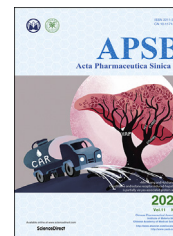




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REVIEW

Can nanoparticles and nano–protein interactions bring a bright future for insulin delivery?



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Abstract Insulin therapy plays an essential role in the treatment of diabetes mellitus. However, frequent injections required to effectively control the glycemic levels lead to substantial inconvenience and low patient compliance. In order to improve insulin delivery, many efforts have been made, such as developing the nanoparticles (NPs)-based release systems and oral insulin. Although some improvements have been achieved, the ultimate results are still unsatisfying and none of insulin-loaded NPs systems have been approved for clinical use so far. Recently, nano–protein interactions and protein corona formation have drawn much attention due to their negative influence on the *in vivo* fate of NPs systems. As the other side of a coin, such interactions can also be used for constructing advanced drug delivery systems. Herein, we aim to provide an insight into the advance and flaws of various NPs-based insulin delivery systems. Particularly, an interesting discussion on nano–protein interactions and its potentials for developing novel insulin delivery systems is initiated.

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1. Introduction

Diabetes mellitus (DM), a kind of metabolic disorder disease, is characterized by the high glycemic levels due to the loss of functions to produce or use insulin^{1–3}. According to statistics, the global diabetic patient number is over 280 million in 2010 and expected to be over 400 million by 2030⁴. With the rapid increase in patient number, the associated costs for treatment of DM and its complications are extremely high, which are estimated to be up to US\$500 billion per year all over the world⁵. Undoubtedly, efficient prevention and therapy of DM is quite meaningful for global economy and human health.

The therapeutic regimen for DM varies according to its different types. DM is generally categorized as two types: type I and type II. Patients suffering from type I DM lose the ability to produce insulin, a blood glucose level-regulating polypeptide which is secreted by β cells of pancreas islets, due to the destruction of β cells^{6,7}. Hence, an insulin replacement therapy is prescribed for type I DM throughout the whole life of patients. In contrast to type I DM, type II is featured with a relative deficiency of insulin or insulin resistance mainly caused by obesity of the patients⁸. Patients with type II DM can produce insulin but the sensitivity of their liver and muscle cells to insulin is low. Reducing fat intake and increasing physical activity are recommended for early-stage type II DM patients to delay the disease progression^{9–11}. Taking oral hypoglycemic drugs to enhance the production and function of insulin is another important way for type II diabetic patients to control glucose level^{12,13}. In most cases, insulin therapy is ultimately required for treating type II DM and physicians and experts prefer to applying insulin therapy as early as possible^{14,15}.

Because of the intrinsic instability of insulin under the physiological conditions, the delivery of insulin, especially *via* the oral route, is extremely challenging. It is well known that the rapid inactivation and degradation of insulin in the gastrointestinal (GI) tract and the low permeation through the intestinal epithelia barrier make the oral delivery of insulin very difficult^{16–18}. Intranasal administration is another possible route for insulin delivery but many concerns have to be addressed before it can be practically used^{19,20}. In addition, microneedle-based insulin delivery systems have also attracted much attention²¹. Nowadays, subcutaneous injection is still the dominating route for delivering insulin in clinic, and frequent injections are required to properly control the glycemic level due to the short-acting property of native insulin. The inconvenience and pains caused by the frequent injections lead to another problem that is the low patient compliance.

In order to improve the delivery efficiency as well as the patient compliance, nanoparticles (NPs) have been used in both subcutaneous and oral formulations to protect insulin from degradation, enhance its epithelial permeation and/or prolong its acting time^{17,22–25}. In the past decade, the nano–protein interactions and the formation of protein corona have been considered as a challenge for NPs-based drug delivery^{26–30}. Meanwhile, such interactions can also be used to decorate NPs for advanced drug delivery^{31–33}. It is foreseeing that nano–protein interactions may also play a role in insulin delivery. Herein, a comprehensive

review of NPs-based insulin delivery is summarized, and a discussion on the nano–protein interactions and the potentials of using such interactions for developing novel insulin delivery systems is initiated (Fig. 1).

2. Traditional insulin delivery and microneedles

Insulin, a protein produced by pancreatic β -cells, is the only blood glucose lowering hormone in organism, which is essential for DM. Generally, oral administration is the most favorable route for patients to take medicine. Unfortunately, insulin cannot be administered orally due partially to its instability and degradation in the GI tract^{34,35}. Besides, the molecular weight of insulin is about 5800 Da, leading to a low drug absorption by intestinal epithelial cells. These above factors together result in an extremely low oral bioavailability of insulin.

Once upon a time, pulmonary delivery of insulin was of great interests. Exubera, the first insulin inhaled formulation, was successfully introduced to the market by Pfizer in 2006 but finally failed in the market because of the high variation in insulin absorption, the complex inhalation technique and the low patient acceptance^{17,36}.

In addition, microneedle-based insulin delivery systems have also attracted much attention of researchers²¹. Unlike insulin injections, insulin in microneedles can be delivered across the skin to systemic circulation by minimally invasive patches, leading to greatly enhanced patients' compliance. There are diverse structures of microneedles, such as solid, hollow, dissolved, degradable and phase transition microneedles. Although some microneedle-based insulin delivery systems (mainly the hollow systems) had entered the clinical trials, none of them has become commercially available so far³⁷. Some concerns, including the safety of materials, the precise insulin deliver and the complicated fabrication process of microneedles, should be further studied and improved until its application in clinic.

Hitherto, subcutaneous injection is still the dominating route for administering insulin in clinic. However, the short half-life of insulin requires repeated injections in one day to efficiently control the glycemic level, which causes inconvenience to patients. To decrease the injection frequency of insulin and enhance the patient compliance, great endeavors have been made to develop advanced insulin delivery systems, in which NPs-based insulin delivery systems are the major research fields.

3. Nanoparticles-based insulin delivery systems

NPs have shown great potentials in sustained and controlled delivery of various theranostic agents^{34,36}. To develop injectable long-acting insulin formulations and oral insulin systems, NPs are commonly utilized due to its capacity to control the release of insulin, protect insulin from degradation and enhance its bioavailability^{34,38,39}. Herein, the potential applications of insulin-loaded NPs *via* subcutaneous or oral delivery route are summarized with emphasis on the latter since oral insulin is always of the greatest importance and interest. In addition, a newly emerging

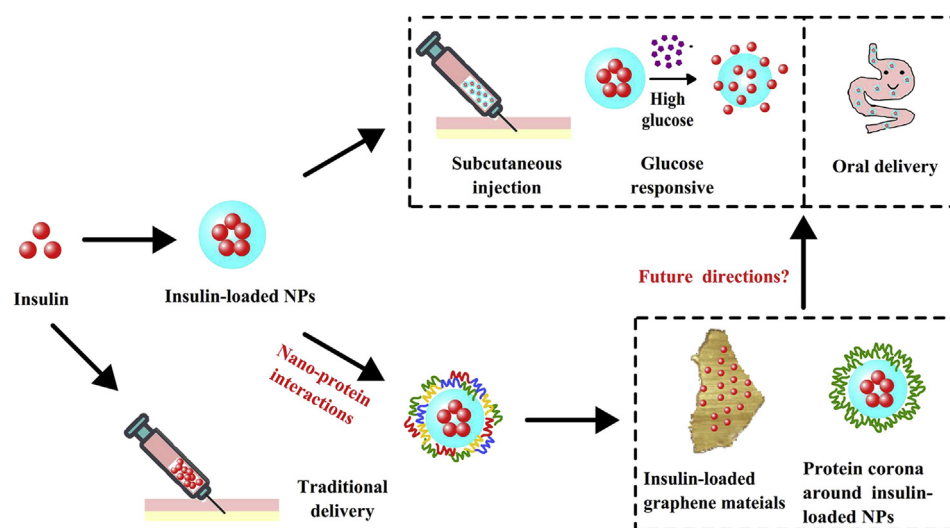


Figure 1 Schematics of the applications of nanoparticles (NPs) and nano-protein interactions in insulin delivery. Nano-protein interactions include the interaction between nanocarriers (such as graphene materials) and insulin and the one between insulin-loaded NPs and the other endogenous proteins.

nanoparticle-based glucose-responsive insulin delivery system will also be discussed.

