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REVIEW ARTICLE

Advancements in oral insulin: A century of research and the emergence of targeted nanoparticle strategies

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Abstract

With the growing prevalence of diabetes, there is an urgent demand for a user-friendly treatment option that minimizes side effects related to the use of subcutaneous injections. Scientists have dedicated over a century to developing an oral dosage form of insulin that can be administrated orally. The oral route of administration is the most desirable route for regularly dosed drugs in terms of safety and patient compliance. However, oral delivery of insulin remains a formidable challenge due to its intrinsically limited ability to cross the intestinal epithelium membrane and susceptibility to enzymatic degradation. This article reviews oral insulin research over the past decade, with a particular focus on surface modifications of nanoparticles (NPs). Various strategies involving controlling surface charges, utilizing protective proteins, and targeting specific receptors with ligands have been explored. Notably, surface modifications of the NPs for targeting specific intestinal receptors have shown promise in enhancing insulin oral absorption and bioavailability. Advanced technologies such as oral microneedles and gene therapy have also been developed, but their safety requires further assessment. Despite encouraging preclinical results across numerous strategies, the current clinical evidence is less optimistic. In summary, the present findings highlight the substantial journey that still lies ahead before achieving successful oral delivery of insulin.

Practical Applications: This review provides a summary of recent progress in oral insulin delivery, particularly highlighting surface-modified functional nanoparticles serving as an effective drug delivery system, which offers valuable information to the

Abbreviations: AAV, adeno-associated virus; AC, acrylic acid; Ag2S, silver sulfide; ALB, albumin; ASBT, apical sodium-dependent bile acid transporter; BCS, Biopharmaceutics Classification System; CMCS, carboxymethyl chitosan; CPPs, cell-penetrating peptides; CS, chitosan; CSAD, chondroitin sulfate sodium alginate derivative; CSK, CSKSSDYQC; DCA, deoxycholic acid; DCDA-CS, dicyandiamide chitosan; DLPC, dimyristoyl phosphatidylcholine; EE, encapsulation efficiency; FA, folic acid; FBG, fasting blood glucose; GI, gastrointestinal; HA, hyaluronic acid; HIPs, hydrophobic ion pairs; HTM, hepatocyte targeting molecule; IA, itaconic acid; IF, intrinsic factor; IG, insulin-guanidine; IGF1, insulin-like growth factor 1; LV, L-valine; MMA, methyl methacrylate; MSNs, mesoporous silica nanoparticles; NLCs, nanostructured lipid carriers; NPH, neutral protamine Hagedorn; NPs, nanoparticles; PAT1, proton-coupled amino acid transporter 1; PCB, poly(carboxybetaine); PEG, polyethylene glycol; PETMP, pentaerythritol tetrakis(3-mercaptopropionate); P-gp, P-glycoprotein; PLA, poly(lactic acid); PLGA, poly(lactic-*co*-glycolic acid); QDs, quantum dots; SB12, sulfo-*N*-lauryltaurine 12; SDC, sodium deoxycholate; SEDDS, self-emulsifying drug delivery systems; SLNs, solid lipid nanoparticles; SOMA, self-orienting millimeter-level microneedle array; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TJs, tight junctions; TMC, trimethyl chitosan; UDCA, ursodeoxycholic acid; WCPs, wild chrysanthemum pollen; WGA, wheat germ agglutinin; ε-CL, ε-caprolactone.

researchers. Due to the limited effectiveness of oral protein drugs caused by biological barriers, innovative technologies and drug delivery systems have been developed to overcome these obstacles and achieve therapeutic goals. This review concluded that surface modifications to nanoparticles can improve insulin stability and permeability, thereby enhancing oral bioavailability. It could assist researchers in developing more effective and patient-friendly oral drug delivery systems.

KEYWORDS
insulin, nanocarrier, nanoparticle, oral drug delivery system, targeted modification

1 | INTRODUCTION

1.1 | Diabetes and insulin

Diabetes is a chronic metabolic disease. Derived from insulin secretion, diabetes is primarily divided into two major categories: type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM).[1] T1DM is characterized by the inability to secrete insulin and is considered an autoimmune disease. It is commonly diagnosed during adolescence. In contrast, T2DM is characterized by insulin resistance and is normally diagnosed in adulthood. The majority of diabetes cases are diagnosed as T2DM. By 2030, the number of T2DM patients is predicted to increase to 4.39 billion worldwide.[2] This may be primarily due to the unhealthy lifestyles that modern individuals adopt. Risk factors for T2DM include consumption of high-fat foods, excessive alcohol intake, and lack of physical activity.[3] These factors can contribute to obesity, which is another high-risk factor for developing T2DM.[4]

Insulin is a protein hormone with a molecular weight of 5800 Da, composed of two chains, α and β, linked by two disulfide bonds.[5] The log p value of −1.6 for insulin classifies it as a category of Class III according to the Biopharmaceutics Classification System, indicating its hydrophilic properties.[6] The current clinical route of insulin administration is subcutaneous injection. However, in addition to disadvantages such as poor patient compliance due to injection pain and the risk of injection site infections, the rapid clearance of subcutaneously injected insulin results in a short-term therapeutic effect.[7,8]

Noninvasive insulin delivery approaches have been extensively investigated, including oral, transdermal, inhalational, ocular, and vaginal routes. However, each of these routes presents unique challenges, resulting in suboptimal insulin bioavailability.[9–11] Among these approaches, oral administration remains the most widely accepted route of drug delivery because of its convenience.[12] Orally administered insulin has been suggested to stimulate endogenous insulin secretion, resulting in a lower risk of hypoglycemia compared to subcutaneous injection.[13] Its potential to preserve and maintain beta-cell function has also been reported.[13] However, oral insulin must overcome the harsh gastrointestinal (GI) environment for absorption. The barriers of mucus and epithelial cells in the GI tract result in its limited bioavailability. Therefore, despite the extensive development of various oral delivery systems over a century since the discovery of insulin, oral insulin has not been available in clinical practice.

