following the administration of different insulin formulations. Oral, oral administration; SC, subcutaneous administration; CS NPs, chitosan nanoparticles. Adapted with permission from ref 28. Copyright 2009 Elsevier.

due to their pH instability, while in the stomach. To overcome these problems, CS NPs were lyophilized and the dried NPs were filled in a gelatin capsule coated with an enteric polymer (Eudragit L100-55).[30] This enteric polymer is pH-sensitive and capable of withstanding prolonged contact with acidic gastric media, yet readily dissolves in a mildly acidic to a neutral environment of the small intestine.[41] This approach can prevent the release of insulin from CS NPs while in the stomach and enhance their absorption on the surface of the small intestine, thus further increasing their bioavailability (Figure 7).

According to the X-ray images, the enteric-coated capsule remained intact in the stomach and started to disintegrate in the proximal portion of the small intestine (Figure 8). Once in the small intestine, the capsule was dissolved completely and the loaded contents propagated through the intestine. With this enteric-coated capsule filled with free-dried CS NPs, the relative bioavailability of insulin in comparison to SC injection was increased to about 21%.[30]

Other Therapeutic Applications

In addition to insulin, this NP platform technology has been successfully investigated for oral delivery of other therapeutic macromolecules, including exendin-4 and heparin.[42,43]

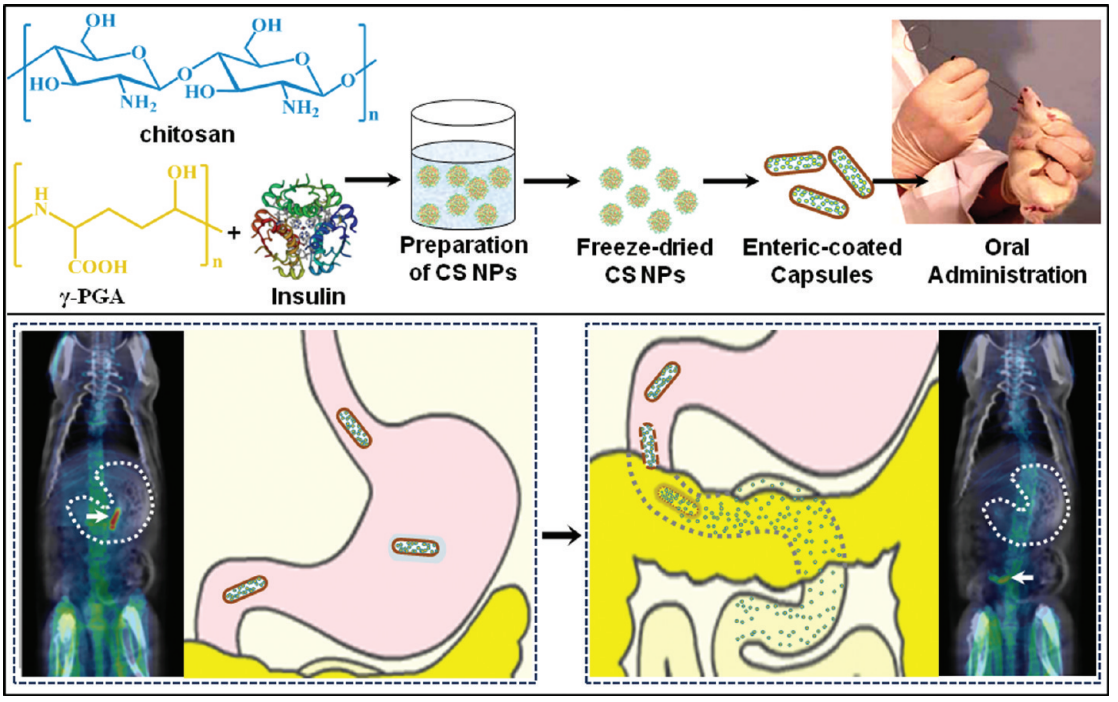

FIGURE 7. Schematic illustrations showing the preparation of enteric-coated capsules filled with freeze-dried CS NPs. The prepared enteric-coated capsules can prevent the release of insulin from CS NPs while in the stomach and enhance their absorption on the surface of the small intestine, thus further increasing their bioavailability. CS NPs, chitosan nanoparticles. Modified with permission from ref 30. Copyright 2010 Elsevier.

