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REVIEW

Oral peptide therapeutics for diabetes treatment: State-of-the-art and future perspectives

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Abstract Diabetes, characterized by hyperglycemia, is a major cause of death and disability worldwide. Peptides, such as insulin and glucagon-like peptide-1 (GLP-1) analogs, have shown promise as treatments for diabetes due to their ability to mimic or enhance insulin's actions in the body. Compared to subcutaneous injection, oral administration of anti-diabetic peptides is a preferred approach. However, biological barriers significantly reduce the efficacy of oral peptide therapeutics. Recent advancements in drug delivery systems and formulation techniques have greatly improved the oral delivery of peptide therapeutics and their efficacy in treating diabetes. This review will highlight (1) the benefits of oral anti-diabetic peptide therapeutics; (2) the biological barriers for oral peptide delivery, including pH and enzyme degradation, intestinal mucosa barrier, and biodistribution barrier; (3) the delivery platforms to overcome these biological barriers. Additionally, the review will discuss the prospects in this field. The information provided in this review will serve as a valuable guide for future developments in oral anti-diabetic peptide therapeutics.

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1. Introduction

Diabetes is a prevalent chronic disease that affects millions of individuals globally. It is reported that, as of 2021, there are approximately 537 million adults with diabetes worldwide, with a prevalence rate of 10.5%[1]. Diabetes can be classified into two distinct forms: type 1 and type 2. Type 1 diabetes is caused by self-immune destruction of pancreatic β cells, which leads to an inability to secrete insulin, requiring lifelong insulin injections as the sole means of regulating blood sugar levels[2–4]. Type 2 diabetes is characterized by relative insulin deficiency or insulin resistance, causing ineffective utilization of blood sugar and elevated levels due to insufficient sensitivity to insulin in the body. As the disease progresses, the decline in pancreatic β cell function results in insufficient insulin secretion, making insulin the key component of treatment for advanced type 2 diabetes[5]. Besides, glucagon-like peptide-1 (GLP-1) analogs, including exenatide, liraglutide, dulaglutide, and semaglutide, are also frequently utilized to treat type 2 diabetes by promoting insulin synthesis and secretion. These peptide therapeutics have been shown to be effective and safe in clinical trials and are widely used in clinical practice[6,7].

The development of oral peptide therapeutics for diabetes has been a dream for researchers (Fig. 1). In 1922, the second year after the discovery of insulin[8], Dr. Joslin attempted to develop oral insulin[9]. Although the result was a failure, it marked the beginning of a century-long quest for oral insulin[10]. Since then, various strategies have been developed to facilitate oral insulin delivery. The first patent for oral insulin was issued in 1965[11]. In 2001, an oral insulin preparation developed by Emisphere was approved for clinical trials by FDA[12,13]. This was the first oral insulin to enter clinical trials. However, the results of phase II clinical trials showed no significant difference compared to the placebo group. In 2014, an oral insulin developed by Oramed (ORMD-0801) was approved for phase III clinical trials[14]. Unfortunately, ORMD-0801 did not meet both the primary endpoint of improving glycemic control and the second endpoint of achieving a mean change of the fasting plasma glucose from baseline. Despite the extensive efforts on oral insulin, oral GLP-1 analog has also been widely explored for diabetes treatment[15,16]. One distinctive characteristic of GLP-1, setting it apart from conventional anti-diabetic medications like insulin, is its glucose-dependent mechanism of action[17]. As a result, the risk of hypoglycemia associated with GLP-1-based treatments is remarkably low[18]. Since the rapid degradation of native GLP-1 *in vivo*, the longer-lasting GLP-1 analogs, such as Exenatide and Semaglutide, have been developed in clinical treatments[15,19,20]. As a significant breakthrough, the world's first oral GLP-1 receptor agonist (GLP-1 RA), Semaglutide tablet, was approved in 2019[21].

Although oral anti-diabetic peptides are preferred, they encounter two significant challenges: highly individualized pharmacokinetic differences and extremely low bioavailability[13,22], which remain unresolved. Variations in patient characteristics, dietary habits, medication routines, and uncertainties in intestinal motility contribute to significant differences in the absorption of oral peptides. Furthermore, the complex physiological barriers in the gastrointestinal tract severely impede the oral absorption of peptides. Fortunately, recent years have witnessed the emergence of numerous new delivery technologies aimed at enhancing the absorption of oral anti-diabetic peptides, instilling hope among diabetes patients. Presently, several oral peptides have either been commercialized or are undergoing clinical trials (Table 1)[21,23–30], and some reviews have summarized the prevailing advancements in oral peptides[31–33]. Our work uniquely concentrates on the oral delivery of anti-diabetic peptides, a niche yet crucial area in diabetic treatment. Unlike the reviews on oral peptide delivery, we delve into the advantages and delivery details of anti-diabetic peptides. Another key aspect of our review is the emphasis on the latest delivery platforms specifically designed for targeting organs integral to the metabolism of anti-diabetic peptides, an area not extensively covered in other reviews. Additionally, we provide a comprehensive summary of the challenges in the clinical translation of these peptides in our conclusion, offering insights into potential solutions and future directions in this field.

2. Advantages of oral administration of anti-diabetic peptide therapeutics

Currently, the primary administration route for anti-diabetic peptides in clinical practice is subcutaneous injection. Although

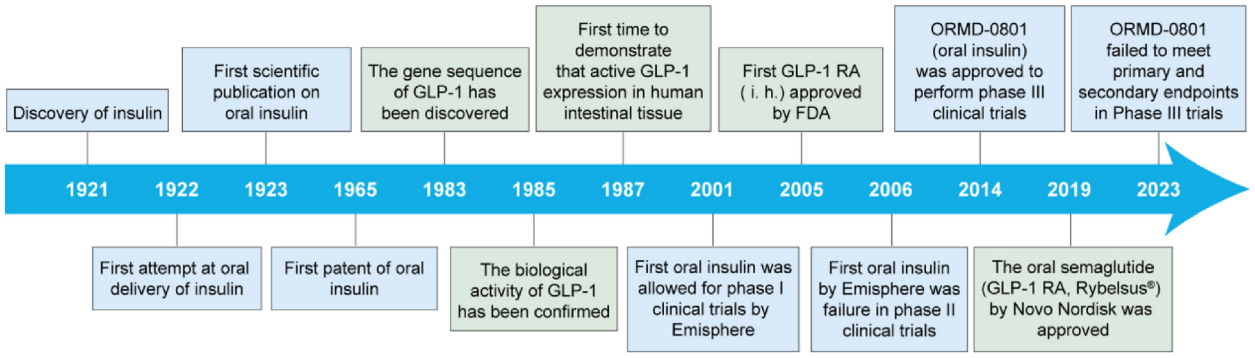

Figure 1 Evolution and milestones of oral insulin and GLP-1 RA therapeutics for diabetes treatment.

Table 1 Oral proteins or peptides currently available in the market or undergoing clinical trials.

Brand name	Active ingredient	Manufacturer	Technology/principle	Stage	Ref.
Lupkynis®	Voclosporin	Novartis	Cyclization modification enhances peptide stability	Launched in 2021	23
Mycapssa®	Octreotide	Chiasma	Promoting drug absorption through SNAC	Launched in 2020	24
Rybelsus®	Semaglutide	Novo Nordisk	Promoting drug absorption through SNAC	Launched in 2019	21
–	Insulin	Diasome	Liposomes loaded with insulin possessing liver-targeting functionality	Phase III	25
–	Insulin	Oramed	POD™: enteric-coated capsules containing enzyme inhibitors and permeation enhancers (EDTA and bile salts)	Phase III	26–28
–	Insulin	Bows pharmaceuticals AG	Dextran-based carrier for insulin	Phase II	29
–	Insulin; Proinsulin; C-peptide	Oshadi	Inorganic silicon nanoparticles, non-covalently bound complex of polysaccharides and insulin	Phase II	30

–, not applicable. SNAC, sodium *N*-[8-(2-hydroxybenzoyl)amino]-caprylate; EDTA, ethylene diamine tetraacetic acid.

subcutaneous injections offer relatively stable blood glucose control, their long-term and frequent usage can result in significant pain, inconvenience, and adverse reactions, such as hypoglycemia, infection, inflammation, and edema. Studies have reported that approximately 60% of diabetes patients struggle to maintain stable blood glucose levels due to their inability to tolerate prolonged repetitive injections[14,34]. In comparison, oral administration offers a safer, more effective, and non-invasive treatment strategy, leading to higher patient compliance. Most drugs are administered orally, and the robust regenerative capacity of the gastrointestinal tract ensures that long-term oral administration does not impose any burden. Moreover, oral delivery eliminates the need for additional devices, enhancing convenience for patients[35]. Considering these advantages, oral delivery of anti-diabetic peptides continues to be a focal point of attention for laboratories and companies.