3.1. Subcutaneous delivery systems

3.1.1. Insulin-loaded long-term delivery systems

Subcutaneous injection is the only practical route for insulin administration since the failure of Exubera, an insulin formulation delivered by inhalation. Basically, frequent repeated injections are required for diabetic patients to effectively control the glycemic level. However, frequent injections cause pain and inconvenience and result in the low patient compliance. The drug delivery systems that can provide a long-term release of insulin have attracted much attention. NPs-based insulin delivery systems have been considered as the promising formulations for sustained and controlled delivery of insulin^{40–42}. For instance, Shilo et al.³⁸ coated insulin onto gold NPs (INS-GNPs) for controlled and prolonged glucose control. After subcutaneous injection into BALB/c mice, INS-GNPs with the size of 50 nm showed longer and stronger hypoglycemic effect than that of INS-GNPs of 20 and 70 nm. In the pharmacodynamics study on diabetic mice, the hypoglycemic effect of INS-GNPs lasted 3 times longer than that of insulin solution. The mechanism of the prolonged effect was proved to be that the insulin conjugation onto GNPs prevented its enzymatic degradation. Similarly, Tomar et al.⁴³ developed insulin-loaded PLA-PEG NPs which showed a prolonged hypoglycemic effect of more than 7 days, and the NPs were proved to be nontoxic.

Our group has also demonstrated the great potentials of NPs in the long-term delivery of insulin. We constructed the insulin-phospholipid complex-loaded PHBHHx NPs (INS-PLC-NPs) which showed a long-term insulin release *in vitro* and very high insulin encapsulation efficiency (89.7%) due to the formation of INS-PLC⁴⁴. Most importantly, upon a single subcutaneous injection into the diabetic rats, INS-PLC-NPs showed a rapid, stable, effective and lasting hypoglycemic effect for more than 3 days (Fig. 2). Such a sustained hypoglycemic effect conferred INS-PLC-NPs a greatly enhanced pharmacological bioavailability relative to free insulin (350%)⁴⁴. In a further study, INS-PLC-NPs

were loaded into a β -glycerophosphate disodium salt/chitosan-based thermo-sensitive hydrogel (NP-CS/GP) to further extend insulin release. We found NP-CS/GP has a hypoglycemic effect for more than 5 days and showed a pharmacological availability of 379.85% relative to the free insulin-loaded hydrogel (INS-CS/GP), and higher (Fig. 3)²².

3.1.2. Glucose-responsive insulin delivery systems

Glucose-responsive insulin delivery system is another newly and major category of subcutaneously injected insulin vehicles with intelligent control of insulin release. As we know, both of hyperglycemic and hypoglycemic are detrimental for diabetic patients^{45,46}. However, because of the terribly narrow therapeutic index of insulin, monitoring and proper controlling of blood glucose all the time are very important but difficult. Accordingly, an intelligent system, glucose-responsive insulin delivery system emerged, which aims at emulating the function of pancreatic β -cells to release demanded insulin in response to high glycemic level and achieving accurate glycemic control seamlessly. In the smart glucose-responsive system, various glucose-responsive materials or strategies have been adopted for bio-inspired reaction⁴⁷. Basically, insulin is embedded in a matrix containing glucose-responsive materials, which changes its structure to launch or stop insulin release in response to the blood glucose level.

In the past years, significant progress in glucose-responsive insulin delivery system has been achieved, which will be discussed according to different-responsive mechanisms in this section: (1) glucose oxidase-based system; (2) glucose binding protein-based system; (3) glucose binding molecule-based system; (4) modified insulin-based system.

3.1.2.1. Glucose oxidase-based systems. Typically, the glucose-responsive systems are based on glucose oxidase (GOD)⁴⁸, and the first GOD-based pH sensitive hydrogel for glucose-responsive delivery of insulin was reported in 1984⁴⁹. GOD is widely used for the quantification of blood glucose due to its high sensitivity in reaction with glucose. Under the catalysis of GOD, glucose is transformed into gluconic acid and byproduct

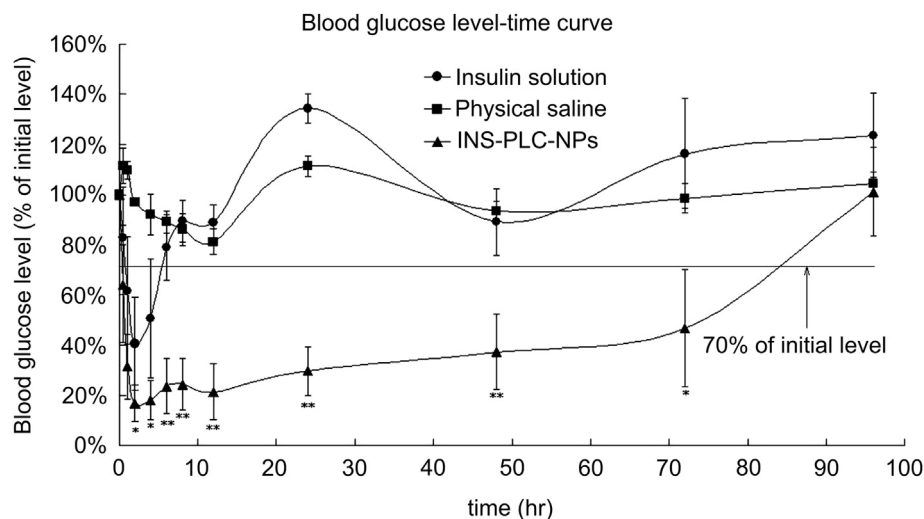


Figure 2 Blood glucose level-time profiles of insulin solution (1 IU/kg), physical saline and INS-PLC-NPs (4 IU/kg) in the diabetic rats model upon s.c. administration ($n = 5$). Significance of INS-PLC-NPs group vs. insulin solution group: * $P < 0.05$, ** $P < 0.001$. Reprinted with the permission from Ref. 44 Copyright © 2012 Elsevier BV.

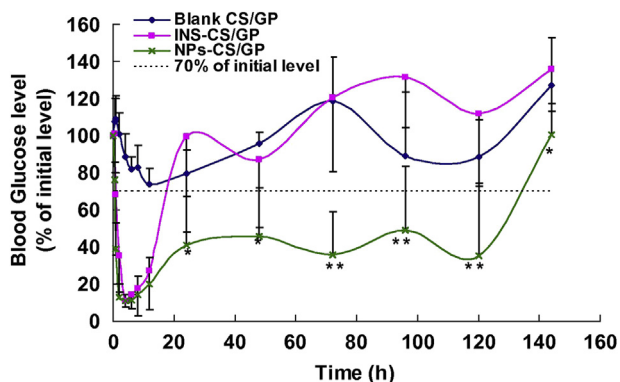
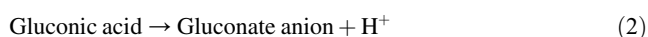
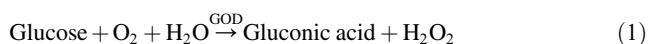


Figure 3 Blood glucose level-time profiles of blank CS/GP, INS-CS/GP (4 IU/kg) and NP-CS/GP (6 IU/kg) in the diabetic rats model upon subcutaneous injection ($n = 5$). Significance of INS-CS/GP group vs. NP-CS/GP group: * $P < 0.05$, ** $P < 0.01$. Reprinted with the permission from Ref. 22 Copyright © 2013 Elsevier BV.

hydrogen peroxide (H_2O_2) as shown in Eq. (1). The hydrogen ion dissociated from gluconic acid will reduce the local pH as shown in Eq. (2)⁵⁰. The protonization and H_2O_2 can serve as activators for the collapsing or swelling of hydrogel⁵¹ or dissociating of nanoparticle matrix, subsequently leading to the release of loaded insulin^{52–55}.



In the GOD-based glucose-responsive nano-carriers for insulin, the mechanism is usually based on the disassembling/degradation of the matrix^{39,56,57}. For instance, Qi et al.³⁹ developed a glucose sensitive microcapsules using hemoglobin (Hb) and GOD cross-linked by glutaraldehyde (GA) in the form of layer by layer. With the increase of glucose level, GOD catalyzed glucose into gluconic acid and H_2O_2 . When the local pH was decreased by the production of gluconic acid, the permeability of Hb/GOD capsule

wall was enhanced and the structure of the multilayer loosed, which affiliated the release of loaded insulin.