1.2 | Ambitions and setbacks with oral insulin

Researchers have made considerable efforts to develop oral insulin formulations since the initial discovery of insulin by Banting and Best in 1921.[14] The first attempt at oral insulin was made the following year. During a week of consecutive oral administration of incrementally dosed insulin preparations, the patient showed no improvement in metabolism.[15] In 1923, adding alcohol to the oral formulation was attempted to increase insulin absorption.[16] However, this also failed to produce positive results. Several clinical trials of oral insulin were conducted between 2001 and 2019. Although some companies abandoned the development of oral insulin after clinical trial setbacks, others, such as Emisphere in the United States, Diabetology in the United Kingdom, and Oramed in Israel, persevered.[17] Emisphere received FDA approval for a phase I clinical trial of its first oral insulin formulation in 2001. Another oral insulin formulation from Emisphere entered phase II clinical trials 5 years later. However, the results were disappointing, as significant differences were observed between the control and treatment groups. This was mainly due to the limited sample size of only eight subjects. In 2014, Oramed's ORMD-0801 received FDA approval for Phase III clinical trials. It had a larger sample size of 710 diabetic patients. However, the results were still disappointing. No superior glycaemic control was observed in patients treated with oral insulin compared to placebo after 26 weeks of treatment.

Significant efforts have been dedicated to achieving oral delivery of insulin. Encapsulation plays a crucial role in protecting insulin from the harsh acidic and enzymatic conditions of the GI tract. This review outlines various strategies employed to safeguard orally administered drugs. Nanocarriers have emerged as effective tools for improving insulin stability and enhancing its permeability across the intestinal barrier. Surface modifications of these nanocarriers, including adjustments in surface electrical properties, protein protective modifications, and targeted alterations, have been summarized.

2 | GASTROINTESTINAL ENVIRONMENT

Following oral ingestion, food or medications traverse the stomach before reaching the intestine. Absorption of nutrients or medications primarily transpires in the small intestine. However, the GI environment comprises diverse pH levels, multiple digestive enzymes, mucus, and various epithelial cells, all of which significantly influence the absorption of protein drugs such as insulin.[18]

2.1 | pH gradients and enteric coating

The pH levels within the GI system exhibit a gradient, ranging from strong acidity in the stomach to near neutrality in the intestine. The stomach environment is rich in a strong gastric acid, hydrochloric acid, resulting in an extremely low gastric pH of about 2.5.[19] In the duodenum, the presence of alkaline substances such as bile causes the pH to rise to around 6. In the jejunum, it rises to ≈7.5, whereas in the colon, it returns to about 6.5.[19] The variable pH environment of the GI tract, particularly the highly acidic environment within the stomach, poses a notable challenge to the stability of orally administered protein drugs as they are susceptible to denaturation under highly acidic conditions. Fortunately, this difficulty can be effectively overcome by enteric coating.

2.2 | Proteinases and inhibitors

The abundance of enzymes in the GI tract is another significant challenge to the stability of orally administered insulin. Proteinases such as pepsin in the stomach and trypsin and chymotrypsin in the small intestine are able to cleave insulin into smaller peptides, rendering it inactive.[20]

Utilizing proteinase inhibitors to temporarily or permanently deactivate target proteinases is a commonly employed strategy in protein drug delivery.[21] A notable example is the oral insulin ORMD-0801. This formulation used a soy-derived trypsin inhibitor to reduce insulin degradation.[22] The Phases I and II clinical trials of ORMD-0801 yielded favorable outcomes. However, it is crucial to acknowledge the potential drawbacks of prolonged enzyme inhibition, which could result in enzyme deficiency and related adverse effects.

2.3 | Mucus barrier and mucosal adhesion systems

The mucus is a viscoelastic gel layer attached to the luminal surface of the GI tract. Intestinal mucus is secreted by goblet cells. It acts as a physiological barrier to protect the underlying epithelial cells from exogenous pathogens.[23] The composition of mucus is mainly water (≥90%), mucin (5%), lipids, and electrolytes.[24] The presence of mucus leads to several obstacles to drug permeation. The viscoelastic nature of mucus allows it to entrap drugs and makes it difficult for drugs to diffuse to epithelial cells.[25]

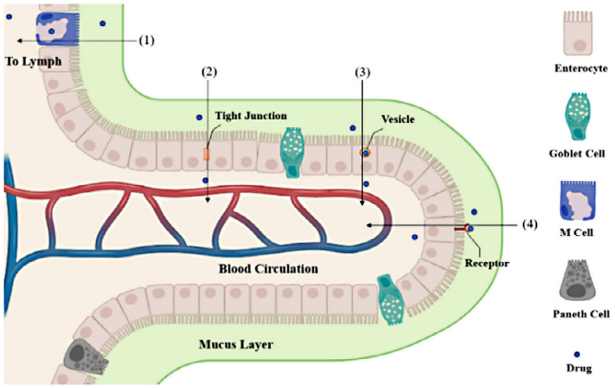

FIGURE 1 Mucus layer and epithelial cell layer; endocytosis mechanisms: (1) transcytosis by M cells to lymph circulation; (2) paracellular transport via tight junctions; (3); endocytosis by enterocytes mediated by vesicles; (4) receptor-mediated transcytosis.

Mucosal adhesion systems have been utilized to enhance the adherence of insulin formulations to the intestinal mucus. Due to the high hydration and negative charge of the mucus layer, hydrophobic or positively charged formulations exhibit enhanced mucosal adhesion properties. Chitosan (CS), a natural and biocompatible polysaccharide, has been extensively investigated as an insulin carrier.[26] Its positive charge has been found to allow it to interact electrostatically with the mucus layer. This may increase the residence time and improve the absorption of insulin. Furthermore, glycoproteins in mucus are rich in cysteine residues. Surface modification of thiol groups on CS can form disulfide bonds with the cysteine residues of these glycoproteins and achieve longer residence time.[27] This approach has been shown to improve the oral bioavailability of insulin by 11.3% in diabetic rats.[28]

2.4 | Epithelial cell barrier and epithelium penetration system

Another physiological barrier within the GI tract is a monolayer of epithelial cells. The intestinal epithelium consists of different functional cell types: enterocytes, goblet cells, and M cells (Figure 1).