In addition to the above advantages, oral anti-diabetic peptide delivery has unique advantages compared to other administration methods as it mimics the endogenous secretion pathways. Illustrated through the paradigm of insulin, under normal physiological conditions, pancreatic β cells synthesize insulin, which is subsequently conveyed to the liver *via* the portal vein. Pancreatic insulin secretion exhibits significant fluctuations, but upon reaching the liver through the portal vein, insulin levels gradually stabilize. It is estimated that approximately 50–80% of the insulin is metabolized in the liver, while the remaining insulin enters the peripheral circulation, establishing a liver-peripheral insulin concentration gradient. Thus, the concentration of insulin in the liver is approximately 2–3 times higher than that found in peripheral tissues[36–38]. Similarly, when insulin is administered orally, it is absorbed from the small intestine through the intestinal capillaries, enters the portal vein, and is then transported to the liver. This administration route exposes the liver to elevated insulin concentrations while maintaining relatively lower insulin levels in peripheral tissues (Fig. 2A). The liver-peripheral insulin concentration gradient serves as a safeguard against the occurrence of hypoglycemia resulting from excessively high peripheral insulin levels. Additionally, owing to the liver's high sensitivity to insulin, the elevated insulin concentration in the portal vein rapidly stimulates the liver to uptake glucose, store glycogen, and inhibit

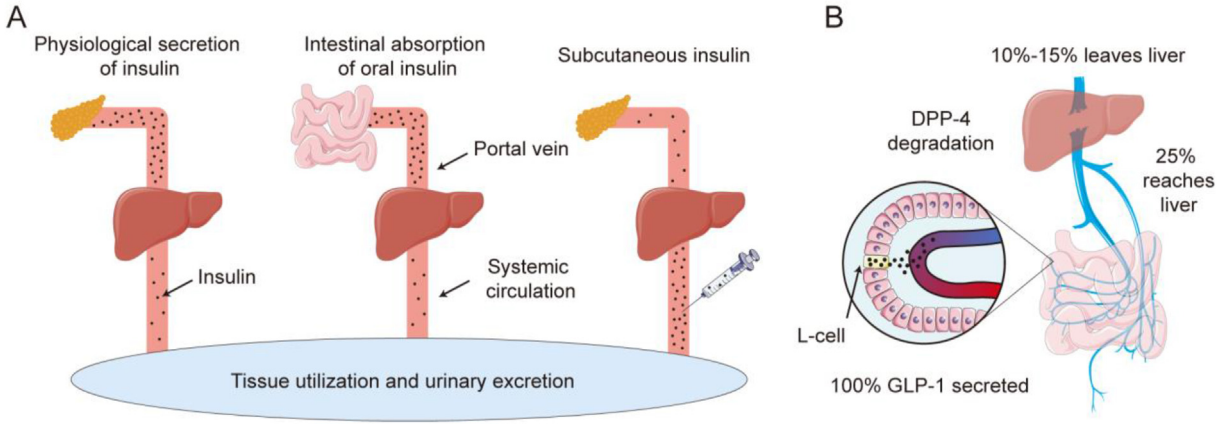

Figure 2 The distribution of insulin and GLP-1 under physiological conditions or after administration. (A) Insulin behavior by physiological secretion, oral, and subcutaneously injectable routes. (B) Secretion and transport of GLP-1.

glycogen breakdown, thereby enhancing blood glucose regulation. In contrast, in non-endogenous insulin secretion processes, the liver's glucose-regulating effects may be delayed, leading to metabolic dysfunction[39]. Similarly, under normal physiological conditions, GLP-1 is secreted by intestinal L cells[40]. Due to its rapid metabolism[15], approximately 25% of GLP-1 reaches the liver, and ultimately, about 10%−15% of GLP-1 enters the bloodstream (Fig. 2B), establishing a liver-peripheral concentration gradient[41]. Oral administration of GLP-1 can simulate this physiological process, thereby achieving a safer and more effective therapeutic approach.

3. Biological barriers for oral anti-diabetic peptide therapeutics

Over the one hundred years, oral peptide formulations have been regarded as the pinnacle of drug delivery in the field of diabetes treatment. This mode of administration holds tremendous promise, but it also presents notable challenges[31,42,43]. A range of biological barriers within the gastrointestinal tract, encompassing harsh pH conditions, enzymes, and the intestinal mucosa barrier, along with post-intestinal distribution barriers[22] within the body after peptides absorption into the bloodstream (Fig. 3), collectively contribute to the significant impediment of oral anti-diabetic peptide absorption, leading to an exceedingly low bioavailability. Existing barriers can be classified into three primary types: pH and enzyme degradation, intestinal mucosa barrier, and biodistribution barrier. The first two barriers are related to absorption, and considerable research has been conducted in this domain. This section offers a summary of commonly employed strategies to overcome absorption barriers, as presented in Table 2[38,44−83].

3.1. *pH and enzyme degradation*

Inherent variances in pH levels across distinct segments of the gastrointestinal tract give rise to a multifaceted pH milieu that can induce alterations in the structure, precipitation, or enzymatic breakdown of peptides[84], consequently diminishing their efficacy in oral absorption. Initially, the generation of gastric acid by cells in the gastric wall establishes an exceedingly acidic milieu within gastric juice (pH 1−2). Within this setting, protein hydrolytic enzymes, such as pepsin and cathepsin, manifest robust activity, rendering peptides highly susceptible to rapid degradation. Subsequently, researches propose that peptides maintain stability solely within a narrow pH spectrum proximate to their isoelectric point[31,85]. Upon entry into the duodenum, characterized by a pH of 4−5.5[86], certain peptides may precipitate due to their proximity to the isoelectric point. Lastly, progression into the small intestine unveils an array of additional protein hydrolytic enzymes, including those secreted by the pancreas, brush-border peptidases expressed by intestinal epithelial cells, cytoplasmic enzymes within intestinal cells[87,88], and potentially, enzymes released by local microorganisms[73,85], all contributing to peptides degradation. In essence, the inhospitable pH milieu coupled with the presence of diverse protein hydrolytic enzymes throughout the gastrointestinal tract renders peptides highly susceptible to degradation or loss of activity at the beginning of oral administration, constituting the primary obstacle in curtailing the oral absorption of peptides. To enhance the stability of peptides within the gastrointestinal milieu, strategies such as the modification with the natural degradable polymer shell (*e.g.* chitosan and dextran) or the encapsulation within enteric-coated matrices have been employed, which offer a degree of protection against degradation and denaturation (Table 2).

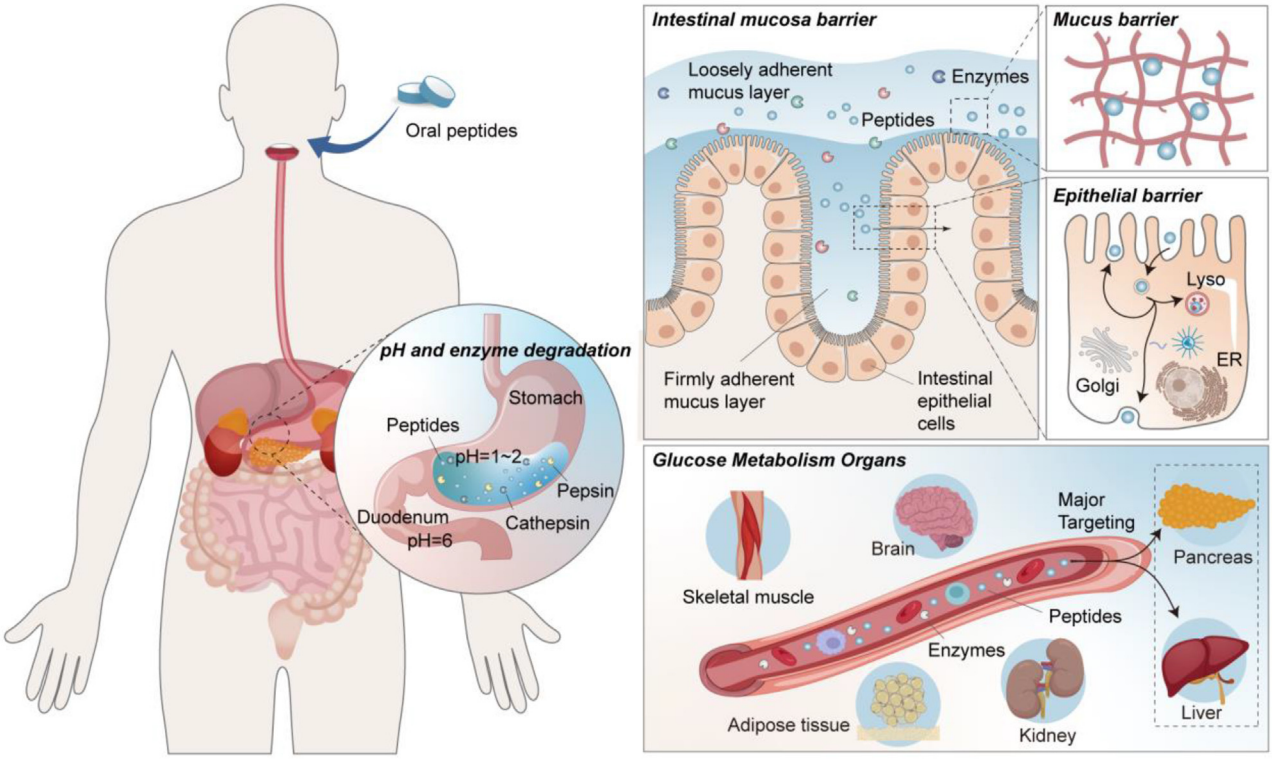

Figure 3 Biological barriers for oral anti-diabetic peptide therapeutics.

Table 2 Strategies to overcome the absorption barriers.