Besides, mesoporous silica was also used for GOD-based glucose-responsive systems^{58–60}. Aznar et al.⁵⁸ prepared a mesoporous silica nano-device capped with cyclodextrin-modified-glucose oxidase (CD-GOD), which could release the loaded cargo in response to the high glucose level. With the increase and accumulation of H_2O_2 , the activity of GOD can be inhibited and inflammation may happen. Therefore, catalase (CAT) was often adopted into GOD-based system⁶¹. Interestingly, the production and consumption of H_2O_2 were also combined with GOD-based glucose-responsive system. Gu and colleagues^{52,62,63} have done much work in this field. For example, they developed a glucose-responsive system⁶², in which GOD and CAT were dispersed to form nanocapsules and then encapsulated into insulin-loaded pH-responsive chitosan microgels. The nanocapsules protected enzymes from denaturation and immunogenicity (Fig. 4). This was a self-regulating valve system. Under normal glycemic condition, microgels released insulin at a basal rate. While under hyperglycemic condition, glucose was catalyzed into gluconic acid and subsequently the amine groups in polymer chains were protonated to increase the charge in gel matrix, leading to microgels swelling and subsequently insulin release. Moreover, glucose sensitive nano-carriers were also combined with microneedle-array patches^{55,64}.

3.1.2.2. Glucose binding protein-based systems. Glucose binding proteins (GBP), usually lectins, can also be used in glucose-responsive systems due to their competitively combination with glucose. Concanavalin A (Con A) is the most widely studied GBP, which is in the form of tetramers exposing four glycoprotein binding sites in a neutral condition⁶⁵. The first Con A-based glucose-responsive insulin delivery system was reported by Che and colleagues in 1979⁶⁶. Under the high glucose conditions, glucose competed for the binding sites on Con A with glycosylated insulin, resulting in the release of insulin in proportion to the glucose level. In further studies, the formulation was optimized with Con A derivatives (e.g., PEGylation Con A⁶⁷), glycosylated insulin (e.g., succinyl amidophenyl mannopyranoside

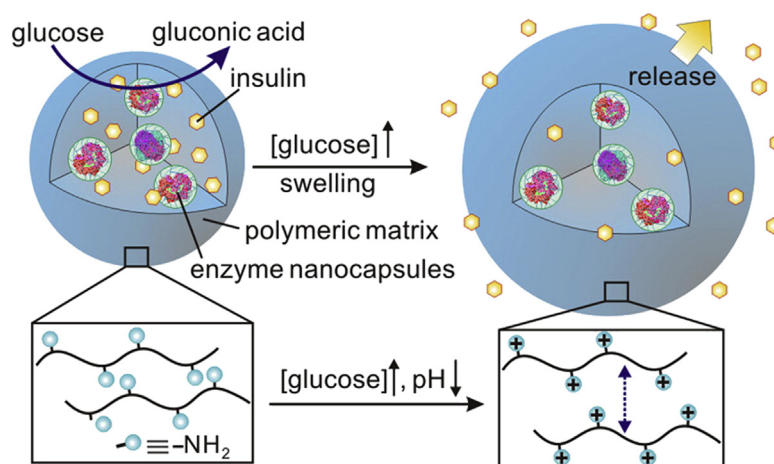


Figure 4 Schematics of the insulin- and enzyme nanocapsules-loaded microgels. Glucose is catalyzed by enzyme into the gluconic acid which in turn leads to the protonation of $-\text{NH}_2$ on the polymer chains. Insulin is ultimately released as a result of the protonation-caused swelling of the microgels. Reprinted with the permission from Ref. 62 Copyright © 2013 American Chemical Society.

insulin^{68,69}) or glycosylpoly(ethylene glycol) insulin⁷⁰ to improve the stability and insulin release.

GBP-based mechanism is generally used for developing glucose-responsive hydrogels^{71–73}. Meanwhile, novel materials were synthesized and adopted to formulate GBP-based glucose-responsive nano-carriers^{74–76}. You et al.⁷⁵ prepared a glucose-sensitive aggregates with Poly(ethylene oxide)-*block*-poly(2-glucosyloxyethyl acrylate) and Con A. Wu et al.⁷⁶ prepared glucose- and pH-responsive mesoporous silica insulin-loaded nanocontainers with Con A gated and mannose functionalized. The insulin release could be triggered by the decrease of pH (<5.5) and increase of glucose concentration.

3.1.2.3. Phenylboronic acid-based systems. Phenylboronic acid (PBA) and its derivatives have been used as glucose binding agents to design the closed-loop insulin delivery systems since 1994⁷⁷ and have achieved great advances so far⁷⁸. PBA and derivatives can form the reversible covalent complexes with glucose containing polyhydroxyl groups and thus can be used as the glucose sensor in glucose-responsive systems. PBA moieties have been widely implanted in crosslinked polymeric matrix loaded with insulin. With the increase of glucose concentration, glucose–PBA complex forms and the hydrophilic phenylborate level increases, leading to the swelling of the matrix and insulin release^{53,79–81}.

In addition, in PBA moieties-contained layer-by-layer particles^{82,83} or core–shell particles^{84–88}, glucose competitively binds to PBA and triggers the release of insulin due to the particle disassembling and degradation. Shi et al.⁸³ fabricated a poly(γ -glutamic acid) (γ -PGA) and chitosan oligosaccharide (CS) polyelectrolyte-based glucose-responsive capsule system. At the high glucose level, the competitive reaction of amino-phenylboronic acid (APBA) with glucose caused the polymer capsules swelling and broke the cross-linking, ultimately resulting in capsules dissociation and insulin release. Meanwhile, decoration of the (γ -PGA-g-APBA)₅ capsules with lactobionic acid (LA) led to the on-off regulation of insulin release.

Also, PBA-modified polymers were combined with glycosylated insulin to formulate glucose-responsive system⁸⁹. Zhao et al.⁸⁹ developed glucose-responsive mesoporous silica NPs

(MSN) co-loaded with insulin and cyclic AMP. The boronic acid-functionalized MSNs were loaded with cyclic AMP inside and decorated with glycosylated insulin (G-Ins) on the exterior surface *via* interactions with PBA. Thus, the competitive binding of glucose with PBA triggered the release of G-Ins and cAMP which could stimulate the secretion of insulin by β -islet cells.

3.2. Oral delivery systems

Oral administration is widely recognized as the most convenient and tolerated drug delivery route for patients. However, as a protein drug, insulin is mainly administered by subcutaneous injection due to the harsh environment in the GI tract. To solve this problem, various functional NPs-based systems have been developed.

3.2.1. Barriers for oral absorption of insulin

The oral absorption barriers that insulin has to overcome in the GI tract mainly include chemical, enzymatic and physical barriers⁹⁰. Basically, most oral drugs transit through the GI tract, adhere to the mucus layer, cross the intestinal epithelium, and finally enter the systemic circulation³⁴. However, the oral bioavailability of insulin is quite low (less than 1%) due to its instability in the GI tract and low epithelial permeation⁹¹.

The remarkable change of pH values from 1.0 to 2.5 in the stomach to ~ 7.5 in the terminal ileum constructs the chemical barrier to the orally administered insulin⁹². The low pH in the stomach is one of the harsh conditions leading to the degradation and low absorption of insulin. The proteases secreted into the GI tract act as the enzymatic barrier, which destruct the orally administered insulin into the inactive small amino acids or peptides. The enzymes involved in the degradation of proteins in the GI tract mainly include the pepsin in the stomach, the pancreatin in the small intestine (such as trypsin, chymotrypsin and elastase), the amino-peptidases in the brush border membrane, and the specific enzymes in the cytosol^{93–95}. Moreover, even if a small amount of insulin can survive from the above proteases, it will be probably degraded by the liver enzymes, known as the first-pass effect.