Enterocytes and M cells are primarily involved in nutrient uptake and transport, whereas goblet cells are responsible for mucus secretion.[15] ≈90% of the intestinal epithelium is made up of enterocytes, between which are tight junctions (TJs).[29]

The primary mechanisms of transcellular transport comprise endocytosis by enterocytes and M cells, receptor-mediated transport, and paracellular transport.[30] The uptake of protein drugs in the intestine is primarily mediated by endocytosis, which includes micropinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis. Paracellular transport across TJs is mainly responsible for transporting hydrophilic small molecules with molecular weights less than 500 Da.[31,32] Several studies have attempted to enhance oral insulin absorption by opening TJs and involving ligands in improving transport through M cells.[33] These strategies slightly improved the insulin

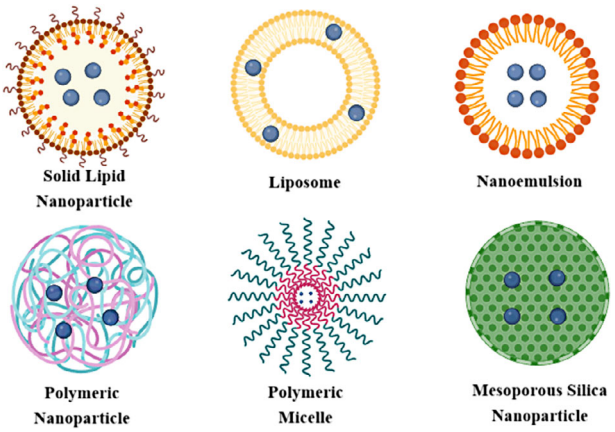

FIGURE 2 Structure of commonly used drug delivery systems.

bioavailability. However, the opening of TJs is associated with the risk of immune responses and autoimmune disease.[34]

Cell-penetrating peptides (CPPs) are a family of peptides initially derived from the human immunodeficiency virus that have been used extensively in the construction of epithelium penetration systems.[35] They have been successful in improving the transcellular transport of oral insulin formulations approximately twofold.[36–38] However, the oral bioavailability of insulin remains relatively low at 2.48%.[36]

3 | ORAL DRUG DELIVERY SYSTEMS

The encapsulation of insulin in specific carriers can offer a considerable improvement in oral bioavailability. These systems are designed to protect insulin from acidic and enzymatic degradation and to improve permeability in the intestine.

Nanocarriers are currently the preferred oral delivery system. Drugs can be encapsulated within the carrier matrix or core, forming particles with sizes below 1000 nm.[39] Nanocarriers or nanoparticles (NPs) are recognized for their high permeability due to their small particle size.[40] This has made them promising candidates for insulin delivery. Depending on the carrier material, nanoscale formulations are categorized into lipid NPs, polymeric NPs, and inorganic NPs. The extensively used drug delivery systems include solid lipid nanoparticles (SLNs), liposomes, nanoemulsions, polymeric NPs, polymeric micelles, and mesoporous silica nanoparticles (MSNs), as shown in Figure 2.[41] These have all shown encouraging results for the oral delivery of insulin in preclinical studies.

3.1 | Lipid-based nanoparticles

Lipid-based NPs are nanoscale solid particles, vesicles, or emulsions composed of lipids.[42] Due to the high lipid content of cell membranes, lipid NPs are considered advantageous for cellular uptake.[43] However, their lipophilicity poses a challenge to the encapsulation of hydrophilic proteins such as insulin.[44]

SLNs and nanostructured lipid carriers (NLCs) are two major types of lipid NPs.[45,46] SLNs typically consist of solid lipids and surfactants, whereas NLCs consist of both solid and liquid lipids.[47] The presence of surfactants allows insulin to be retained in the aqueous phase of NPs. SLN and NLC formulations deliver insulin in solid forms, therefore offering improved stability in the GI tract compared to liposomes, niosomes, and nanoemulsions. However, the solid nature may limit their uptake by intestinal epithelial cells.[48] An SLN formulation for oral insulin achieved a relatively low bioavailability of about 5% in rats.[49]

Liposomes are spherical vesicles comprising lipid bilayers, within which insulin can be enclosed in the hydrophilic core. However, liposomes are perceived to exhibit low stability within the GI tract.[50] Studies have been conducted attempting to improve the stability of liposome formulations by loading bile salts into the lipid bilayer.[51] Zhang et al. introduced biotin (vitamin B7) onto the surface of liposomes to mimic vitamin absorption in the small intestine. It increased the bioavailability of oral insulin to 8.32%.[52] This significant enhancement in bioavailability can be attributed to the biotin modification, as the unmodified liposome only exhibited a bioavailability of 3.30%.

Niosomes are vesicles consisting of nonionic surfactants and cholesterol.[53] Nonionic surfactants are used extensively in drug delivery systems due to their reported lower toxicity and cost-effectiveness.[54] They also serve as potent P-glycoprotein efflux inhibitors, thereby enhancing drug absorption.[55] An oral insulin formulation has been developed with trimethyl chitosan (TMC)-coated niosomes, employing Span 60 as the surfactant. This formulation achieved a high encapsulation efficiency (EE) of 80%, improved stability under simulated gastric conditions, and enhanced intestinal permeability.[56]

Nanoemulsions are transparent or translucent liquid formulations composed of water, oil, and surfactants.[57] Water-in-oil (w/o) nanoemulsions are capable of entrapping insulin in the aqueous core for insulin delivery.[58] The bioavailability of oral insulin w/o nanoemulsions has been reported to be almost ten times that of free insulin solution.[59] However, due to the high water content of the mucus layer, w/o nanoemulsions are likely to undergo a phase transition before reaching the epithelial cells in the intestine. This may result in an untimely release of insulin and less-than-ideal absorption.