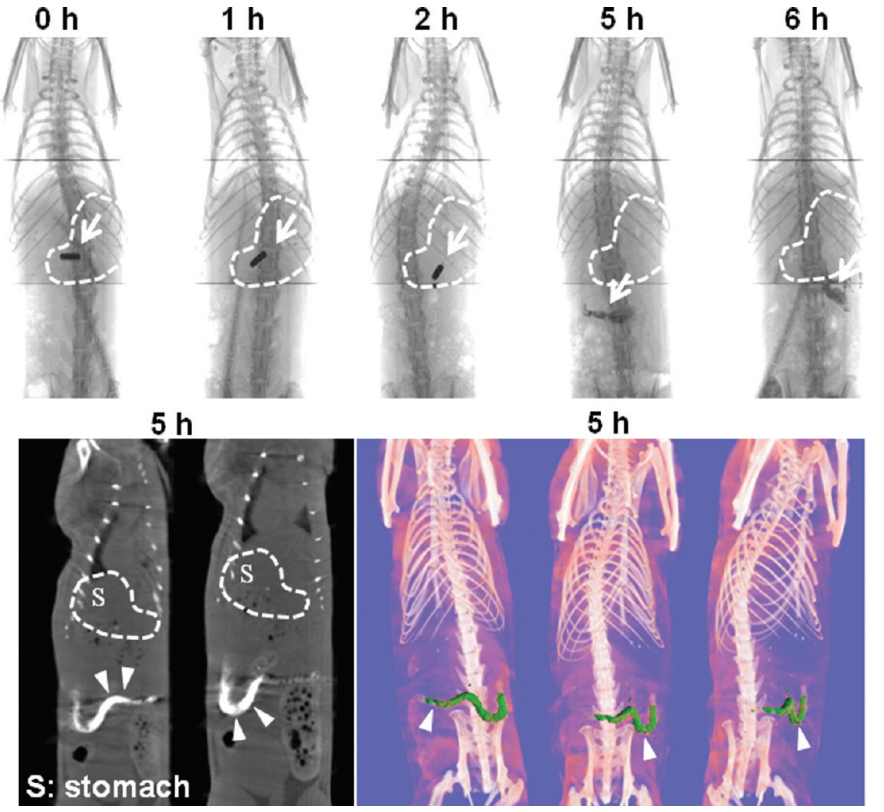

FIGURE 8. Sequential plain X-ray images (upper panel), CT-scan images (lower-left panel) and 3D-volume-rendering images (lower-right panel) of rats orally treated with the barium sulfate-filled capsule (indicated by arrows). The released barium sulfate was located in the intestine (indicated by arrowheads). Adapted with permission from ref 42. Copyright 2011 Elsevier.

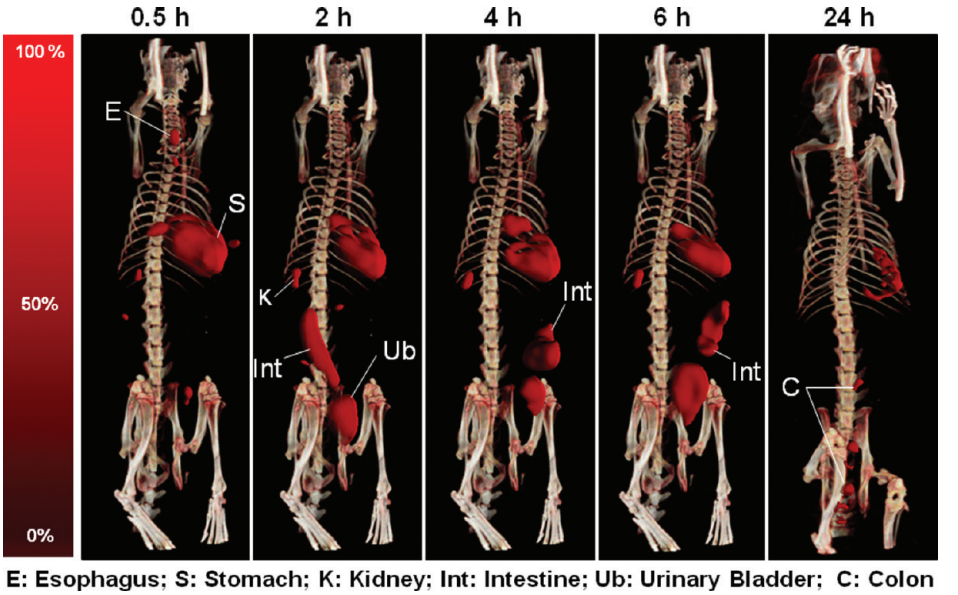

FIGURE 9. Three-dimensional (3D)-volume-rendering SPECT/CT images of ^{123}I-exendin-4 distributed in rats at distinct time points after oral administration of CS NPs. CS NPs, chitosan nanoparticles; ^{123}I-exendin-4, ^{123}iodine-labeled exendin-4. Adapted with permission from ref 42. Copyright 2011 Elsevier.