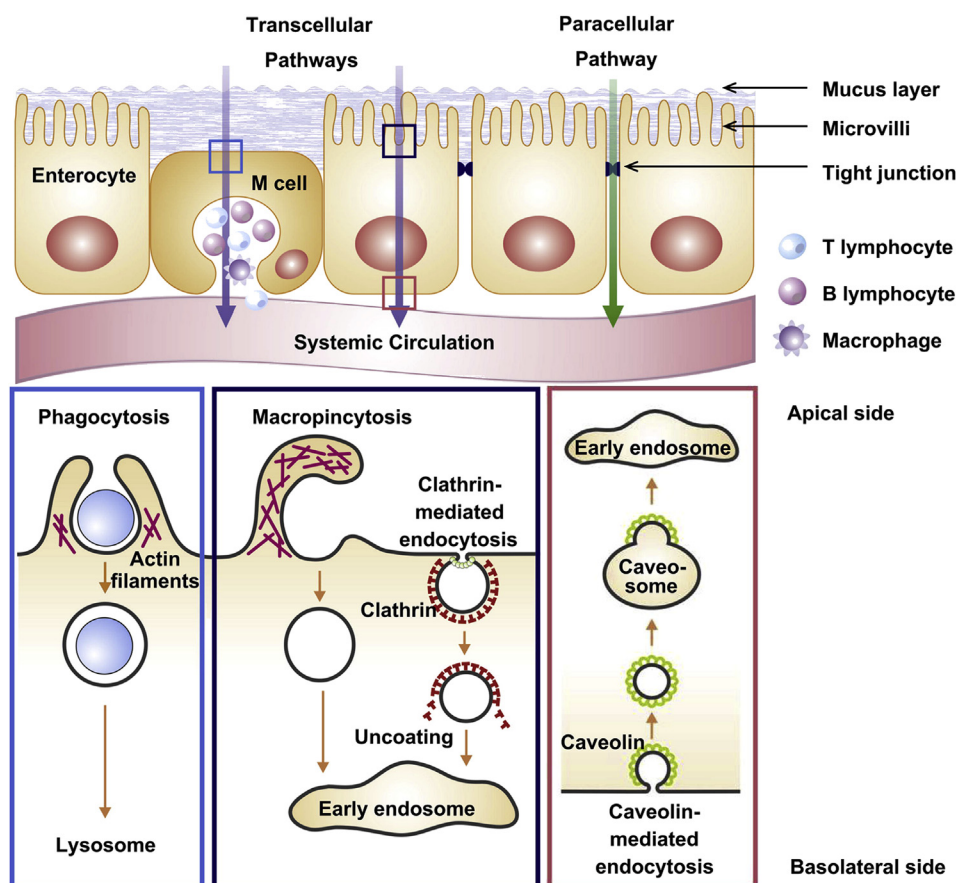


Figure 5 Schematics of the main mechanisms for NPs uptake by intestinal epithelium, including paracellular pathway and transcellular pathway. Inserts show the simplified processes of varied transcellular transport, including phagocytosis, macropinocytosis and clathrin/caveolae-mediated endocytosis. Reprinted with the permission from Ref. 34 Copyright © 2011 Elsevier BV.

The physical barriers in the GI tract are another challenge for the oral absorption of insulin. The mucus layer secreted on the intestinal epithelium is semipermeable and only allows the permeation of nutrients, water and small molecules. In this regard, insulin, a polypeptide with a molecular weight of ~ 5800 Da, is hard to cross the mucus layer. In addition, the negative charge of insulin molecules (isoelectric point is ~ 4.5) in the small intestine (pH ~ 6.8) is also unfavorable for their transport across the mucus layer which is known to be negatively charged. The intestinal epithelial cells constitute another physical barrier for the oral absorption of insulin. It has been reported that only the hydrophilic drugs with a molecular weight of below 200 Da can pass through the intestinal epithelium by the paracellular route, and that only the lipophilic small molecules smaller than 700 Da are allowed to pass through the intestinal epithelium by the transcellular route^{34,96}. Undoubtedly, insulin is difficult to be taken up by the epithelial cells due to its large molecular weight. The above-mentioned barriers work together and ultimately lead to the extremely low oral bioavailability of insulin. Therefore, many researchers have been working to solve this problem recent years.

3.2.2. Nanotechnology for enhancing oral absorption of insulin

The past decades have witnessed the progress of NPs-based insulin delivery systems which showed great potentials to bypass the absorption barriers and enhance the oral bioavailability of insulin^{97–99}. Encapsulated into the NPs, insulin can be protected

from the chemical and enzymatic degradation^{100,101}. Besides, polymeric NPs can significantly improve the uptake by small intestinal epithelial cells and promote the absorption *via* paracellular pathway³⁴. Furthermore, the insulin-loaded NPs can also be taken up by M-cells, a kind of special enterocytes in the GI tract¹⁰². Generally, NPs facilitate the transport of insulin across the intestinal epithelium by both paracellular and transcellular pathways (Fig. 5)^{35,102}. Paracellular pathway is characterized as an approach to get across the tight junctions between adjacent cells. In contrast, transcellular pathway is an approach to directly get across the epithelial cells *via* phagocytosis, macropinocytosis or endocytosis.

3.2.2.1. Nanoparticles overcoming chemical and enzymatic degradation.

At the early stage, efforts were focused on enhancing insulin resistance to chemical and enzymatic degradation with the help of NPs encapsulation. Ramachandran et al.¹⁰³ prepared the insulin-loaded PEGylated calcium phosphate NPs which showed a negligible release of insulin under the gastric acidic condition (6.5%) and a complete release in the intestinal neutral pH. But only the high resistance to acidity is far from enough since the released insulin in the intestinal site will still encounter the enzymatic degradation and epithelial uptake limitation. Yu et al.¹⁰⁴ constructed a more complicated system, in which the insulin-loaded polymer-lipid hybrid NPs (INS-PLGA-lipid-PEG NPs) were transformed into microparticles by spray freeze-drying and loaded in enteric capsules. The enteric capsules

could prevent insulin from the gastric harsh conditions, and the NPs released in the intestine could increase the cellular permeability and uptake. Consequently, the insulin absorption was greatly enhanced and the oral bioavailability of the NPs system in diabetic rats was about 12% relative to the subcutaneously injected insulin. Some other pH sensitive materials were also used for oral delivery of insulin, such as poly(*N*-isopropylacrylamide) (NIPAAm) polymer derivatives¹⁰⁵ and hydroxypropylmethyl cellulose phthalate (HPMCP)¹⁰⁶.

NPs co-loaded with insulin and enzyme inhibitor is another strategy for overcoming the enzymatic barrier and enhancing oral absorption of proteins^{107,108}. Su et al.¹⁰⁷ prepared a functional NPs system by mixing CS with anionic γ PGA-DTPA. The γ PGA-DTPA could inhibit the proteases significantly and enhance the paracellular permeability of NPs by disrupting the intestinal tight junctions. Additionally, the CS/ γ PGA-DTPA NPs were also pH-responsive and in favor of intestinal absorption of insulin. Based on the above strategies, the insulin relative oral bioavailability of the functional NPs was increased to approximately 20%.

3.2.2.2. Nanoparticles overcoming the physical barriers. The mucus layer together with the intestinal epithelial cells constructs the physical barriers for insulin oral absorption. In order to overcome this issue, NPs systems decorated with mucoadhesive or absorption enhancers are developed. Dange et al.¹⁰⁹ prepared Ins-NPs with hybrid polyester poly(ϵ -caprolactone) and polycationic acrylic polymer Eudragit® RS. With an insulin entrapment efficiency of 96% and the mucoadhesive property, the polymeric NPs preserved insulin bioactivity and showed a significant intestinal uptake.

Cell penetrating peptides (CPP) have been widely used to improve protein permeability across the intestinal mucus layer and epithelia^{23,110–112}. Low molecular weight protamine (LMWP) is such a CPP¹¹³. Sheng et al.²³ conjugated insulin with LMWP and encapsulated the conjugate into the *N*-trimethyl chitosan chloride-coated PLGA NPs (MNPs). MNPs showed the mucoadhesive property in the intestine, which shortened the distance between insulin and the epithelium and protected insulin from enzymatic degradation. After released from MNPs, the conjugate showed a high permeation across the mucus layer and epithelium. When orally administered to diabetic rats, the MNPs showed a fast onset, a long-lasting hypoglycemia effect and a relative bioavailability of $17.98 \pm 5.61\%$. Particularly, the oral delivery of insulin or the conjugate (50 IU/kg) did not show any hypoglycemic effect, demonstrating the great protection effect of MNPs on insulin²³. In another study, *N*-(2-hydroxypropyl) methacrylamide copolymer (pHPMA) derivative was used as a dissociable “mucus-inert” hydrophilic coating to encapsulate *N*-trimethylated chitosan (TMC)-based insulin core¹¹⁴. The NPs exhibited remarkable permeability across the mucus layer and epithelia barriers, and the co-loaded TMC could open the tight junctions between epithelial cells. After orally administered to the diabetic rats, the pHPMA-coated TMC-based NPs showed a relative bioavailability of 8.56%, 2.8-fold higher than that of the uncoated TMC-based NPs. CPP was also grafted onto chitosan, forming a novel material for insulin encapsulation and oral delivery (CPP-g-chitosan)¹¹⁵.