Self-emulsifying drug delivery systems (SEDDS) are formed from an oil phase, surfactants, and cosurfactants.[60] They are capable of forming oil-in-water emulsions when exposed to an aqueous phase. Due to the hydrophilic nature of insulin, it is challenging to dissolve it in the oily core. Therefore, strategies have been developed to increase its lipophilicity. One approach is to form hydrophobic ion pairs (HIPs), in which insulin forms electrostatic interactions with counterions containing a hydrophobic portion, resulting in lipophilic complexes.[61] Insulin-guanidine was selected by Claus et al. to form HIPs because of its two additional cations compared to insulin.[62] The SEDDS produced typically range in size from 200 to 350 nm, with an absolute bioavailability (relative to intravenous injection) of 0.55% in healthy rats.[62]

3.2 | Polymeric nanoparticles

Polymeric NPs are nanocarriers with polymers as their framework. The polymers can be divided into two main types: natural and synthetic. Commonly employed natural polymers consist of CS and alginates (Alg), whereas synthetic polymers predominantly include poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA).[63] These polymers have received FDA approval due to their proven biocompatibility.[64]

CS can be the vehicle for insulin due to its desirable physicochemical and biological properties.[65] It is positively charged and therefore has the ability to interact with negatively charged mucus, thereby enhancing mucosal adhesion. Moreover, its ability to open TJs has been suggested to improve epithelial permeability. Derivatives of CS, such as TMC and carboxymethyl chitosan (CMCS), have also been developed. An insulin nanosystem based on CS and CMCS reported an insulin bioavailability of up to 18% in diabetic rats.[66]

Hydrogels are polymeric formulations characterized by a framework of natural or synthetic polymers that can swell into a mesh-like structure upon hydration.[67] Their pH and thermal responsiveness enable them to regulate the release of oral drugs. Incorporating into hydrogels can prevent insulin degradation under the low pH of the stomach, as exemplified by the carboxymethyl-β-cyclodextrin and carboxymethyl chitosan-based hydrogel developed by Yang et al.[68]. This hydrogel formulation effectively reduced fasting blood glucose by about 30% and holds promise for metabolic improvement after 28 days of treatment in T2DM mice. In another study, an insulin-loaded hydrogel was prepared with methyl methacrylate and itaconic acid.[69] Oral administration of this formulation reduced blood glucose levels in diabetic rats to below half of baseline levels after 6 h.

Micelles are drug delivery systems composed of amphiphilic molecules that form hydrophobic cores and hydrophilic surfaces.[70] One insulin-loaded micelle system, whose backbone was synthesized from a combination of hydrophilic polyethylene glycol (PEG) and hydrophobic polymer PMHC 18, achieved long-term stability at room temperature. The micelles, stored at room temperature for 2 weeks, continued to demonstrate blood glucose-lowering effects in diabetic rats similar to those of fresh micelles.[71] In contrast, reverse micelles represent a controversial drug delivery system. Lipid-based reverse micelles loaded with insulin were prepared by Chu et al.[72]. They were dispersed in an oily solvent containing surfactant, sodium deoxycholate (SDC), and sulfo-*N*-lauryltaurine 12 (SB12). The micelles had the ability to reassemble into lipid NPs in the aqueous GI environment, further enhancing their stability. SDC was introduced to assist in the stabilization of the formulation during reassembling, whereas the SB12 ligand was involved in enhancing insulin uptake through the proton-coupled amino acid transporter 1 (PAT1) pathway. The oral bioavailability of insulin achieved with this formulation was 5.6%.

3.3 | Inorganic nanoparticles

Inorganic nanocarriers are drug delivery platforms that utilize materials such as silica and gold as their structural framework. Over recent years, these inorganic substrates have gained substantial traction in pharmaceutical research owing to their exceptional drug-loading capabilities.[73]

MSNs have become one of the most popular inorganic drug delivery systems. MSNs modified with PEG have been reported to improve the stability of insulin in the GI environment.[74] MSNs modified with CPPs have been reported to slightly increase the oral bioavailability of insulin by 2.5%.[36] However, concerns have been raised about the hepatotoxicity, renal toxicity, and neurotoxicity of inorganic nanocarriers.[75] This highlights the need for careful assessment of their long-term.

Another inorganic nanoscale formulation is known as quantum dots (QDs), which are nanocrystals.[76] QDs based on silver sulfide (Ag2S) were used to deliver metformin.[77] The resulting accumulation in the liver and improved metformin bioavailability indicate their potential for insulin delivery. In the study by Hunt et al., CS and glucose copolymers were coated onto insulin-loaded Ag2S QDs.[78] The oral bioavailability of this formulation in healthy mice was found to be 4%. T2MD rats treated with the QD formulation showed controlled body weight over a 6-week period, whereas those treated with subcutaneous insulin injections showed a 30% increase in body weight. Additionally, the safety of the QD formulation was evaluated in healthy baboons, and no adverse events were reported.

4 | SURFACE MODIFICATION OF NANOPARTICLES

NPs exhibit the ability to preserve insulin stability under acidic and enzymatic conditions while enhancing its permeability across the intestinal barrier. Over the past decade, there has been a growing emphasis on surface modifications of NPs aimed at augmenting insulin absorption. Prominent strategies involve alterations in surface electrical properties, protein-based protective modifications, and targeted adjustments. These endeavors have yielded functional nanocarriers, resulting in notable enhancements in the oral bioavailability of insulin, as summarized in Table 1.

4.1 | Surface coating

Functional coatings are strategies to improve the stability and intestinal permeability of oral formulations by applying functional materials to the surface of NPs. Hydrogels, gelatin, and pollen have also been employed for surface coating.

Hydrogel coating is capable of preventing the sudden release of insulin in an acidic environment. Arginine-insulin-loaded liposomes have been incorporated into cysteine-modified Alg hydrogels.[50] At pH 1.2, the liposomes within the hydrogels released only 10% of insulin in 3 h, whereas conventional liposomes released 40%. In diabetic mice, liposomes encapsulated in hydrogels showed a higher oral bioavailability than those not encapsulated (11% vs. 7%).

Gelatin-coated NPs also showed high stability in an acidic gastric environment. Gelatin, a hydrophilic protein, was chosen for coating due to its high biocompatibility and cost-effectiveness.[90] Kumar et al.

TABLE 1 Insulin dose used and bioavailability reported, focusing on functional nanoparticles (NPs), modifications, and characterizations.