Exendin-4, a 39-amino-acid peptide, shares several gluco-regulatory activities with the mammalian incretin hormone glucagon-like peptide-1 (GLP-1) such as glucose-dependent enhancement of insulin secretion, suppression of glucagon

secretion, reduction of gastric mobility and food intake.[44] The FDA has approved exenatide, a synthetic version of exendin-4, as an adjunctive therapy for type 2 diabetic patients.[45] The reported bioavailability of exenatide administered via distinct noninjection routes has been found to be minimal.[46]

Figure 9 shows the biodistribution of the ^{123}I-labeled exendin-4 orally delivered by CS NPs, examined by SPECT/CT; as shown, exendin-4 was absorbed into the systemic circulation and found in the kidneys and urinary bladder. Oral administration of the capsule containing exendin-4-loaded CS NPs showed a maximum plasma concentration at 5 h after treatment; the bioavailability, relative to its SC counterpart, was found to be approximately 15%.[42] The absorbed exendin-4 could then stimulate the insulin secretion and provide a prolonged glucose-lowering effect.

Heparin, a highly negatively charged glycosaminoglycan, is a potent injectable anticoagulant. Clinically, heparin has been used to prevent deep vein thrombosis and peripheral arterial embolism and reduce the incidence of myocardial infarction and death in patients with unstable angina.[47] Our previous study described a NP system shelled with CS for oral delivery of heparin; the NPs were prepared by a simple ionic gelation method to form CS/heparin complexes without chemically modifying heparin. No significant anticoagulant activity was detected after oral administration of the free-form heparin solution in a rat model, while administration of CS NPs orally was effective in the delivery of heparin into the bloodstream; the absolute bioavailability was 20.5%.[43]

Concluding Remarks

Despite tremendous research efforts to overcome the difficulties in oral absorption of proteins, a clinically effective and safe delivery system remains an elusive challenge. The pH-responsive NPs shelled with CS reported by our group are clearly a promising vehicle for oral delivery of protein drugs such as insulin. However, a few obstacles must be addressed for these CS NPs to become a reality for oral insulin delivery. For instance, these CS NPs must demonstrate their superiority over the injectable insulin in terms of better management of the blood glucose level and lesser occurrence of diabetic complications. Additionally, their long-term efficacy must be demonstrated in large animals and in humans.

Another challenge in using mucoadhesive CS NPs, with prolonged retention in the intestinal tract, for oral protein delivery is maintaining the physicochemical and biological stability of the delivered drugs. Immobilizing enzyme inhibitors within CS NPs can render this benefit and increase the bioavailability of orally administered proteins. The presence of both reactive amino and hydroxyl groups on CS provides opportunities for chemical modification to incorporate various enzyme inhibitors, while maintaining its TJ opening activity. Moreover, CS is insoluble in the neutral/basic pH environment due to the deprotonation of its amino groups, leading to the loss of its mucoadhesive characteristic and its TJ opening activity such as in the jejunum and ileum, as mentioned earlier. Chemically modified CS can be prepared to improve its physiochemical properties such as aqueous solubility and mucoadhesive and intestinal permeation capabilities. If the above challenges are successfully addressed, the CS-based NP system for oral delivery of protein drugs may soon become a reality. Hopefully, this Account provides readers with further insight into the great potential of a CS-based NP platform for a diverse array of applications in oral protein delivery for therapeutic treatment.

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BIOGRAPHICAL INFORMATION

Hsing-Wen Sung received his Ph.D. degree in Biomedical Engineering from the Georgia Institute of Technology in 1988. He currently serves as a Tsing Hua Chair Professor of Chemical Engineering at National Tsing Hua University (NTHU), Taiwan. The several research awards that he has received include Fellow of the American Institute for Medical and Biological Engineering and Fellow of the International Union of Societies for Biomaterials Science and Engineering. His main research interests focus on biomaterials applied for tissue engineering and drug/gene delivery.

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FOOTNOTES

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