Ligand decoration of NPs is another strategy for enhancing insulin absorption in GI tract. For example, it was reported that some plant lectins showed different carbohydrate specificity and mucoadhesive property, facilitating NPs transport across the cellular barriers¹¹⁶. Wheat germ agglutinin (WGA) is reported to

be greatly specific to intestinal cell lines^{117,118}. Accordingly, WGA can be decorated onto drug carriers, mediate the adhesion interactions between WGA grafted drug carrier and WGA receptor, thus generating improved drug absorption in GI tract^{119,120}. Compared with the undecorated nano-sized carriers, the WGA-decorated insulin carriers showed a much greater relative pharmacological bioavailability after oral administration^{121,122}. Another work demonstrated that the vitamin B12-NPs conjugate had different *in vitro* and *in vivo* properties and hypoglycemic effect depended on the molecular weight of dextran in NPs (Conjugate 70 kD > Conjugate 10 kD > Conjugate 200 kD)¹²³. The efficacy of vitamin B12-NPs conjugates were further optimized using different degree of cross-linking and different linkage. It was reported that NPs with the low cross-linking (4.0%) and carbamate linkage were superior carriers which showed a relative pharmacological availability up to 29.4%¹²⁴.

3.2.2.3. Multi-functional nanoparticles for oral delivery of insulin. As discussed above, various strategies have been used to overcome oral absorption barriers for insulin. When combining different strategies together, a multi-functional nano-carrier is expected to achieve the highly enhanced oral drug delivery. For instance, He et al.¹⁰⁶ developed a core-shell NPs-based insulin delivery system with an extremely high encapsulation efficiency (97%) and drug loading capacity (67%). The NPs, consisting of an insulin/L-penetratin (CPP) complex core and a hyaluronic acid (HA) coating, were protected by enteric microcapsules (MCs, Fig. 6a). In this system, the enteric microcapsules offered protection for insulin against the acidic stomach environment and allowed fast drug release in small intestinal. Moreover, the HA coating on the NPs could enhance the permeation of NPs across the intestinal epithelium, resulting in the significantly improved insulin absorption in the intestine. The *in vivo* results showed that this multifunctional system had a high relative oral bioavailability (11%) and thus could reduce the blood glucose level efficiently (Fig. 6b and c). Some typical literatures reporting the NPs-based insulin delivery systems are summarized in Table 1.

4. Nano-protein interactions-based insulin delivery systems

4.1. Nano-protein interactions and its negative impact on drug delivery

NPs have shown great potentials in improving insulin delivery, but a recently proposed issue of nano-protein interactions stands like an obstacle for the practical use of NPs. It has been clearly demonstrated that when NPs are in contact with protein-containing biological fluids, such as serum, the proteins will rapidly interact with NPs forming the protein corona (Fig. 7)^{26,31,130–132}. Basically, the physicochemical properties of NPs, such as size and morphology, will be changed upon the formation of protein corona. The significant size increase of NPs is a common result of protein corona formation and the size of NP-protein complex will become stable ultimately^{32,133}. Maiorano et al.¹³⁴ found that, the size of AuNPs of different original sizes quickly increased in cell culture medium with 10% fetal bovine serum (FBS) and reached a plateau at about 48 h, and different culture medium (PRIM and DMEM) showed different effect on the protein corona formation. Due to protein adsorption, the NPs

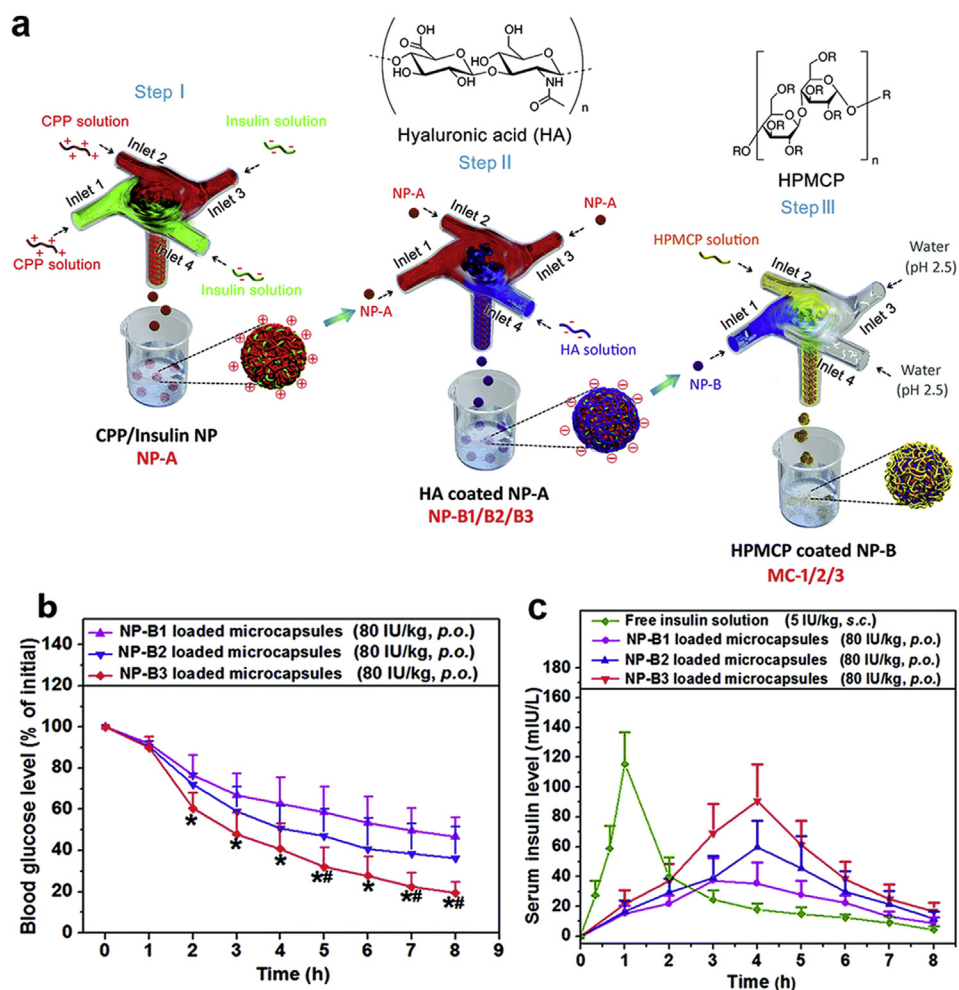


Figure 6 (a) Schematics of the procedures for preparing CPP/insulin nanoparticle (NP-A) core, HA-coated NPs (NP-B1/B2/B3) and HPMCP coated NP-B (MC-1/2/3). (b) Blood glucose level-time curves of diabetic rats after oral administration of MC-1/2/3 (80 IU/kg). * $P < 0.05$ vs. NP-B1, # $P < 0.05$ vs. NP-B2. (c) Serum insulin level of diabetic rats following oral administration of MC-1/2/3 (80 IU/kg). Subcutaneous injection of insulin solution (5 IU/kg) is the control. Data are means \pm SD ($n = 6$). Reprinted with the permission from Ref. 106 Copyright © 2018 Royal Society of Chemistry.

surface is replaced with protein corona leading the substantial change of NPs surface morphology. Generally, the protein corona surrounding the NPs can be observed by transmission electron microscopy (TEM)²⁶, scanning electron microscopy (SEM), and atomic force microscopy (AFM)^{32,135}.

Most importantly, the *in vivo* fate of NPs is substantially changed due to nano-protein interactions^{30,133,135–137}. Usually, after entering into systemic circulation, the proteins adsorbed on the surfaces of NPs are non-specific. According to previous reports, the major negative effects of the non-specific nano-protein interactions on NPs delivery are mainly shown in pharmacokinetics and safety^{132,138}. Firstly, the opsonization effect caused by opsonins (*e.g.*, complements, fibrinogen and immunoglobulins) adsorption onto NPs leads to the fast clearance of drug-loaded NPs from systemic circulation, resulted in the rapid loss of therapeutic effects¹³⁹. This is a big challenge for NPs drug delivery systems. Secondly, the protein corona covers the ligands or functional groups on the surface of NPs which are designed for targeted drug delivery, consequently leading to the loss of their targeting ability^{30,140}. Thirdly, the increased size of NPs may enhance the risk of capillary blockage. For example, in our previous study, the size

of GO/rGO increased to about 2 μm after incubated with the diluted serum¹³⁶. Last but not the least, the potential toxicity induced by nano-protein interactions cannot be ignored^{26,138,141,142}.