Formulations	Modification	Contribution	Size (nm)	Zeta-potential (mV)	Dose (IU/kg)	Bioavailability (%)	Ref.
PBCA/CS/Alg	Negatively charged	Mucus permeation improvement	218	−20.6	50	8.8	[79]
DLPC NPs	Zwitterion	Cell membrane permeability improvement	107.5	−6	50	4.76	[80]
DSPE-PCB Capsules	Zwitterion	Cell membrane permeability improvement	30	−41	20	42.6	[81]
A1(CN-DEX)	Protein	Stability improvement	267	−21.6	40	12.5–20.2	[82]
Glu-APD	Peptide	Oligopeptide transporter targeting	153	6.92	50	10	[83]
CMCS-PBA-LA	Amino acid	Oligopeptide transporter targeting	190	N/A	75	7.55*	[84]
FA-CS/NPs	Vitamin	FA receptor targeting	288	21.69	50	17	[85]
NP HA-SH	Thiol	Mucin targeting	102	−37	70	11.3	[86]
DC-LIP	Cholic acid	Bile acid transporter targeting	145	+21.6	50	16.1	[87]
WGA SLNs	Lectin	M cells targeting	70	−13	50	7.11	[88]
CSK-NPs	CSK	Goblet cells targeting	184.5	24.7	50	7.05	[89]
MSN-NH2 @COOH/CPP5	CPP	Cell membrane permeability improvement	100–150	−0.49	100	2.48	[36]

Note: Dose: insulin dose administered orally to diabetic rats. Bioavailability%: relative bioavailability equivalent to a subcutaneous injection of 5 IU kg^{-1} insulin. N/A: data not reported. *: pharmacological availability.

Abbreviations: Alg, alginates; CSK, CSKSSDYQC; CN-DEX, casein and dextran complexes; CMCS, carboxymethyl chitosan; CS, chitosan; CPPs, cell-penetrating peptides; DLPC, dimyristoyl phosphatidylcholine; DC-LIP, deoxycholic acid-modified liposomes; FA, folic acid; PCB, poly(carboxybetaine); SLNs, solid lipid nanoparticles; WGA, wheat germ agglutinin.

developed gelatin-coated insulin NPs based on porous copper-metal organic frameworks.[91] The coated NPs released insulin more slowly (50%) than the uncoated (80%) over a 6-h period at pH 3.5. This suggests that the coating can prevent premature release of insulin and provide better control release. The hypoglycemic effect of this formulation was unclear, as it was not tested on animals.

Pollen encapsulation has been found to improve oral insulin bioavailability through various mechanisms. CS and γ-polyglutamic acid were used as scaffolds for insulin-loaded NPs, which were subsequently loaded into wild chrysanthemum pollens (WCPs) to create formulation PNMs@insulin.[92] The robust wall of the pollen particle was expected to ensure the stability of PNMs@insulin in a low pH environment, and the spiky structure of the WCPs may assist capture by intestinal villi, thereby enhancing insulin absorption. Notably, healthy mice administered empty WCP capsules showed controlled blood glucose levels after glucose or food intake, suggesting a hypoglycemic effect of the pollen itself.[92] PNMs@insulin achieved a high oral insulin bioavailability of 43.75% in diabetic mice, significantly higher than that of the CS NPs (14.1%). WCP encapsulation may thus represent a promising approach for insulin delivery.[92]

4.2 | Surface charge control

The surface charge of NPs can be modified to improve the oral bioavailability of insulin. Due to the overall negative charge possessed by mucins, negatively charged nanocarriers can avoid electrostatic interactions with the mucus layer, thereby achieving higher permeability and increased insulin absorption.[93] Conversely, positively charged carriers are predicted to adhere to the mucus layer, prolonging the residence time of the formulation in the mucus layer and improving insulin absorption.[94] Neutral nanocarriers are suggested to penetrate the mucus layer rapidly and promote uptake by epithelial cells.[93]

4.2.1 | Positively and negatively charged modification

One study reported that NPs with either negative or positive surface charges showed similar efficacy in insulin delivery.[79] Cheng et al. prepared negatively charged NPs with Alg and positively charged NPs with CS.[79] Their zeta potentials were −20.6 and +27.6 mV, respectively. Although negatively charged NPs showed significantly higher mucus permeability (80% vs. 30%), their differences in intestinal permeability were minimal. Furthermore, the two NPs showed similar oral insulin bioavailability in diabetic rats, around 8%–9%.

Another study reported contrasting results: The electronegative NPs exhibited higher insulin bioavailability compared to the electropositive NPs. Wang et al. prepared electronegative and electropositive NPs based on CMCS and CS.[66] The electronegative NPs demonstrated 1.3 times greater intestinal permeability than the electropositive NPs. Moreover, the electronegative NPs

exhibited increased insulin plasma exposure (1.5-fold) and bioavailability (1.8-fold) compared to the electropositive NPs.

4.2.2 | Electroneutral nanocarriers

Zwitterions are compounds carrying both positive and negative charges and have been used to fabricate electroneutral nanocarriers.[95] Typical examples of zwitterions are phosphatidylcholine and carboxybetaine.[96] NPs constructed with zwitterions have been suggested to show high intestinal epithelial permeability as they mimic the surface properties of viral capsids. They are less likely to interact electrostatically with the mucus layer and are readily taken up by cells.

Shan et al. developed insulin NPs based on PLA and the zwitterion dimyristoyl phosphatidylcholine (DLPC).[80] These NPs showed a size of 107.5 nm and a zeta potential of −6 mV. They were compared with fast penetrating NPs (F127 NPs). DLPC NPs showed similar mucus permeability to F127 NPs but 1.57 times higher permeability in the HT29-MTX-E12 (E12) monolayer cell model. Furthermore, the insulin bioavailability of DLPC NPs, 4.76%, was higher than that of F127 NPs, 2.84%.

Han et al. developed an insulin micelle system modified with the zwitterion poly(carboxybetaine) (PCB).[81] PCB micelles diffused faster in mucus and were taken up by Caco-2 cells to a greater extent than nonionically neutral PEG-modified micelles. Their high cellular uptake was largely attributed to receptor-mediated endocytosis. The formulation was substantially transported into cells due to PCB targeting the PAT1. However, direct oral administration of the PCB NPs was found to be pharmacologically inactive, possibly due to instability in the stomach. Therefore, they were freeze-dried and encapsulated in enteric capsules for oral administration. PCB NPs then showed preferable bioavailability in diabetic rats compared to PEG NPs (42.6% vs. 8.35%). The impressive bioavailability may also contribute to the small particle size of the system.[97] Further research on this strategy has not been reported subsequently.