Barrier	Strategy employed	Example of agents	Primary function	Ref.
pH and enzyme degradation	Colon targeting; Enteric coating; Microcapsules;	HPMC; Eudragit; Gelatin; α-cyclodextrin	Preventing denaturation and the low level of proteases	44–47
	Protease inhibitors	Aprotinin; Na-glycocholate; Bacitracin; Camostat mesylate	Enzyme inhibition	48–50
	Natural degradable polymer shell;	Chitosan; Dextran; Alginate	Low toxicity and enhanced sustained release	51–53
	Nanorobots;	–	Rapid passage through the stomach under propulsive forces;	54–57
	Microneedles		Physically piercing tissue barriers to evade the gastrointestinal tract	58–60
Mucus barrier	Mucus adhesion	Natural/synthetic biodegradable polymer NPs	Facilitate close contact through electrostatic interactions, thereby increasing drug residence time	60,61
		PEGylation	Increase hydrophilicity to enhance adhesive capabilities	62,63
		Virus-mimicking	The surface charge is slightly negative, and the outer shell is hydrophilic	64,65
		Hydrogels	The swollen hydrogel adheres to the intestinal mucosa	38,66,67
	Mucus penetration	NPs with small size	Diffusing faster in the mucus by having a diameter smaller than the average pore size of the mucous mesh structure	38,68
		Hydrophilic modification	Hydrophilic surface helps reduce hydrophobic interactions and promotes mucus penetration	69–72
Epithelial barrier	Absorption enhancers	Fatty acids; Surfactants; EDTA	Perturbed cell membranes, improved cell bypass transport, and selectively opened tight junctions	73–75
	Hydrogels	Super porous hydrogel; pH-sensitive hydrogels	The expansion of the hydrogel exerts mechanical pressure to open the tight junctions	38,67,75
	NP carrier systems	Liposomes; Nanospheres; SLN; SNEDDS	Selectively opening tight junctions	76–78
	Modification of specific groups	Ligands (immunoglobulins, transferrin, lectins, biotin, folate, and vitamin B12);	Ligands can be recognized, thereby improving cell internalization	74,79,80
		CPP (penetratin, transportan, oligoarginine, and TAT)	Endocytosis, cell membrane perturbation and channel generation may be the potential modes of action	77,81–83

–, not applicable. HPMC, hydroxypropyl methyl cellulose; NPs, nanoparticles; SLN, solid lipid nanoparticles; SNEDDS, self-nanoemulsifying drug delivery systems; CPP, cell-penetrating peptides; TAT, transactivator of transcription.

3.2. *Mucus barrier and epithelial barrier*

In addition to pH and enzymatic factors, the mucus layer situated within the gastrointestinal tract exerts a substantial influence on the absorption of peptides[73,89]. Notably, the intestinal mucus layer presents a complex and multifaceted barrier system, encompassing the hydrophobic mesh barrier[88], physical-chemical barrier[73], and dynamic drug remodeling barrier[51].

Intestinal mucus is a gel-like structure composed of mucins lining on the surface of intestinal epithelial cells. Its pore size ranges from approximately 20 to 1800 nm[68], facilitating lubrication and protection of intestinal epithelial cells, thereby preventing direct exposure to the intestinal lumen environment. However, when peptides interact with the mucus layer, they encounter the following obstacles: (1) the mucin mesh surface consists of densely packed hydrophilic and hydrophobic regions, leading to hydrophobic interactions between mucin and peptides. This interaction significantly hinders the diffusion of peptides within the mucus, forming a hydrophobic mesh barrier[88]. (2) Mucus can form various low-affinity interactions with peptides present on the mucus surface, restricting the diffusion of

peptides and hindering their upstream transport, creating a physical−chemical interaction barrier[73]. (3) The mucus can be divided into two layers: the loosely adherent mucus layer and the firmly adherent mucus layer. The loosely adherent mucus layer constantly secretes and sheds mucins, leading to mucus renewal every 4−5 h[62,90]. Although this rapid clearance of adherent bacteria or pathogenic substances contributes to intestinal health, it also limits the retention time of peptides in the intestines[91], forming a physical filtration barrier that significantly reduces the uptake of peptides and establishing a barrier for dynamic peptide reconstruction[51], resulting in lower transport efficiency. Currently, several strategies have been employed to overcome the mucus barrier, as summarized in Table 2. Specific natural/synthetic biodegradable polymer nanoparticles (NPs) and hydrogels demonstrate mucus-adhesive properties, thereby augmenting the retention of anti-diabetic peptides and increasing absorption. Besides, the reduction of the carrier's size and the establishment of a hydrophilic surface can also facilitate rapid mucus penetration, ultimately enhancing absorption[68].

Situated beneath the intestinal mucus layer lies the compact structure of the intestinal epithelium, composed of a continuous layer of cells. The peptides necessitate transport into the bloodstream *via* either the transcellular or paracellular pathways. In consideration of the structural features of intestinal epithelial tissue and the lipophilicity of cell membranes[92], hydrophilic peptides primarily rely on the actively-mediated transcellular route. However, owing to their substantial molecular weight and the absence of intrinsic active transmembrane transport mechanisms, direct cellular entry becomes challenging[93,94]. Moreover, in the majority of instances, following their transmembrane ingress into enterocytes, peptides are conveyed to lysosomes, wherein they undergo swift degradation by hydrolases, resulting in their subsequent inactivation[95−98]. Furthermore, peptides may also undergo luminal secretion, subsequently re-released onto the membrane surface[99], thereby diminishing peptide absorption. Additionally, the composition of the cellular cytoplasm, comprised of myriad proteins, cytoskeletal elements, and organelles, impairs the efficiency of peptide diffusion towards the basal side, thereby influencing their efflux process. At present, the majority of orally administered peptides grapple with the predicament of facile cellular uptake juxtaposed with challenging efflux—a situation aptly characterized as "easy entry, difficult exit"[100]. Despite existing research efforts, the enhancement of efflux processes for peptides remains an ongoing challenge. Ligand-modified carriers are a favorable choice for enhancing transcellular efficiency and have been extensively employed in peptide delivery[101]. Besides, additional strategies, such as absorption enhancers, also have the capacity to facilitate the transcellular transport of peptides. The details of these strategies are summarized in Table 2.

3.3. *Biodistribution barrier*

After successfully navigating through the challenges posed by pH, enzymes, mucus, and epithelial barrier, peptides can be transported adequately into the bloodstream. Nonetheless, the intricate biological distribution of peptides constitutes a long journey, wherein multiple factors hinder peptides access to organs crucial for glucose metabolism, including the liver, pancreas, skeletal muscle, adipose tissue, kidney, and brain.

Upon entering the bloodstream, diverse proteases and peptidases distributed in the blood, tissues, and intercellular spaces promptly initiate the degradation of peptides, leading to a short half-life within the body[22,42,102]. Specifically, GLP-1 (7−37), one of the active forms of native GLP-1, undergoes rapid breakdown mediated by dipeptidyl peptidase-4 (DPP-4) and is efficiently eliminated by the kidneys within 1−2 min. Additionally, oral peptides must undergo first-pass elimination in the liver. And, due to the high polarity, peptides can easily enter the renal glomerulus, leading to renal excretion. These factors above collectively contribute to an extremely short half-life of peptides within the body. Furthermore, during peptide transport, the immune system participates in their degradation[103]. The reticuloendothelial system (RES), abundant in macrophages, promptly engulfs drug delivery systems upon entering the circulatory system, leading to the swift clearance of peptides. Additionally, the liver functions as the primary site for insulin and GLP-1 clearance, with Kupffer cells efficiently clearing them to prevent adverse reactions, such as hypoglycemia resulting from peripheral hyperinsulinemia. Hepatocytes emerge as pivotal target cells for the majority of peptides entering the bloodstream *via* the hepatic portal vein. The phagocytic activity of Kupffer cells poses a significant obstacle to the efficacy of peptides. Hence, evading the phagocytic system and augmenting hepatocyte uptake of oral peptides are imperative for peptide therapy in diabetes treatment. It has been reported that pegylation diminishes the immunogenicity and antigenicity of proteins[104,105], thereby preventing clearance by the immune system. RES-blockade strategy can transiently and reversibly impede hepatic clearance, amplifying the accumulation of nanomedicines in tumors and enhancing their antitumor effects, exhibiting favorable biocompatibility[106]. Furthermore, environmentally responsive biomaterials release peptides on demand at appropriate locations, while targeted formulations release peptides at specific sites, both contributing to the sustained therapeutic effects of peptides[107,108]. Ultimately, it is worth noting that individual differences may introduce uncertainty in the delivery, absorption, and effectiveness of peptides, making it an important factor that should not be overlooked.

4. Strategies for oral anti-diabetic peptide therapeutics

In the realm of developing oral anti-diabetic peptides, a myriad of strategies have been devised to enhance their absorption. The strategies encompass the utilization of absorption enhancers, ionic liquids (ILs), NPs, and microneedles (MNs)[84,109]. These innovative techniques have demonstrated their effectiveness in surmounting multiple gastrointestinal barriers, facilitating the efficient delivery of peptides into the bloodstream, thereby enhancing their overall transport efficiency. Furthermore, recent investigations have accentuated a growing emphasis on directing peptides toward organs closely associated with glucose metabolism, particularly the liver and pancreas[110−112]. This approach is aimed at optimizing glucose utilization within the body by leveraging a more nuanced understanding of endogenous insulin/GLP-1 secretion patterns and biological distribution[107,108]. In this section, we present an exhaustive summary of the various strategies employed to enhance the absorption of anti-diabetic peptides. Additionally, we highlight the emerging trend of targeted delivery to the liver and pancreas for maximizing therapeutic effects. These ongoing refinements hold significant promise in augmenting treatment options for individuals afflicted with diabetes.