4.2. Potentials of using nano-protein interactions

Despite the undesired negative effects discussed above, nano-protein interactions and protein corona formation can also be used in developing advanced NPs-based delivery systems³¹.

Firstly, pre-coating NPs with endogenous proteins or antibodies is a strategy for endowing NPs with targeting property^{143–145}. For example, Tonigold et al.¹⁴⁴ pre-coated NPs with anti-CD63 by adsorption method which kept the targeting property by resisting the uptake of monocyte derived dendritic cells. In another study, Au NPs pre-conjugated with albumin showed increased bio-distribution in brain and lung compared to Au NPs decorated with citrate and apolipoprotein E¹⁴⁶. Besides, low-density lipoproteins (LDL)-decorated NPs showed a specific affinity for tumors, which was applied for targeted drug delivery for cancer therapy¹⁴⁷.

Table 1 Examples of NPs-based insulin delivery systems.

Nano-carrier	Size; Zeta potential	Entrapment efficiency (%)	Drug loading	Dose	Efficacy	Ref.
Insulin-coated gold NPs	20–70 nm; –	–	–	s.c.: 6 mg/mouse	Effect lasting for 6 h	38
Poly(ethylene glycol) capped poly(lactic-co-glycolic acid) NPs	140–170 nm; –14.5 mV	66	–	s.c.: 2 IU/kg	Effect lasting for ~6 h	42
Poly(ethylene glycol) (PEG) and polylactic acid (PLA)-based copolymeric NPs	181.9 nm; –	58.5	313.4 IU/g	s.c.: 25, 50 IU/kg	Effect lasting for more than 7 days	43
Insulin phospholipid complex-loaded biodegradable PHBHHx NPs	182.4 nm; –36.93 mV	87.19	–	s.c.: 4 IU/kg	Pharmacological availability (PA) 350.29%; effect lasting for ~83.5 h	44
NPs loading insulin and glucose enzymes coated by chitosan or alginate	340 nm/293 nm; 10.6 mV/–11.5 mV	54.1/77.9	7.9%/11.4%	s.c.: –	Effect lasting for 10 days without peaks of hyperglycemia or hypoglycemia	52
Enzyme (GOx and CAT) nanocapsules loaded in chitosan microgels	12 nm; –	59.7	44.6%	s.c.: 40 mg/kg	Effect lasting for 24 h	62
Microparticles composed of chitosan, Con A and dextran	2.5 µm; –	92.2	9.1%	–	<i>In vitro</i> : glucose responsive insulin release	74
Poly(ethylene glycol)- <i>b</i> -Poly(acrylic acid-co-acrylamidophenylboronic acid) (PEG- <i>b</i> -(PAA-co-PAAPBA)) micelles	130 nm; –	–	29%	–	<i>In vitro</i> : glucose responsive insulin release	85
p(3-acrylamidophenylboronic acid- <i>b</i> -diethylene glycol methyl ether methacrylate) NPs	Submicron-sized; –37.2 mV	~70	~15%	s.c.: 0.4 mg/kg	<i>In vitro</i> : glucose- and temperature-sensitive insulin release <i>In vivo</i> : effect lasting for 48 h	86
Cetyl palmitate-based solid lipid NPs	361 nm; –3.4 mV	43	–	<i>p.o.</i> : 50 IU/kg s.c.: 2.5 IU/kg	PA 1.6%; effect lasting for 24 h	99
INS-loaded polymer–lipid hybrid NPs	176 nm; –31.1 mV	92.30	2.40%	<i>p.o.</i> : 40 IU/kg s.c.: 5 IU/kg	RBA 12.42%; effect lasted for 24 h	104
Insulin/L-penetratin complex NPs coated with hyaluronic acid	103.7 nm; –19.7 mV	97	67%	<i>p.o.</i> : 80 IU/kg s.c.: 5 IU/kg	BAR 11%; PA 3.7%; blood glucose level (BGL) reduced by 60% in 8 h	106
CS/γPGA-DTPA NPs (CS: chitosan; γPGA: poly(γ-glutamic acid); DTPA: diethylene triamine pentaacetic acid)	246.6 nm; +37 mV	75.70	16.30%	<i>p.o.</i> : 30 IU/kg s.c.: 5 IU/kg	BAR 19.7%; prolonged reduction in BGL	107
Polyester poly(ε-caprolactone) and a polycationic non-biodegradable acrylic NPs	358 nm; +41.8 mV	96	–	<i>p.o.</i> : 50 IU/kg (PK); 100IU/kg (PD) s.c.: 10 IU/kg	BAR 13.21%; reducing the glythemia–time curve area by 38%	109
TMC-based NPs coated with pHPMA (TMC: trimethyl chitosan; pHPMA: <i>N</i> -(2-hydroxypropyl) methacrylamide copolymer)	163 nm; –3.35 mV	54.10	24.50%	<i>p.o.</i> : 50 IU/kg s.c.: 5 IU/kg	BAR 8.56%; maximal BGL reduced 36% at 4 h; effect lasting for 10 h	114
Cell-penetrating peptide (CPP) grafted chitosan NPs	316 nm; +42 mV	–	–	<i>p.o.</i> : 30 IU/kg s.c.: 5 IU/kg	BAR 19.6%; BGL reduced 30% in 4 h and the effect lasted for 12 h	115
Lectin-modified solid lipid NPs	75.3 nm; –13.1 mV	40.18	–	<i>p.o.</i> : 50 IU/kg s.c.: 2 IU/kg	BAR 7.11%; PA 6.08%	121
Vitamin B12–nanosphere conjugate	160–250 nm; –	45–70	2%–4%	<i>p.o.</i> : 20 IU/kg s.c.: 0.4 IU/kg	PA 11.4–26.5%; effect prolonged for many hours	123

(continued on next page)

Table 1 (continued)

Nano-carrier	Size; Zeta potential	Entrapment efficiency (%)	Drug loading	Dose	Efficacy	Ref.
Vitamin B12 coated dextran NPs	192 nm; –	63.50	3%	<i>p.o.</i> : 20 IU/kg <i>s.c.</i> : 0.4 IU/kg	PA 29.4%; BGL reduced 70%–75% and effect lasting for 54 h	124
PEGylation and Con A-based targeted NPs	196.3 nm; –25.6 mV	44.60	–	<i>p.o.</i> : 20 IU/kg <i>s.c.</i> : 0.4 IU/kg	A delayed (2–4 h) reduction of BGL and effect lasting for 24 h	125
FA-PEG-PLGA NPs	260 nm; –	87	6.50%	<i>p.o.</i> : 50 IU/kg <i>s.c.</i> : 5 IU/kg	BAR 19.62%; PA 20.40%; effect lasting for 24 h	126
FA-CS NPs	288 nm; +21.90 mV	>80	–	<i>p.o.</i> : 50 IU/kg <i>s.c.</i> : 5 IU/kg	BAR: 17.04%; significant decrease in BGL in 6 h and down to baseline at 24 h	127
Cp1-11 peptide/insulin-loaded NPs	237.2 nm; –	90.43	28.06%	<i>p.o.</i> : 50 IU/kg <i>s.c.</i> : 4 IU/kg	PA 9.83%; BAR 15.62%; BGL reduced to 55.1% in 2 h and maintained 60% for 8 h	128
Zein-carboxymethylated short-chain amylase/CS nanocomposites	311.32 nm; 43.77 mV	89.60	6.80%	<i>p.o.</i> : 50 IU/kg <i>s.c.</i> : 5 IU/kg	PA 14.12%; BAR 15.19%; effects lasting for up to 8 h	129

–Not applicable.

In addition, it has been demonstrated that the specific albumin corona preformed surrounding NPs can serve as a protective coating for NPs system, prolonging the blood circulation time and improving the biostability^{32,33,148}. Coating NPs with BSA corona, the drug release from NPs was greatly slowed and the stability of NPs in mice liver homogenate was enhanced compared with that of naked NPs. These results could be attributed to the physico-chemical barrier effect of the protein corona³². Furthermore, the *in vivo* pharmacokinetics study showed that, the half-life of

albumin corona-coated NPs was 6-fold higher than that of naked NPs after intravenously injected into rats, and reversely the clearance rate was declined by 2.5-fold³³. Therefore, protein corona has the potential for constructing prolonged drug delivery systems.