In summary, these findings indicate that the surface electrical characteristics of NPs exert limited influence on the oral bioavailability of insulin. NPs bearing negative, neutral, and positive charges all exhibit markedly enhanced insulin absorption relative to free insulin solution.

4.3 | Protein protective nanoparticles

Proteins have been used to improve the stability of oral insulin formulations by preventing enzymatic degradation.

Albumin (ALB) coating has been employed to safeguard against protease degradation, thus enhancing the pharmacokinetic properties of protein drugs.[98] The ALB modification resulted in large NPs with a size of 300.8 nm and a zeta potential of +28.9 mV.[99] ALB NPs remain stable under simulated gastric conditions and release almost 100% of insulin under simulated intestinal conditions. The positively charged ALB NPs were reported to be attracted to the negatively charged glycocalyx of epithelial cells, thereby enhancing insulin absorption. The permeability of ALB NPs in the Caco-2/HT29-MTX/Raji B cell model was found to be 3.6 times greater than that of non-ALB-modified NPs. However, in vivo studies have not yet been conducted, leaving the efficacy of this formulation uncertain.

Casein and dextran complexes (CN-DEX) have also been utilized to enhance the stability of NPs. CN-DEX-coated NPs were prepared by a precipitation method using maize prolamin as a support framework.[82] This formulation was found to be stable in an HCl solution at pH 2 and showed resistance to proteases such as pepsin and trypsin. It also showed prolonged hypoglycemic effects (up to 36 h) and high insulin bioavailability, ranging from 12.5% to 20.2% in T1DM mice. The improved insulin absorption was largely attributed to the inclusion of cholic acid in the NPs. In the presence of cholic acid, formulations are able to achieve enhanced absorption via the bile acid pathway.[100]

4.4 | Targeted modification

Through the utilization of ligands to modify NPs, specific receptors on the epithelium can be targeted. This strategy significantly enhances receptor-mediated endocytosis, thereby resulting in enhanced efficacy of orally administered insulin.[101]

4.4.1 | Oligopeptides/amino acid-modified nanoparticles

Oligopeptides are small peptide molecules consisting of 2 to 20 amino acids. NPs modified with oligopeptides have been shown to protect against enzymatic degradation.[83] They also lead to improved drug absorption through mechanisms similar to protein uptake.

Bai et al. used oligopeptides consisting mainly of glutamic acid to modify a PLGA scaffold, resulting in an effective insulin delivery system.[102] The size of the NPs (152.83 nm) was smaller than protein-modified NPs (≈300 nm); however, the EE was low at 23.86%. This low EE may lead to drug wastage and challenges in large-scale production.[103] Cellular uptake of oligopeptide NPs was found to be 7.8 times higher than PEG NPs, which was attributed to oligopeptide transporter-mediated internalization. Their oral insulin bioavailability was reported to be 10.0%, almost twice that of PEG NPs. In addition, long-term in vivo safety studies in mice treated with the oligopeptide NPs for 40 days showed no toxicity.

Other oral insulin NPs have demonstrated improved pharmacological availability when modified with L-valine (LV).[84] This LV modification on nanocarriers was found to enhance insulin uptake through oligopeptide transporters. The LA NPs resulted in a 60% reduction in blood glucose levels in diabetic rats. The reported pharmacological availability of insulin was 7.55%.

Strategies that attempt to increase insulin uptake by mimicking oligopeptide absorption achieve limited insulin bioavailability, typically not surpassing 10%. In contrast, targeted strategies that emulate

vitamin-mediated transcellular pathways have reported higher insulin bioavailability.[85]

4.4.2 | Vitamin modified nanoparticles

Vitamins, particularly the B family of vitamins, have been studied for the modification of NPs. This is due to the widespread vitamin receptors in the small intestine, such as folic acid (FA, VB9) receptors and intrinsic factor (IF), as well as sodium-dependent multivitamin transporters (SMVT).[104–106] Vitamin-modified NPs are able to target these receptors, thereby increasing drug absorption.

Surface-modified CS NPs using FA were employed to deliver insulin. They showed a larger size, ≈288 nm.[85] FA NPs target FA receptors on epithelial cells in the small intestine.[104] FA-mediated endocytosis was reported to play an important role in the cellular uptake of the FA NPs. This oral insulin formulation maximally reduced blood glucose levels in diabetic rats by ≈50%, with a high oral insulin bioavailability of 17%.

Biotin-modified nanocarriers reported increased bioavailability of oral insulin. Both biotinylated CS NPs and biotinylated liposomes demonstrated significantly improved intestinal permeability and hypoglycemic effects.[52,107] The reported oral bioavailability was 4.6% and 8.23%, respectively. The enhanced drug absorption was facilitated by SMVT-mediated endocytosis.[105]

Vitamin B12 (VB12)-modified insulin NPs have also shown enhanced transcellular transport and improved hypoglycemic effects. These NPs were prepared by conjugating VB12 with chondroitin sulfate sodium alginate derivative vectors.[108] VB12 NPs are able to target IF in enterocytes.[106] The VB12 NPs were small in size, ≈52 nm, with a zeta potential of −37 mV and an EE of 34%. They showed significantly higher permeability in a Caco-2 cell monolayer compared to unmodified NPs. Oral administration of the VB12 NPs resulted in a 54% reduction in blood glucose levels in diabetic rats. In contrast, unmodified NPs reduced blood glucose levels by only 25%.

FA modification appears to be a favorable surface modification for NPs as FA NPs exhibit high oral insulin bioavailability compared to other VB-modified NPs.