4.1. *Delivery platforms for improved oral absorption*

4.1.1. *Absorption enhancer*

Absorption enhancers represent a class of compounds designed to transiently enhance the permeability of the intestinal epithelial barrier, thus facilitating oral peptide absorption. Notable

constituents of this category include fatty acids, fatty acid salts, bioadhesive polymers (such as chitosan and its derivatives), and surfactants[113,114].

Fatty acids, abundantly generated during the digestion of triglycerides within the gastrointestinal tract, exhibit the capacity to enhance paracellular permeability by modulating the tight junctions between epithelial cells[115]. Among these, sodium caprate stands out as the most widely employed fatty acid enhancer and holds the distinction of being the first FDA-approved fatty acid absorption enhancer for pharmaceutical use[113]. Its application in oral insulin 338 (I338) resulted in a bioavailability of 1.5%−2.0%, with no reported toxicity during an eight-week study period[116]. Mechanistically, sodium caprate activates phospholipase C, instigating an increase in inositol triphosphate levels, subsequently leading to calcium release and elevation of intracellular calcium concentration[31,117−119]. This cascade culminates in calmodulin-dependent actin contraction, resulting in the opening of tight junctions and enhanced paracellular transport (Fig. 4A). Sodium caprate has also been observed to facilitate drug absorption through plasma membrane perturbation, as evidenced by ATP leakage from Caco-2 cells[117]. This perturbation, which is concentration-dependent, remains reversible and mild in the gastrointestinal tract, underscoring its safety as an absorption enhancer.

Sodium *N*-[8-(2-hydroxybenzoyl)amino]-caprylate (SNAC), a derivative of sodium caprylate, shares similar amphiphilic and surface-active characteristics. SNAC has found widespread use in promoting the absorption of macromolecular drugs. In 2019, Semaglutide tablet (Rybelsus®) was approved for use with SNAC to enhance oral absorption and prevent stomach degradation. Co-administration of 2.5−40 mg of semaglutide with 300 mg of SNAC led to improved blood glucose control, with the bioavailability of semaglutide increasing over time, reaching 1.4% around 2 h post-administration. Investigations into SNAC's absorption-enhancing mechanism suggest that it can form non-covalent complexes with peptides, augmenting their lipophilicity and promoting transmembrane absorption (Fig. 4B). Previously, we have discovered that SNAC can interact with insulin and form tight complexes, influenced by concentration, ratio, and pH[120]. As pH increases, SNAC's carboxyl groups (pKa = 5.08) predominantly exist as carboxylates (−COO−), which favor complex formation with insulin's free amino groups. SNAC/insulin complexes are internalized into cells through passive diffusion and remain intact during transport in the cytoplasm. Additionally, the complex accelerates the exocytosis of insulin towards the basolateral side, thereby enhancing its intestinal permeability. Some studies have suggested that high doses of SNAC may affect cell membrane integrity. Brayden et al.[121] demonstrated that high SNAC concentrations (50 mg/mL) led to complete loss of *trans*-epithelial electrical resistance (TEER) and a 36-fold augmentation in [^{3}H]-mannitol permeability in Caco-2 monolayers, while Hess et al.[122] found that SNAC (33−66 mmol/L) increased 6-carboxyfluorescein (6-CF) transport but not [^{3}H]-mannitol, with no decrease in TEER values. However, when SNAC was introduced at a concentration of 165 mmol/L in the jejunal mucus, TEER decreased, concomitant with an increase in the permeability coefficient of [^{3}H]-mannitol, similar to Brayden's findings. Importantly, the loss of membrane integrity does not necessarily indicate tight junction disruption, and direct evidence of SNAC disrupting tight junctions and opening paracellular pathways is lacking[122]. The increased membrane fluidity of Caco-2 epithelial cells by SNAC aligns with surfactant-induced membrane perturbation. Compared to sodium caprate, SNAC's greater distribution of hydrophilic functional groups in the salicylamide region suggests lower efficiency in domain insertion into phospholipid membranes. This difference may explain why higher SNAC concentrations are required for improved penetration when contrasted with sodium caprate. Notably, SNAC's absorption-enhancing effect may vary depending on the peptides involved, warranting further investigation. Recent work by Novo Nordisk introduced an innovative mechanism of action for SNAC. According to their proposal, SNAC forms a complex around semaglutide within the

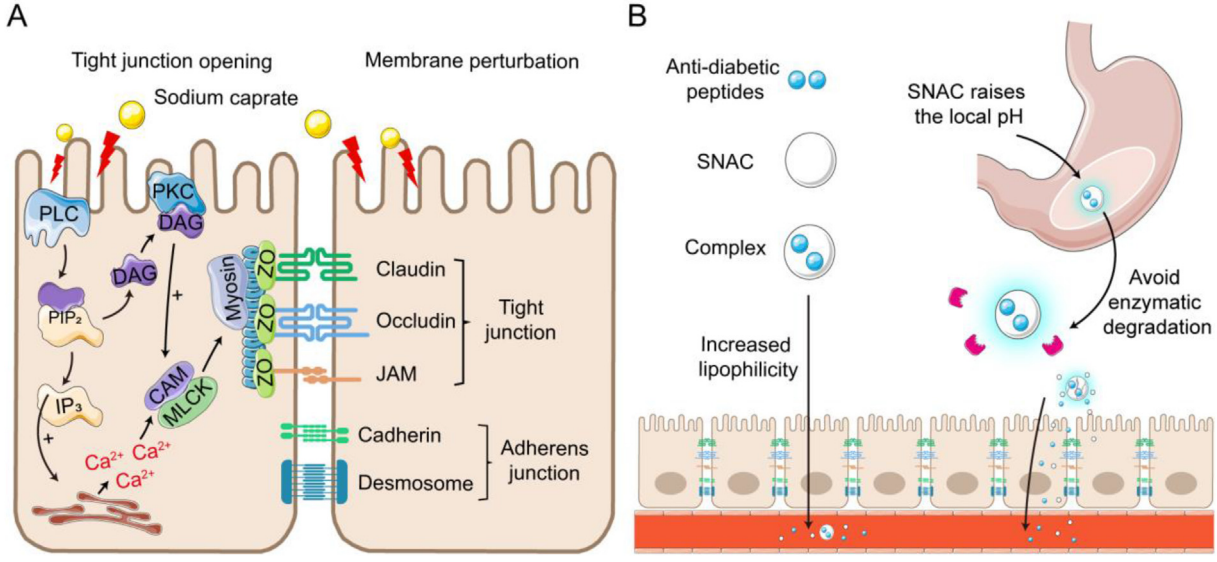

Figure 4 The mechanism of action of sodium caprate and SNAC. (A) Sodium caprate enhances the absorption of anti-diabetic peptides by opening tight junctions and causing membrane perturbation. (B) SNAC enhances the absorption of anti-diabetic peptides by forming complexes with anti-diabetic peptides and increasing its lipophilicity (left); by raising the local pH of the anti-diabetic peptides to reduce enzymatic degradation (right).

gastric environment, resulting in a temporary local pH increase that protects semaglutide from pepsin and enhances dissolution (Fig. 4B)[123]. Moreover, SNAC induces the generation of semaglutide monomers, increases the fluidity of the gastric epithelial membrane without impacting tight junctions, and facilitates semaglutide's entry into the systemic circulation. Regardless of the mechanism at play, SNAC's significant absorption-promoting effect on peptides and its good *in vivo* safety have captured substantial interest from the industry.

Chitosan (CS), derived from the deacetylation of chitin, is another absorption enhancer with a track record of improving peptide oral absorption[124,125]. Insulin-loaded core/shell CS-alginate NPs, effectively safeguard insulin from enzymatic degradation, resulting in an insulin bioavailability of 8.11%[126]. Kim et al.[127] conducted optimizations in the ionotropic gelation process involving CS and phytic acid (PA), achieving an impressive encapsulation efficiency of 97.1%, ultimately yielding a relative bioavailability of insulin at 10.6%. The absorption-promoting attributes of CS can be attributed to its charge properties. Possessing positive charges under mildly acidic conditions due to the presence of glucosamine residues, CS adheres robustly to the negatively charged mucus layer *via* electrostatic interactions, reducing mucus clearance. Additionally, the positive charges on CS can interact with negatively charged serine residues in cell membrane proteins, leading to structural changes and reversible opening of tight junctions, thereby enhancing paracellular transport. Experiments involving CS treatment have demonstrated reduced translocation of zonula occludens-1 (ZO-1) from the membrane fraction, indicating increased paracellular permeability[128]. Based on these remarkable absorption-enhancing properties, CS has emerged as a promising material for consideration in pharmaceutical formulations.