Besides, protein corona can also be used for improving drug loading by self-assembly. In the study of Kah et al.¹⁴⁹ showed that the gold NPs precoated with serum proteins had the higher drug loading capacity (~5–10-fold) for DNA and doxorubicin than covalently conjugated NPs did. Their further study found that¹⁵⁰ the amount of proteins in the corona could regulate the payload release behavior. The DNA release was triggered with the amount increase of human serum or human serum albumin, which could be attributed to the enhanced degree of protein exchange in corona. Protein corona can also be used in other fields, such as antibacterial therapy and tissue engineering^{151–153}. Therefore, nano–protein interactions could be a potential strategy for improving drug delivery in different directions.

4.3. Potential insulin delivery systems based on nano–protein interactions

Similarly, for insulin delivery, nano–protein interactions may provide unique approaches. Herein, two potential strategies to use nano–protein interactions for advanced insulin delivery will be discussed below, including adsorption of insulin on nanographene sheets and formation of protein corona on insulin-loaded NPs.

4.3.1. Interactions of graphene-based nanomaterials with insulin

Graphene-based nanomaterials (GBNs) have great potential applications in drug delivery due to their unique structure and properties^{154–156}. The most typical GBNs include graphene oxide (GO) and its reduced form rGO. The huge specific area of GBNs confer them a great capacity to efficiently load many therapeutic molecules *via* various interactions, such as hydrophobic force,

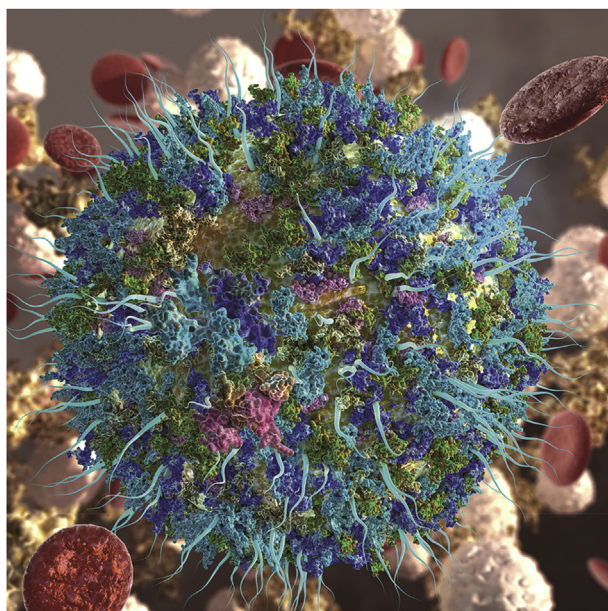


Figure 7 A nanoparticle surrounded with protein corona upon its dynamic interactions with biological fluids. Reprinted with the permission from Ref. 132 Copyright © 2017 American Chemical Society.

hydrogen bonding, π - π conjugation and electrostatic interactions^{157–160}. Insulin can also be loaded onto GBNs *via* nano-protein interactions between GBNs and insulin, forming the sustained and controlled insulin release systems^{158,161}. Unlike the encapsulation of insulin by the traditional NPs, the loading of insulin on GBNs is largely dependent on the physical interactions between GBNs and insulin, such as π - π conjugation and electrostatic interaction. In the case of GO, the electrostatic interaction plays essential roles in insulin loading. It has been shown that the insulin loading on GO is high at pH < 5.4 based on the strong electrostatic forces between positively charged insulin (PI = 5.4) and negatively charged GO¹⁶². In the case of rGO, the insulin loading is shown as pH-independent and largely dependent on the π - π stacking interaction between rGO and insulin^{158,161}.

Insulin is susceptible to low pH and GBNs are found to be able to enhance its stability in low pH medium. Turcheniuk group¹⁶² prepared GO and GO-magnetic particles (GO-MP_{dop}) to load insulin. In their study on *Xenopus laevis* oocytes model, after incubated at pH 2.0 for 5 h, GO-insulin and GO-MP_{dop}-insulin showed almost unchanged meiotic resumption rate while insulin group showed greatly decreased rate, indicating the protection effect of GO on insulin. In addition, the photothermal conversion or electrochemical properties of GBNs confer the insulin-loaded GBNs a pulsatile release property triggered by NIR irradiation or pH change^{161,162}.

Teodorescu et al.¹⁶¹ took advantages of the electrochemical or photothermal conversion properties of rGO to develop an advanced insulin delivery system. They prepared a gold electrode modified with insulin-impregnated rGO, which was designed to combine with flexible skin patches for insulin transdermal delivery. In this system, insulin release could be triggered *via* electrochemical action. Under the potential pulse of 0.8 V, 70 ± 4% of human insulin was released within 30 min (the time of normal insulin secretion *in vivo*), and the bioactivity of released insulin was proved to be well preserved in this process (Fig. 8). Besides, rGO has been proved to be a great near infrared (rNIR)-absorbing photothermal agent which possesses rapid light-to-heat conversion

ability under NIR irradiation and has been used as photothermally triggered drug delivery vehicles^{163–165}. In another work, Teodorescu et al.¹⁵⁸ developed a photothermally triggered insulin delivery system with rGO-modified hydrogels which were designed as a patch for transdermal insulin delivery. The hydrogels were made of poly(ethylene glycol) dimethacrylate (PEGDMA) and impregnated with rGO and finally loaded with insulin *via* physical adsorption overnight. The insulin loading capacity was enhanced with the increase of rGO content in PEGDMA-rGO hydrogels and saturated at 80% when rGO contents reached 0.8 mg. Under the laser intensity of 0.7 W/cm² (980 nm), the hydrogel could reach the temperature of 70 °C within 10 min, which was high enough to interfere with the interaction between insulin and rGO, leading to insulin dissociation from the hydrogel. Moreover, under such high temperature, the hydrogels remained stable and the released insulin kept its bioactivity.

GO has also been used as a drug carrier for proteins (including BSA and insulin)^{159,162,166}. For instance, Zhou et al.¹⁶⁶ loaded an insulin-derived peptide (EALYLV) onto PEG-modified graphene oxide to inhibit the aggregation of human islet amyloid polypeptide (hIAPP) which accumulated in the pancreatic islets of diabetic patients. In another study, insulin was loaded onto GO or GO decorated with 2-nitrodopamine-coated magnetic particle matrices (GO-MP_{dop}), which showed high loading capacity of 100 ± 3% and 88 ± 3% in pH < 5.4, respectively¹⁶². The insulin loaded on GO matrices was proved to be stable under the acidic condition of pH 2, but insulin was released upon exposed to the media with higher pH values. At pH 7, more than 20% of insulin was released in 90 min. This study demonstrated the potential of applying GO for insulin delivery *via* oral route in diabetic treatment. Some other studies have also demonstrated that the photothermal conversion property of GBNs could be utilized in formulating controlled drug release systems^{167–170}. Therefore, GBNs-insulin interaction provides a potential approach for the controlled insulin delivery triggered by the changes of temperature or pH.

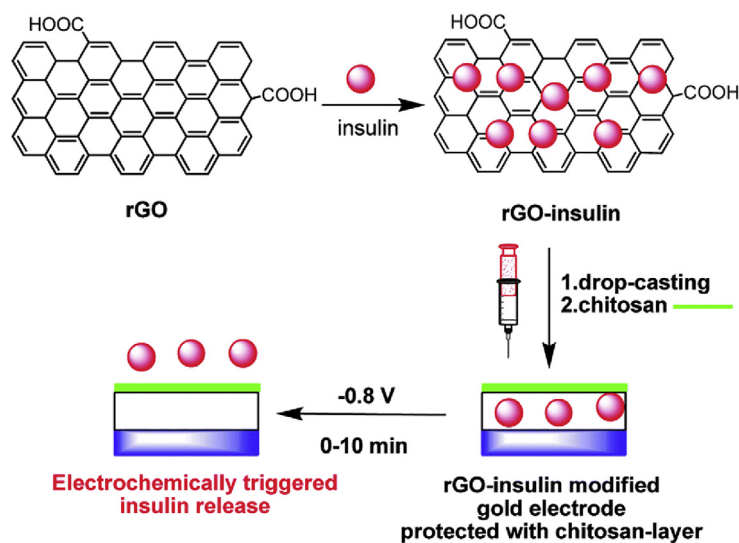


Figure 8 Schematic representation of the formation of insulin-loaded rGO (rGO-insulin), gold electrode modified with rGO-insulin and protected with chitosan-layer, and electrochemically triggered insulin release. Reprinted with the permission from Ref. 161 Copyright © 2015 Royal Society of Chemistry.