4.4.3 | Thiolated nanoparticles

Thiol-modified NPs are able to target mucin by forming disulfide bonds with glycoproteins in mucin, thereby prolonging the retention of the formulation in the intestine and improving insulin absorption.[27]

CS was surface-modified with pentaerythritol tetrakis (3-mercaptopropionate) to generate thiolated insulin NPs. After oral administration, this formulation accumulated in the intestinal mucus of diabetic rats and reached its peak glucose-lowering effect at 3 h.[109] This hypoglycemic effect was found to be greater than free insulin. Another study involving CS NPs coated with thiol groups and hyaluronic acid showed twice the oral bioavailability of insulin compared to those without thiol groups.[86] They showed enhanced retention within the mucus layer with an insulin bioavailability of 11.3%.

It is notable that both of the formulations exhibited burst release of insulin under simulated gastric conditions, possibly due to the introduction of thiol groups. Burst release of insulin could lead to a risk of uncontrolled hypoglycemic effects.[110] Therefore, enteric capsules may be required to improve their stability in the stomach.

4.4.4 | Cholic acid-modified nanoparticles

The introduction of cholic acid into oral drug delivery systems has been shown to improve drug absorption. Cholic acid-modified NPs can undergo endocytosis mediated by the apical sodium-dependent bile acid transporter (ASBT) in the ASBT-enriched ileum and be transported to the liver for enhanced absorption.[111] Moreover, cholic acids may be able to protect NPs from lysosomal degradation.[112]

Ma et al. developed UC-CMs@ins based on ursodeoxycholic acid-modified amphiphilic copolymers of acrylic acid and ε-caprolactone.[113] The sizes and zeta potentials of the UC-CMs@ins were 223.78 nm and +4.7 mV, respectively. The cellular uptake of this formulation was shown to be primarily mediated by ASBT-dependent endocytosis, as inhibition of the ASBT pathway resulted in a 70.2% reduction in insulin cellular uptake. The reported oral insulin pharmacological bioavailability was 26.7% in diabetic mice.

Other oral insulin formulations containing cholic acid have also been reported to increase the oral bioavailability of insulin. CS and deoxycholic acid-modified liposomes showed a lysosomal protection effect and achieved a high oral bioavailability of 16.1% in diabetic rats.[87] In another study, insulin was conjugated with dihydroxy conjugated bile salt to form HIP nanocomplexes. A bioavailability of 14.13% was observed after colonic administration in diabetic rats.[114]

The above evidence suggests that ASBT targeting mediated by cholic acid may represent another promising strategy to enhance oral insulin bioavailability.

4.4.5 | Specific cell-targeted nanoparticles

Oral drug delivery systems targeting specific intestinal epithelial cells, such as M cells and goblet cells, have received considerable attention.

M cells in Peyer's patches express multiple receptors.[115] Transcytosis through M cells is one of the primary pathways for intestinal uptake of protein. Proteins transported by M cells are able to enter the lymphatic circulation.[116] Wheat germ agglutinin (WGA) was used to modify SLNs for M cell targeting. The modified SLNs were about 70 nm in size and had a zeta potential of −13 mV.[88] WGA NPs resulted in an increase in oral insulin bioavailability in healthy rats, rising from 4.99% for unmodified NPs to 7.11%.

Oral insulin formulation targeting goblet cells has also demonstrated an increase in insulin bioavailability. The CSKSSDYQC (CSK) peptide-modified TMC NPs reported a size of 342 nm with an EE

of 55.4%.[89] Increased insulin internalization was observed in the HT29-MTX cell model, indicating improved insulin delivery. In diabetic rats, insulin bioavailability increased to 5.66% compared to 3.69% for unmodified NPs. Furthermore, polymeric micelles formed with glutamic acid copolymers and modified with CSK also reported improved insulin absorption. They exhibited a size of 184.58 nm with a high EE of up to 83.51%.[117] A CSK-mediated improvement in cellular uptake was demonstrated, with insulin bioavailability increased from 3.17% to 7.05%.

Targeted delivery platforms for specific cells appear to improve oral insulin uptake. However, the number of these cells is significantly low compared to enterocytes, which limits oral insulin absorption. This may be one reason why the bioavailability of orally administered insulin in such delivery systems was lower than in receptor-targeted delivery systems.

4.5 | Cell-penetrating peptides modified nanoparticles

CPPs have emerged as a valuable tool for enhancing the transcellular transport of NPs, although the exact mechanism of CPP-mediated internalization remains controversial.[38]

In one study, NPs were modified with SAR6EW, a novel CPP. The presence of SAR6EW resulted in significantly improved cellular uptake in Caco-2 cells.[118] The cellular uptake mechanism mainly involved micropinocytosis. Chen et al. achieved similar results by developing R8 NPs. They used dicyandiamide chitosan as a vehicle and coated it with R8 and HA.[37] The addition of R8 significantly improved the hypoglycemic effect in diabetic rats. The key mechanisms involved in cellular uptake were lipid rafts and macropinocytosis. Other NPs were developed using MSNs as the vehicle and modification with CPP5.[36] It resulted in a remarkable 4.5-fold increase in cellular uptake and a 2.0-fold increase in cellular transport. Caveolae-mediated endocytosis played a key role in the uptake mechanism. However, it reported a relatively low bioavailability of 2.48%.

Since not all of the studies involving CPPs reported insulin bioavailability, and the reported study showed a low bioavailability of 2.48%, the efficacy of CPP modification remains uncertain, thus potentially offering limited improvement in insulin delivery.

5 | ORAL INSULIN FRONTIERS

5.1 | Oral insulin in clinical trials

Although a substantial effort has been dedicated to developing oral insulin formulations, and some formulations have progressed to clinical trials, challenges remain in demonstrating favorable efficacy in diabetic patients.

Nodlin™ is an oral insulin developed by NOD Pharmaceuticals. It was insulin-loaded NPs packaged in bioadhesive enteric capsules.[119] The phase I trial of Nodlin conducted in 2012 reported positive results.[119] In a 4-day study involving 12 healthy volunteers, Nodlin demonstrated glucose-lowering effects similar to those of subcutaneously injected neutral protamine Hagedorn insulin (ChiCTR-TRC-12001872). Although the biopotency of the formulation was relatively high at 37%, there were significant variabilities, with a standard deviation of 90%. The composition of Nodlin was not disclosed.