In conclusion, incorporating absorption enhancers is a common strategy to improve oral peptide absorption. Absorption enhancers with diverse mechanisms of action can be combined and administered at lower doses to synergistically enhance absorption. Ideally, absorption enhancers should be simple, easy to manufacture, and possess a high safety profile to enable oral absorption of peptides, without changing the clinically validated pharmacokinetic profile of the drug. Compared to other strategies with complex preparation processes, the absorption enhancer strategy is more widely used in oral delivery systems for anti-diabetic peptides. Furthermore, the efficacy of absorption enhancers is intricately tied to their localized concentration at the absorption site, with notable variations in their functional performance across distinct sites. Ideally, a delivery system should facilitate the targeted transportation of both the peptides and the absorption enhancer to the precise absorption site. This strategic co-localization is pivotal, as it enables the potentiated effect of the absorption enhancer to manifest prominently, facilitated by their concurrent release at therapeutically efficacious concentrations. Notably, a discernible pattern emerges, with multiple enhancers demonstrating superior absorption-facilitating attributes within the colon and ileum regions compared to the proximal small intestine. This observation underscores the potential advantages of directing co-delivery of peptides and enhancers to the distal small intestine or colon, a strategic approach that may optimize pharmaceutical regimens and dosage formulations.

4.1.2. *Ionic liquid*

ILs are compounds composed of large organic cations, such as alkyl imidazolium salts, alkyl pyridinium salts, alkyl quaternary ammonium salts, alkyl quaternary salts, heterocyclic aromatic compounds, and derivatives of natural products, combined with organic or inorganic anions. These substances remain in a liquid state at room temperature and typically possess a melting point below 100 °C. Through the pairing of different ions, ILs exhibit a wide range of physical and chemical properties, including viscosity, hydrophobicity, density, and biodegradability. Consequently, they find extensive use in biocatalysis, protein stabilization, penetration enhancement, and solubilization[129]. In the domain of oral peptide delivery, ILs play a crucial role in enhancing drug absorption by improving drug solubility, stability, and permeability.

One noteworthy class of ILs is choline and geranate (CAGE) ILs, which has emerged as a promising class of biocompatible solvents for both oral peptide delivery. For instance, Banerjee et al.[130] developed an efficient oral insulin formulation using CAGE ILs, resulting in a significant reduction of up to 45% in blood glucose levels in non-diabetic rats. In addition to CAGE, other choline-based ILs, such as $CGLY_{2:1}$ derived from choline and glycollate, have been utilized for the delivery of peptides[131]. The enhancement of peptide absorption through ILs is predicated on their ability to improve peptide stability and gastrointestinal permeability. ILs effectively prevent peptide aggregation, as demonstrated by Reslan et al.[132], who found that the dihydrogen salt ILs (CDHP) remarkably inhibited the decomposition and polymerization of peptides such as Herceptin. At a CDHP content of 53%, it demonstrates strong inhibition of heparin aggregation during the initial stage, resulting in a relative monomer content of up to 90% compared to the control group. Besides, ILs could form a protective shell-like structure around peptides, preserving their structural integrity[133]. Additionally, ILs enhance peptide stability by inhibiting digestive enzymes. For example, Agatemor et al.[134] demonstrated that CAGE ILs effectively curtailed the activity of DPP-4, thus augmenting the stability of GLP-1. ILs can also overcome the gastrointestinal barrier by reducing mucus viscosity, facilitating peptide diffusion within the mucus layer, and improving absorption through the perturbation of tight junctions. Moreover, ILs can overcome the gastrointestinal barrier by diminishing mucus viscosity, facilitating the diffusion of peptides within the mucus layer, and improving absorption through the perturbation of tight junctions. For instance, Banerjee et al.[130] developed an acid-resistant capsule composed of CAGE ILs, encapsulating insulin. They found that the anionic constituents within ILs interact with the mucus layer to reduce its viscosity, while cations act on enterocytes, opening tight junctions and promoting insulin uptake through the bypass pathway. However, it's essential to note that the effects of ILs can vary depending on their constituent ions and their relative proportions. The composition of ILs significantly dictates their ultimate impact on absorption, making it a critical consideration in prescription design.

In essence, ILs play a pivotal role in significantly enhancing peptide absorption due to their unique ion compositions. Their advantage lies in their uncomplicated procedures and their ability to concurrently overcome multiple absorption barriers, a feat challenging for other absorption-enhancement methods. Presently, the application of ILs in oral anti-diabetic peptides is constrained by concerns regarding their biocompatibility, particularly the safety of prolonged administration[129,135]. To address potential toxicity concerns, natural metabolites are incorporated as constituents of ILs. The components, choline, and geranate, integral to GAGE, are FDA-acknowledged as generally regarded as safe (GRAS) ingredients. They elicit a concentration-dependent

recovery in the TEER of Caco-2 cells within 24 h[130]. Similarly, $CGLY_{2:1}$ demonstrates a favorable safety profile following oral administration[131]. However, relying solely on these *in vivo* safety data is insufficient to underpin the clinical translation of ILs. Therefore, further clinical trials are imperative to corroborate therapeutic efficacy and ascertain long-term toxic effects.

4.1.3. Nanoparticles

NPs represent a promising avenue for improving the oral absorption of anti-diabetic peptides[136,137]. Capitalizing on their nanoscale dimensions, NPs possess the capability to effectively surmount the multiple absorption barriers[138]. Previously, several reviews have comprehensively summarized the different types of NPs in enhancing peptide absorption[139–141]. In this review, our focus is on examining the impact of physicochemical properties (*e.g.* size, surface charge, and rigidity) and ligand modification of NPs for anti-diabetic peptide delivery (Fig. 5). These explorations would contribute to a deeper understanding and improved design of NPs for enhanced peptide delivery.

The physicochemical attributes of NPs, including size, surface charge, and rigidity, constitute fundamental determinants of their effectiveness in overcoming mucus and epithelial barriers during absorption[68,142]. Notably, modulating particle size emerges as a straightforward method to enhance mucus penetration. Smaller NPs exhibit enhanced mucus permeability compared to larger counterparts, owing to the constraints posed by mucus pore size (with an average of approximately 200 nm)[143]. However, extremely small NPs result in inefficient uptake, given their inadequacy in overcoming membrane tension and deformation resistance. Furthermore, in scenarios where cellular uptake relies on specific interactions like ligand-receptor binding like ligand-receptor binding, these diminutive NPs do not provide an adequate surface area for a substantial ligand-receptor interaction, thereby resulting in diminished absorption efficiency[144]. Notably, an array of studies substantiates that the optimal range for cellular uptake falls within 30−60 nm, irrespective of core composition or surface charge[145]. This range may be extended to 500 nm with variations in particle properties and cell models[31]. Beyond 500 nm, NPs within the intestinal milieu are more susceptible to capture by M cells, consequently fostering heightened transport through lymphatic vessels[146].

Surface charge also plays a vital role in particle's absorption. Coating NPs with hydrophilic materials, including Polyethylene glycol (PEG), Pluronic F127, and bovine serum albumin (BSA), can establish a hydrophilic surface endowed with a neutral charge, reducing interactions with mucin and enhancing mucus penetration[68]. Lai et al.[69] noted that densely packed, low molecular weight PEG-modified NPs diffused rapidly in mucus, akin to their diffusion in water. We have observed that the Pluronic F127-modified NPs exhibited more efficient mucus permeability and improved oral insulin delivery efficiency compared to unmodified counterparts, resulting in a 2.5-fold increase in hypoglycemic effect[70]. Besides,

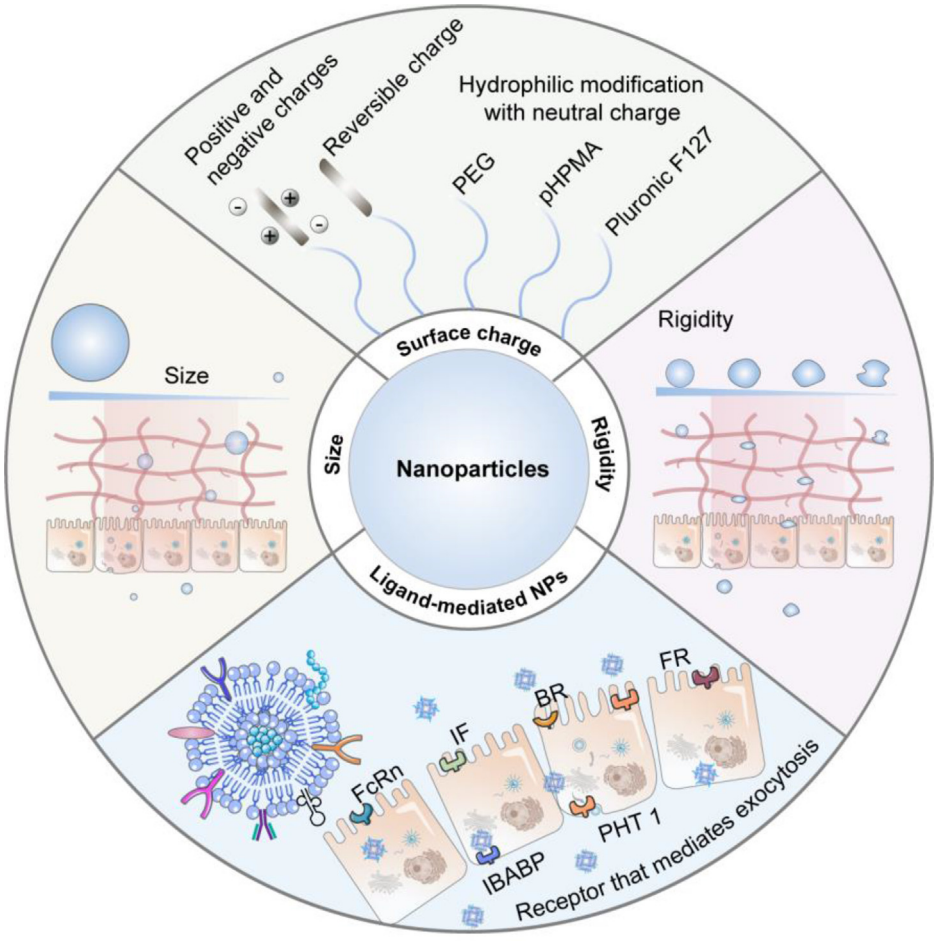

Figure 5 Schematic diagram of strategies for enhancing NP absorption: appropriate physicochemical attributes and ligand modifications facilitate NPs in overcoming absorption barriers.