4.3.2. Formation of protein corona on insulin-loaded NPs

The formation of protein corona may also be used for developing novel oral insulin formulations. In an interesting work, Wand et al.¹⁷¹ developed the insulin-loaded cationic liposomes (CLs) which were coated with BSA corona (PcCLs, Fig. 9a). The PcCLs had a neutral charge and a hydrophilic surface and thus showed the strong penetration into the mucus layer (Fig. 9b). Moreover, the positively charged CLs could be exposed and directly interacted with the epithelium since the BSA corona would be hydrolyzed by enzymes within mucus layer (Fig. 9c). The *in vivo* data showed that the hypoglycemic effect of PcCLs was efficient and could last for 12 h after intrajejunal (i.j.) administration (Fig. 9d). Accordingly, the insulin bioavailability of PcCLs was up to 11.9% (Fig. 9e). This work provides an interesting idea of how to use protein corona for enhancing the oral absorption of insulin, although some key issues still need to be addressed, such as the stability of protein corona against various digestive enzymes in the upper area of small intestine. In our previous work, we showed that the digestive enzyme corona could rapidly form around the insulin-loaded cationic NPs (CNPs) *in vitro*¹³⁵. More importantly, the CNPs could protect the loaded insulin from enzymatic degradation and the epithelial cell uptake of CNPs was significantly inhibited in the presence of the digestive enzyme corona. This finding implies that digestive enzyme corona can reduce the degradation and absorption of CNPs in the upper GI tract, leading to a large number of CNPs delivered to the lower area of GI tract where the CNPs have a great chance to be absorbed due to the

presence of M-cells and the released insulin can also be absorbed in the intact form due to the extremely low enzyme activity.^{135,172} This is another potential approach of using protein corona for oral delivery of insulin-loaded NPs. The discussions above indicate that protein corona has great potentials in enhancing insulin oral delivery, which may be the new direction of oral insulin research and development in the future.

5. Problems and possible solutions

Despite the great advances of NPs-based insulin delivery systems over the traditional ones, no nano-formulations have been approved so far since many problems remain with regard to NPs. First, the potential systemic toxicity of NPs, especially the synthetic ones, is still unclear. Second, the oral bioavailability of insulin-loaded NPs is still far from enough although it has been significantly enhanced compared to insulin solution. Third, the complexity and the delayed response to the change of blood glucose level make the glucose-responsive NPs hard for commercialization. In addition, the nano-protein interaction and corona formation after NPs exposure to biological fluids is an uncertain and complicated factor influencing the *in vivo* fate of NPs.

As the other side of a coin, nano-protein interactions may provide potential approaches for solving these problems. The insulin-loaded GBNs have shown the potentials to provide both the basic insulin release and the desired burst release triggered by

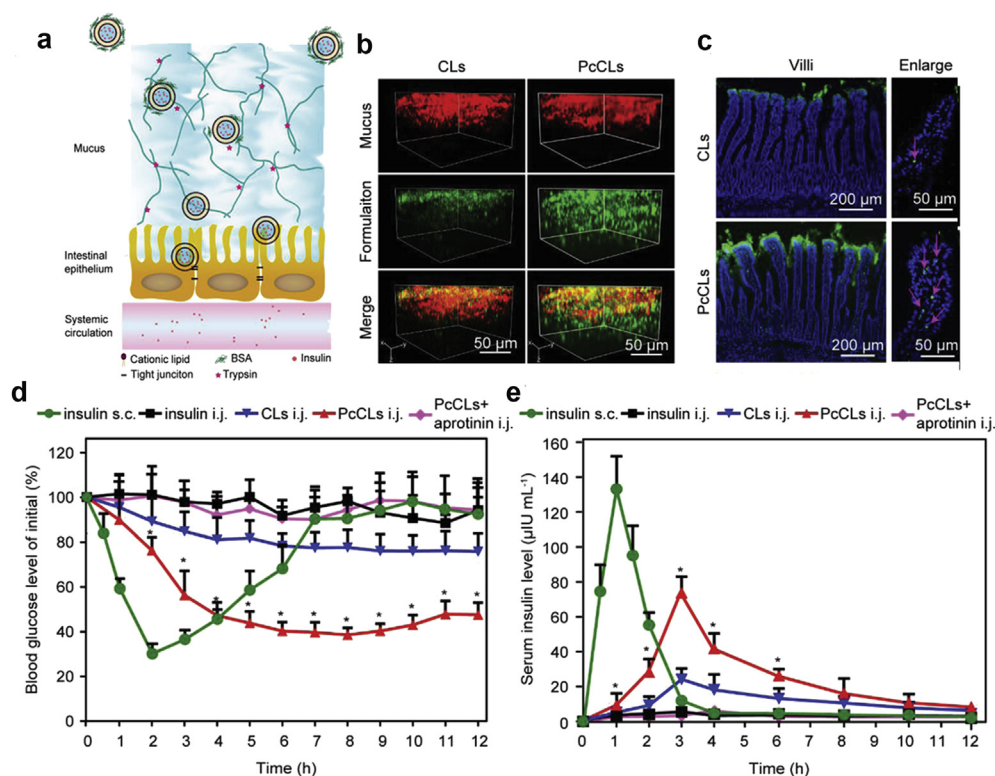


Figure 9 (a) Schematics of the process of PcCLs transport through the mucus layer and epithelium. (b) 3D images of CLs and PcCLs penetration into mucus (green: formulations; red: mucus). (c) Distribution of CLs and PcCLs in small intestinal villi (green: formulations; blue: intestinal villi nuclei). Red arrows indicate the absorption points. (d) Blood glucose level-time curves of diabetic rats after administration of insulin solution (s.c. 5 IU/kg and i.j. 75 IU/kg), CLs, PcCLs and PcCLs mixed with aprotinin (i.j. 75 IU/kg; s.c.: subcutaneous; i.j.: intrajejunal). (e) Corresponding serum insulin level-time curves. Data are mean \pm SD, $n = 6$, $*P < 0.05$ vs. CLs group. Reprinted with the permission from Ref. 171 Copyright © 2019 John Wiley and Sons Ltd.

the photothermal effect. But the use of GBNs brings new concerns on their toxicity and degradation. Another nano-protein interactions-based possible approach is the formation of protein corona surrounding the insulin-loaded NPs to enhance their oral absorption. The specific protein corona, if stable in the GI tract, can improve the mucus penetration and epithelium adhesion of NPs and ultimately enhance the absorption of NPs in the small intestine. Alternatively, the non-specific digestive enzyme corona is not beneficial for the absorption of NPs in the upper GI tract but may enhance the absorption of the intact NPs or the released insulin in the lower area of the GI tract. Utilization of nano-protein interactions may be the new direction of oral insulin research and development in the future.

6. Conclusions and perspectives

Insulin therapy is of great importance for diabetic patients to control their blood glucose level. However, the frequent injections bring not only inconvenience but also pains. For many years, great efforts have been made to improve the insulin delivery. Although certain improvements are achieved, the current therapy is still unsatisfying enough. In this regard, various functional NPs loaded with insulin are developed and have shown significant advance over the traditional formulations. Unfortunately, none of them can be approved for clinical use due to the substantial concerns, such as the unclear toxicity, the still low oral bioavailability and the interference from the nano-protein interactions. Recently, scientists have started to consider how to use the nano-protein interactions for developing advanced insulin delivery systems. The insulin-loaded GBNs for photothermally triggered on-demand insulin release and the protein corona formation surrounding the insulin-loaded NPs for enhanced oral bioavailability have shown the potentials to improve the insulin delivery. It is undoubted that there is still a long way to go to achieve the goal of biomimic and/or oral insulin delivery. On the other hand, the efforts to achieve the goal will not be stopped despite of the great challenges. Utilizing the nano-protein interactions may be a potential approach for designing novel insulin delivery systems in the future.

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Author contributions

Qiang Peng and Ting Zhang designed the research and obtained funding. James Zhenggui Tang, Xiaofan Fei, Yanping Li, Yi Song, and Zhiyong Qian contributed to the discussion and review. Ting Zhang and Qiang Peng wrote the manuscript. Qiang Peng and James Zhenggui Tang revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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