Diasome Pharmaceuticals has developed an orally administered insulin, HDV-I, that targets liver cells. Insulin was encapsulated in liposomes, which were less than 150 nm in size.[120] The surface of these liposomes was modified with a hepatocyte targeting molecule for hepatic cell-specific targeting. It was expected that these liposomes would be absorbed through the intestine into the portal vein and taken up by liver cells, mimicking natural insulin delivery. In a small-scale clinical trial involving six patients with T2DM, HDV-I showed superior hypoglycemic properties compared with placebo.[120] However, its effect on glycaemic control after lunch and dinner was not as pronounced as after breakfast. Its efficacy compared with subcutaneous injection of insulin was also unknown. The large-scale phase II/III study of HDV-I, which included 230 T2DM patients, appears to have commenced in 2008 (NCT00814294). However, the recruitment status and results have not been reported to date.

One of the most promising oral insulin products is Oramed's ORMD-0801. Despite encouraging results in Phase II trials, unfortunately, it failed in a Phase III clinical trial. ORMD-0801 consists of insulin encapsulated in enteric capsules along with protease inhibitors (soybean trypsin inhibitor) and permeation enhancers (sodium ethylenediaminetetraacetate). It functions by resisting insulin degradation in the GI tract and increasing insulin absorption by opening the TJs. In 2019, it was evaluated in an 84-day phase IIb study in 373 patients with T2DM, which reported a 0.6% reduction in baseline HbA1c levels after treatment.[121] In another Phase II study in 8 patients with T1DM, ORMD-0801 also showed the ability to lower blood glucose levels (NCT00867594). However, in early 2023, Oramed reported disappointing results from a Phase III clinical trial.[122] In this randomized, double-blind study involving 710 patients with T2DM, no significant improvement in glycaemic control was observed after 26 weeks of ORMD-0801 treatment compared to the placebo.

Although the initial laboratory experiments yielded promising results, the outcomes from clinical trials involving patients have been less favorable. The treatment that was anticipated to be effective did not perform well in the final testing phase. Consequently, significant further research is required before oral insulin delivery can be deemed successful.

5.2 | Advanced technology in oral insulin

Advances in technology are driving exploration into new approaches for dealing with diabetes. Novel automated drug delivery devices, such as the self-unfolding device and gastric auto-injector, have been developed. Among these, oral microneedle devices are particularly capturing attention (Figure 3). Moreover, there is hope that gene therapy could potentially cure diabetes (Figure 4).

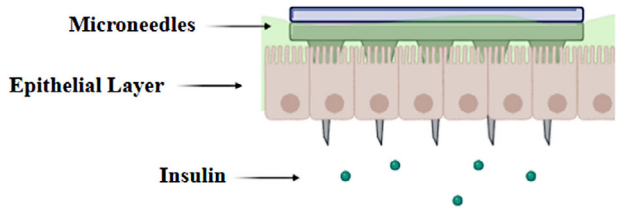

FIGURE 3 Insulin-loaded microneedles.

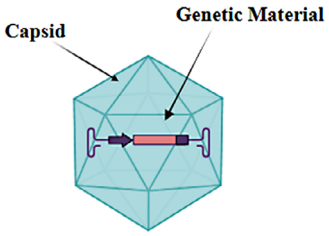

FIGURE 4 Gene-encapsulated adeno-associated virus (AAV) vector.

5.2.1 | Oral microneedles

Microneedles for oral insulin delivery have received increasing attention. Microneedles are an efficient approach that has been extensively studied for transdermal and oral protein drug delivery.[123] A novel technique involves pH-responsive robotic capsules containing insulin-loaded microneedles.[124] These orally delivered capsules can be dissolved in the small intestine, allowing the microneedles to penetrate the epithelial layer and inject insulin. A promising insulin bioavailability of 50% was reported.[124]

Another novel technology is the self-orienting millimeter-level microneedle array.[125] It mimics the natural reorientation of a leopard tortoise that facilitates self-adjustment to the desired upright posture, which allows the device to be stabilized in the stomach and enables microneedle penetration. Similar glucose-lowering effects to subcutaneously injected insulin have been reported.

However, these microneedle injections may involve higher risks of infection compared to subcutaneous injections due to the abundance of microorganisms in the GI tract.

5.2.2 | Gene therapy

Gene therapy has shown great potential for the treatment of diabetes. It normally involves DNA, mRNA, or microRNA.[126] Viral vectors such as adenoviruses lentiviruses have been studied as carriers for gene therapy. Insulin-like growth factor 1 (IGF1), encoded by the IGF1 gene, is critical for beta cell replication and function. Gene therapy using adeno-associated virus (AAV) vectors carrying the IGF1 gene successfully rescued beta cells in diabetic rats.[127]

As T1DM is an autoimmune disease, reducing autoimmunity might be beneficial for glycaemic control in T1DM patients. In a study, obese mice received treatment with the gene of anti-inflammatory cytokine IL-10 delivered by recombinant AAV. The results showed that insulin resistance was alleviated, and glucose-induced insulin release was restored in the mice.[127] This implies that immunomodulatory gene therapy could potentially mitigate pancreatic cell damage and reinstate beta cell function in individuals with diabetes.

6 | CONCLUSION

Diabetes, a widespread chronic condition significantly affecting patients' quality of life, is primarily managed through inconvenient subcutaneous insulin injections, leading to adherence issues. Despite a century-long pursuit, oral insulin research has not yet yielded significant success, mainly due to the susceptibility of insulin to degradation in the GI system. This review has highlighted that extensive studies on the GI environment have led to the development of various oral insulin delivery systems, with nanocarrier delivery systems receiving particular attention. Surface modification of NPs, focusing on protective and targeting designs, has been a key area of research. Targeting strategies aim to deliver insulin to specific cells or receptors within the intestinal tract, achieving relative oral bioavailability rates of up to 17%. Emerging technologies, such as oral microneedles and gene therapy, show promise but require further safety assessments. Despite promising preclinical results, the clinical performance of these approaches has been underwhelming, with a notable candidate failing in the phase III trial. Effective oral insulin administration thus remains a distant goal, highlighting the ongoing challenges in this field.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest, financial or otherwise.

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