we employed BSA to coat cationic liposomes with a hydrophilic surface and neutral charge. The Mean square displacement (MSD) of neutral charge liposomes increased by 21 times, further yielding an oral insulin bioavailability of 11.9%[71]. Recently, biomimetic NPs with high-density charges and overall charge neutrality have shown significant mucous permeability[72]. The MSD was 5-fold and 100-fold greater than PEG NPs and cationic NPs, respectively. Moreover, lyophilized insulin-loaded NP powder within enteric-coated capsules achieved an oral insulin bioavailability of up to 42.6%. Surface characteristics of NPs also assume a pivotal role in traversing epithelial barriers. Typically, NPs with positively charged or hydrophobic surfaces facilitate augmented cell membrane contact probability and duration, thus prompting effective endocytosis[147]. Recent findings indicate that anionic NPs induce tight junction relaxation and augment intestinal permeability *via* integrin binding and myosin light chain kinase (MLCK) activation[148]. In essence, adept utilization of NP size and surface charge effects engenders superior absorption outcomes.

Rigidity characterizes NP deformability and also impacts their absorption. Optimal deformability of drug carriers with moderate rigidity facilitates traversing biological hydrogels and cell membranes. Conversely, rigid NPs cannot deform, while soft ones encounter hindrance from mucin-fibril interactions. Bao et al.[149] synthesized short nanotubes (SNTs) and crosslinked short nanotubes (CSNTs), with soft SNTs showcasing rapid permeation compared to stiff CSNTs. Molecular dynamics (MD) simulations disclosed rotational dynamics enhancement within the shear flow and the mucus network. We designed liposomes with varying rigidities by manipulating phospholipid phase transition temperatures. Moderate stiffness liposomes (Young's modulus is approximately 15 MPa) displayed superior mucus penetration and cell uptake efficiency. MD simulations and stimulated emission depletion (STED) microscopy experiments evidenced semi-elastic NPs morphing into ellipsoids, enabling rotation-facilitated swift penetration and elevating insulin oral bioavailability to 13.65%[150]. Zheng et al.[151] noted distinct elastic properties in zwitterionic hydrogel NPs resulting in significantly varying oral insulin absorption in diabetic rats. Among these, the hardest NPs (HNPs) with a Young's modulus around 165.2 MPa exhibited optimal elasticity, affording the most effective hypoglycemic impact and elevating insulin oral bioavailability to approximately 15%. Overall, the advantages of rigidity are noteworthy, becoming more integral in future NP design endeavors.

In the aforementioned examples, it becomes evident that appropriate physical properties primarily aid NPs in overcoming the mucus and epithelial cell barriers during absorption. Nonetheless, these barriers often exhibit contradictory and conflicting requirements for particle design: (1) reducing the size of NPs can enhance their capability to penetrate mucus, yet smaller NPs might encounter challenges in cellular uptake; (2) hydrophilic materials or charge-neutral modifications enhance mucus penetration but prove less effective in cellular internalization. As a result, a range of NPs targeting multiple barriers have been developed, with the application of dissociable coating materials emerging as a strategy to address these multiple challenges. As mentioned in the preceding text, we employed BSA as a coating for cationic liposomes. The resultant liposomes exhibited a neutral surface charge, leading to improved permeability in intestinal mucus[71]. Wu and colleagues[152] devised charge-reversible NPs comprised of cationic peptides and anionic phosphoserine. The overall neutral charge of these NPs facilitates mucus penetration, after which the hydrolysis of phosphoserine exposes the cationic peptides, thereby promoting cellular uptake. Compared to NPs modified with cell-penetrating peptides (CPP), the charge-reversible NPs have increased the oral bioavailability of insulin by 1.9-fold. In another study, Shan and colleagues[153] formulated NPs coated with poly(N-(2-hydroxypropyl) methacrylamide) (PHPMA), which disengaged from the particle surface during mucus penetration, subsequently enhancing cellular entry. During osmosis, the protein corona is shed, unveiling the cationic core and facilitating cellular uptake. NPs with a dissociable coating demonstrate a 20-fold increase in absorption within mucus-secreting epithelial cells compared to free insulin. Moreover, the oral bioavailability of insulin delivered *via* these NPs is 2.1 times higher than that of unmodified NPs. Furthermore, fine-tuning the rigidity of NPs serves as a straightforward and effective approach to overcoming multiple barriers. We contrasted the mucus penetration and cellular uptake capabilities of NPs with varying rigidities, revealing that moderately elastic NPs could adeptly traverse the dual barrier[154]. Furthermore, in the work mentioned earlier by Bao et al.[149], the potential of applying NP rigidity to overcome multiple barriers was similarly observed. To conclude, while contradictions and conflicts exist between the mucus and epithelial cell barriers, the application of dissociable coating materials proves effective in resolving these challenges. Additionally, selecting an appropriate NP rigidity emerges as another efficacious method. As the *in vivo* fate of NPs is further refined, more optimized strategies are likely to be proposed in the future.

Due to the limited capability of conventional NPs for cellular transcytosis and tight junction opening, ligand-mediated targeted delivery strategies have been widely applied when designing the *in vivo* fate of NPs[155]. Currently, receptors or transporters commonly used in the intestines include neonatal Fc receptor (FcRn), transferrin receptor (TfR), intrinsic factor (IF), biotin receptor (BR), and folate receptor (FR), among others[156,157]. By functionalizing ligands on the surface of carriers that specifically bind to receptors or transporters on the intestinal epithelial cells, the transport efficiency of NPs can be improved. Pridgen et al.[158] modified the fragment crystallizable (Fc) fragment of IgG on NPs, enabling them to be taken up by specifically binding to FcRn on the intestinal epithelial cells. Fc-modified NPs show approximately 10 times higher absorption efficiency *in vivo* compared to unmodified ones. Similarly, Fc-modified exenatide NPs prepared by Shi et al.[159] exhibited strong transcellular transport capabilities and sustained hypoglycemic effects (up to 12 h) after oral administration. Ke et al.[160] modified VitB12 on the surface of chitosan derivative NPs for insulin delivery. Compared to unmodified NPs, VitB12-modified NPs reduced insulin retention in the intestinal lumen (0.59-fold) and increased its absorption in epithelial tissue (4.8-fold). These studies indicate that ligand modification significantly promotes the endocytic behavior of NPs, enhancing the oral absorption of peptides. However, through in-depth research on the fate of NPs, it has been discovered that targeting apical membrane receptors alone through ligand design leads to limited absorption efficacy, as most NPs cannot effectively escape lysosomes and end up being degraded. Consequently, researchers have developed many NPs with lysosome avoidance and basal lateral release capabilities. NPs modified with the Fc ligand demonstrate certain lysosome escape capability. Under acidic conditions, the Fc ligand exhibits high affinity to FcRn, thus avoiding lysosomal degradation[161]. Besides lysosome avoidance, another crucial factor for successful exocytosis is targeting the basal lateral membrane. In recent years, studies have found that surface modification of carriers with cholic acid or

deoxycholic acid enables efficient endocytosis and exocytosis through different receptors on the membrane. We prepared deoxycholic acid-modified CS NPs (DNPs), which bind to the apical sodium-dependent bile acid transporter (ASBT) on the surface of ileum cells, and are subsequently taken up into the cells. They further disrupt the lysosomal membrane structure to achieve escape. Additionally, the DNPs in the cytoplasm can bind to the ileal bile acid-binding protein (IBABP) and be transported to the basal lateral side of the cells. Through the intestinal bile acid transport pathway, DNPs improve insulin transcytosis efficiency by 12.86 times, ultimately achieving an oral bioavailability of 15.9% for insulin[162]. Furthermore, we designed a dual ligand-modified nanocarrier (PG-FAPEP) that targets both apical and basal membranes to mediate complete transcytosis of NPs[163]. In this design, folic acid is used to mediate NP endocytosis by coupling with the core PLGA. Simultaneously, a tripeptide (aspartic acid-phenylalanine-glycine) that specifically binds to the proton-coupled oligopeptide transporter (PHT1) is synthesized to target the basal lateral membrane. PG-FAPEP rapidly penetrates the mucus through the charge effect of the tripeptide and triggers the proton sponge effect in lysosomes to achieve escape. The modified carrier's transcytosis efficiency reaches 19.7%, which is 11.6 times higher than that of unmodified PLGA carriers. After oral administration, the relative bioavailability of insulin in rats reaches as high as 14.3%. These strategies effectively address the exocytosis deficiency observed in single ligand-modified NPs.

Overall, through extensive research on NPs, it has been established that modulating the physical properties and ligand modifications of NPs is an effective strategy to enhance the oral absorption of this class of formulations. Numerous scientific studies have contributed to the gradual design of NPs to optimize their fate in the body, resulting in improved outcomes in overcoming mucus barriers and epithelial cell barriers. However, the current research on the design of NPs with specific physical properties, particularly shape and stiffness control, is still limited. Materials and technical reports in these fields are scarce, such as the shape of NPs, which is one of the essential properties of NPs that we have not mentioned but is very important. The inability to provide safe organic materials limits their application in oral peptide delivery. Future advancements are eagerly anticipated to develop more mature technologies for the controlled design of NPs with diverse physical properties, thereby effectively harnessing their advantages. Regarding ligand-modified NPs, they have demonstrated the ability to partially control the fate of NPs in the body. However, the majority of current research predominantly focuses on the cellular uptake efficiency of these carriers, with limited investigation into their intracellular fate and exocytosis mechanisms. Therefore, further exploration of the intracellular transport processes of drug carriers is necessary to elucidate the correlation between ligand modifications and intracellular transport pathways. This deeper understanding will serve as a guide for the design of drug carriers with highly efficient transcellular activity, ultimately leading to improved oral bioavailability of protein and peptide-based anti-diabetic drugs.

4.1.4. Microneedle-based platforms

MNs are intricately designed needle-like structures created using micro-electromechanical technology, typically characterized by diameters smaller than a few tens of micrometers and lengths ranging from 25 to 2000 μm[164]. MN technology holds the potential for facilitating the oral delivery of peptides, because it can directly address the challenges encountered by traditional oral formulations, such as biochemical barriers, gastrointestinal mucus barriers, and gastrointestinal cellular barriers, thus effectively enhancing drug absorption.

Traverso et al.[58] conducted pioneering proof-of-concept experiments in pigs, illustrating the ability of MNs to effectively overcome barriers presented by the gastrointestinal mucus and epithelium, thereby enhancing the oral bioavailability of biomacromolecules. Furthermore, the device demonstrated safety and tolerability during the experimental period. Notwithstanding safety evaluations, concerns persist regarding the potential toxicity associated with metal materials. Consequently, biodegradable or dissolvable MNs have been developed for biomedical purposes. A luminal unfolding MN injector (LUMI) oral capsule has emerged as a result[165]. Once the LUMI capsule reaches the small intestine, the external capsule degrades, enabling three folded arms inside to spring open and propel a 1 mm-long microneedle (Fig. 6E), loaded with insulin, into the intestinal wall. Upon activation, the capsule splits, and the non-degradable elastomeric core of LUMI is expelled from the gastrointestinal tract (Fig. 6A). LUMI demonstrates a faster onset of action compared to subcutaneously injected insulin, resulting in a systemic absorption efficiency of 10% within 4 h. Currently, this technology is undergoing investigation in clinical trials. However, despite the optimization of forces exerted on the small intestinal wall by LUMI technology to ensure safer functionality, concerns regarding potential intestinal dilation caused by MN persist. Moreover, the thinness of the intestinal wall, ranging from 0.1 to 2 mm, presents a risk of perforation. Given the abundance of intestinal microbes, even minor damage from MNs poses a significant risk of systemic infection. Another limitation is the requirement for LUMI to pass through the stomach, which is subject to a 1−4 h gastric emptying time. This poses challenges for insulin delivery, as timing in relation to food consumption is crucial. Fortunately, the research team has addressed these concerns in their subsequent work. Drawing inspiration from the passive reorientation ability of tortoises, they developed a self-orienting millimeter-scale applicator (SOMA) based on the shell of a leopard tortoise (Fig. 6F)[166]. SOMA comprises spring-loaded MNs loaded with insulin. The shape and density distribution of SOMA were optimized to ensure the consistent orientation of the MNs upon landing in the stomach, followed by rapid adjustment to an upright position to securely adhere to the stomach wall (Fig. 6B). Subsequently, the MNs penetrate the gastric tissue, releasing insulin into the bloodstream. *In vivo* results have demonstrated that oral administration of SOMA produces glucose-lowering effects similar to subcutaneous insulin injection, with comparable insulin levels in the bloodstream. Achieving injection in the stomach, SOMA offers improved safety and efficacy. Compared to the intestinal wall, the gastric wall, which is 4−6 mm thick, provides a broader protective layer and more space for drug insertion. Furthermore, gastric tissue exhibits rapid regeneration, and the flowable seal of the mucus barrier temporarily closes any defects in the inner wall. Considering the known variations in gastric emptying, delivering the dose to gastric tissue instead of the small intestine may provide more predictable dosing times and advantages for delivering anti-diabetic drugs.

MNs need to make targeted contact with the tissue wall to penetrate and deliver drugs, but this process is often hindered by digestive fluids or food in the gastrointestinal tract. To overcome this obstacle, Zhang et al.[167] developed a magnetic-responsive MN robot. These MN robots consist of a magnetic substrate, detachable connectors, and a tip component. Encapsulated in

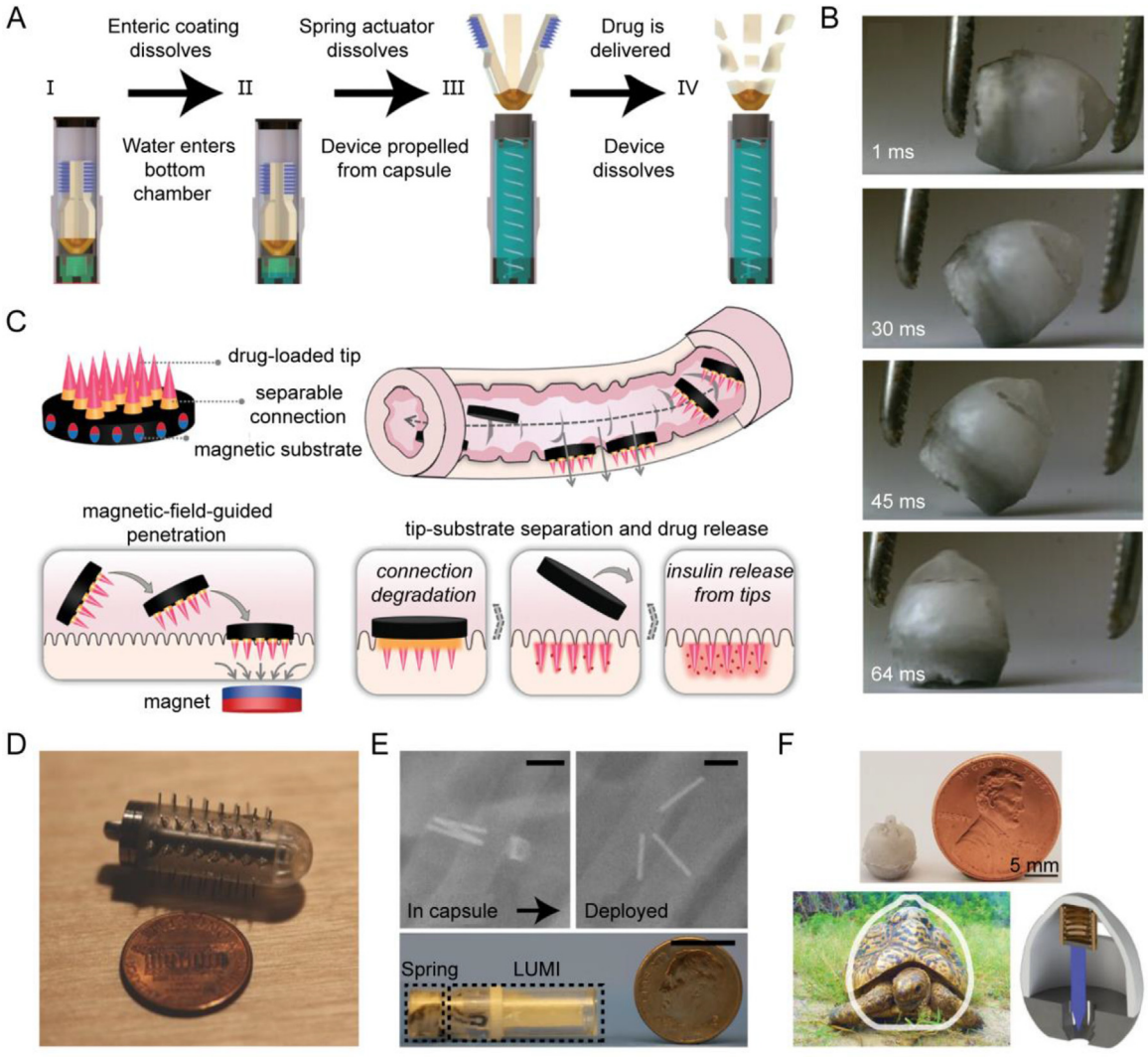

Figure 6 Oral microneedle devices and their *in vivo* localization and injection. (A) LUMI actuation scheme. (B) Self-orients of SOMA. (C) Schematic illustrations of the oral magneto-responsive MN robots. Under the influence of a magnetic field, the MN robot navigates and penetrates the intestinal wall to facilitate drug delivery. (D) Custom-made equipment used for estimating MN safety and transit time. (E) LUMI capsule actuation *in vivo* in the swine (top) and Encapsulated LUMI (bottom). Scale bars: 1 cm. (F) Comparison of the shell shape between the leopard tortoise (*S. pardalis*) and SOMA, along with a schematic representation of SOMA's actuation. Reprinted with the permission from Refs. 165, 166, 167, and 58. Copyright © 2019 Springer Nature, 2019 Science, 2021 John Wiley and Sons, and 2015 Elsevier.

commercial enteric capsules, they can be orally administered and released upon entering the small intestine. Guided by a magnetic field, the MN robots traverse the digestive fluids and penetrate the intestinal wall (Fig. 6C). After penetration, the detachable connector degrades, separating the tip from the substrate and leaving the tip inside the small intestine to release the insulin, while the magnetic substrate is expelled from the body. The blood glucose levels of the treated miniature pigs are expected to normalize within approximately 2 h, demonstrating promising therapeutic efficacy. Further clinical research on these products is eagerly awaited. Overall, Oral physical platform devices designed for in-body injection offer several advantages, including easy traversal of multiple absorption barriers, comparable efficacy to subcutaneous injections, and the ability to establish liver-peripheral insulin concentration gradients specific to oral administration, thereby minimizing the risk of hypoglycemic side effects associated with subcutaneous injections. However, the challenge of in-body retention of these physical devices needs to be addressed. While existing research has demonstrated the potential for platform devices to be eliminated from the body, concerns remain regarding their comparative safety when compared to fully biocompatible materials, particularly with regards to gastrointestinal perforation and inflammatory reactions. Furthermore, the effectiveness of oral MNs in achieving in-body injection warrants further investigation, given the potential hindrance posed by digestive fluids and food residues.

4.2. Delivery platforms for major metabolic organs-targeted delivery

Over the past century, research endeavors have predominantly concentrated on enhancing oral anti-diabetic peptides absorptive potential[168–170]. In recent years, there has been a shift towards exploring the targeted delivery of anti-diabetic peptides, such as

insulin and GLP-1, through oral routes to organs associated with glucose metabolism, particularly the liver and pancreas (Fig. 7).

The liver serves as a crucial target organ for insulin action. Hepatocytes, the primary cell type in the liver responsible for various physiological functions, such as glucose metabolism, storing and releasing glucose, as well as synthesizing and secreting bile[171]. Moreover, the surface of hepatocytes is characterized by a high density of insulin receptors (IRs), boasting approximately 200,000 receptors per hepatocyte, which makes hepatocytes the main site of insulin action[172,173]. However, upon entering the liver, oral anti-diabetic peptide formulations first reach the hepatic sinus[146], a primary site for substance exchange and metabolic functions. The liver, being a representative RES organ, features Kupffer cells and liver sinusoidal endothelial cells (LSECs) in the hepatic sinus, both capable of engulfing formulations, particularly those with a size exceeding 100 nm[146,174,175]. This poses a challenge for these formulations to effectively reach hepatocytes. Therefore, achieving targeted delivery to hepatocytes becomes imperative to enable insulin to exert its effects and elicit biological responses effectively. Studies have demonstrated that the utilization of oral insulin delivery strategies, endowed with hepatocytes-targeting attributes, holds the capacity to augment hepatic insulin accumulation while re-establishing the critical liver-peripheral insulin gradient. In this modality, insulin exerts its influence on hepatic insulin receptors, thereby facilitating glucose uptake and glycogen storage, ultimately contributing to the maintenance of euglycemia and the mitigation of hypoglycemic episodes[176,177]. Toward this objective, we developed two delivery systems with this feature. One is a virus-mimetic multifunctional nano-carrier with a surface ligand-switchable, named Pep/Gal-PNP. This carrier, exhibiting pH-responsive characteristics, efficiently traverses the intestinal mucosa barrier and selectively delivers insulin to the liver by binding to the asialoglycoprotein receptor (ASGPR) on the surface of hepatocytes under physiological blood pH conditions (Fig. 7A–C)[108]. This selective targeting results in a significantly greater hepatic insulin utilization when compared to peripheral tissues (19.9% vs. ~7%), culminating in a remarkable 7.2-fold increase in hepatic glycogen production. The other system, termed Pep-PMS, exhibits the capacity to surmount the epithelial barrier and achieve substantial accumulation within the space of Disse (the space between LSECs and hepatocytes), facilitating its interaction with IRs on the cell membrane of hepatocytes (Fig. 7D and E). Remarkably, Pep-PMS releases insulin in a glucose-responsive manner on multiple occasions, thereby ensuring a sustained reduction in blood glucose levels for up to 12 h[107]. Zhang et al.[178] introduced a cholic acid modification group on the surface of oral insulin NPs, enabling precise delivery of encapsulated insulin to the target site, hepatocytes, through the enterohepatic circulation pathway. This strategy successfully increased the bioavailability of insulin to 26.9%. Bao et al.[179] integrated cholic acids into the core of oral insulin delivery NPs (CN-DEX) and surface-modified them with dextran. The inclusion of dextran serves a dual purpose: it shields insulin from hydrolysis in the gastrointestinal tract and undergoes gradual degradation as CN-DEX passes through the mucus layer, unveiling cholic acid molecules, which can leverage bile acid transporters to facilitate intestinal absorption and hepatocytes-targeting. Additionally, the oral hepatocytes-targeted liposome technology, developed by the United States-based Diasome Company, has progressed into Phase III clinical trials.

The pancreas, serving as the primary site for insulin secretion, emerges as a pivotal target organ in the realm of diabetes therapy. It is noteworthy that a multitude of ion channels and receptors orchestrating the intricate regulation of insulin secretion are localized on the surface of pancreatic β cells. Among these, the GLP-1 receptors emerge as the preeminent target, prominently featured in contemporary research. The interaction of GLP-1 RA with its receptor triggers the release of insulin, which effect is glucose-dependent, maintaining blood glucose levels in balance without the risk of hypoglycemia and facilitating weight loss.

Due to the widespread distribution of GLP-1 receptors within the pancreas, GLP-1 RA possesses the ability to selectively target and accumulate within pancreatic tissues[180]. However, GLP-1 RA is susceptible to degradation during transit to the pancreas. Consequently, the majority of research efforts focus on enhancing its stability by amino acid substitution, fatty acid side chain modifications, and PEGylation, among others[181]. Furthermore, exploiting the targeting capabilities of GLP-1, the conjugation of GLP-1 as a ligand to carrier molecules allows for pancreatic targeting. Notably, Finan et al.[182] demonstrated the functional targeting of GLP-1 receptors by conjugating GLP-1 with estrogen. In comparison to the unconjugated GLP-1 control, the synergistic interplay between these two compounds corrected obesity (more than twice the weight loss, $P < 0.001$), hyperglycemia ($P < 0.001$), and dyslipidemia ($P < 0.05$) in mice while mitigating adverse reactions in other tissues. Apart from active targeting, passive pancreatic targeting through the mesenteric lymphatic system has been shown to be advantageous for anti-diabetic peptide therapy. Lin et al.[183] engineered phase-transitional nanoemulsions (EXT@PC/NEMs) for oral delivery of Exenatide (EXT). EXT@PC/NEMs are capable of passive targeting to the pancreas through the mesenteric lymphatic system, resulting in a relative pharmacokinetic bioavailability of 23.8 ± 5.0% (Fig. 7F). Additionally, this mode of pancreatic targeting has the potential to enhance blood glucose control. Chuang et al.[184] adopted a NP approach composed of CS and poly(γ-glutamic acid) (CS/γPGA NPs) for the oral co-administration of bovine insulin and exendin-4 in a combinatorial therapeutic regimen. CS/γPGA NPs facilitated intestinal bypass penetration and the transport of bovine insulin and exendin-4 to the liver and pancreas, leading to approximately 4-fold and 3-fold increases in glucose absorption in the heart and skeletal muscle compared to control groups. Additionally, it is worth noting that recent research has indicated the potential action of insulin on the pancreas. Lee et al.[185] developed oral insulin NPs, named Bile-acid-polymer (pBA) NPs, These NPs preferentially accumulate in the pancreas of mice, exhibiting high affinity with the bile acid receptor TGR5 on pancreatic islet cells (Fig. 7G and H). This interaction activates the secretion of GLP-1 and endogenous insulin, restoring blood glucose levels in mice and pigs with type 1 diabetes.

In summary, the targeted delivery of anti-diabetic peptides to metabolic organs, especially the liver and pancreas, holds the promise of achieving a safer and more effective reduction in blood glucose levels. Additionally, the associated research has illuminated the promise of this therapeutic paradigm. Nonetheless, it is important to note that the current investigations into oral antidiabetic peptide targeting therapy remain relatively limited, with a paucity of studies focusing on other metabolic organs integral to blood glucose regulation, such as white adipose tissue, brown adipose tissue, and skeletal muscles, which have been largely marginalized in the context of diabetes treatment. In light of this, future research should endeavor to expand the purview of therapeutic targets to encompass these

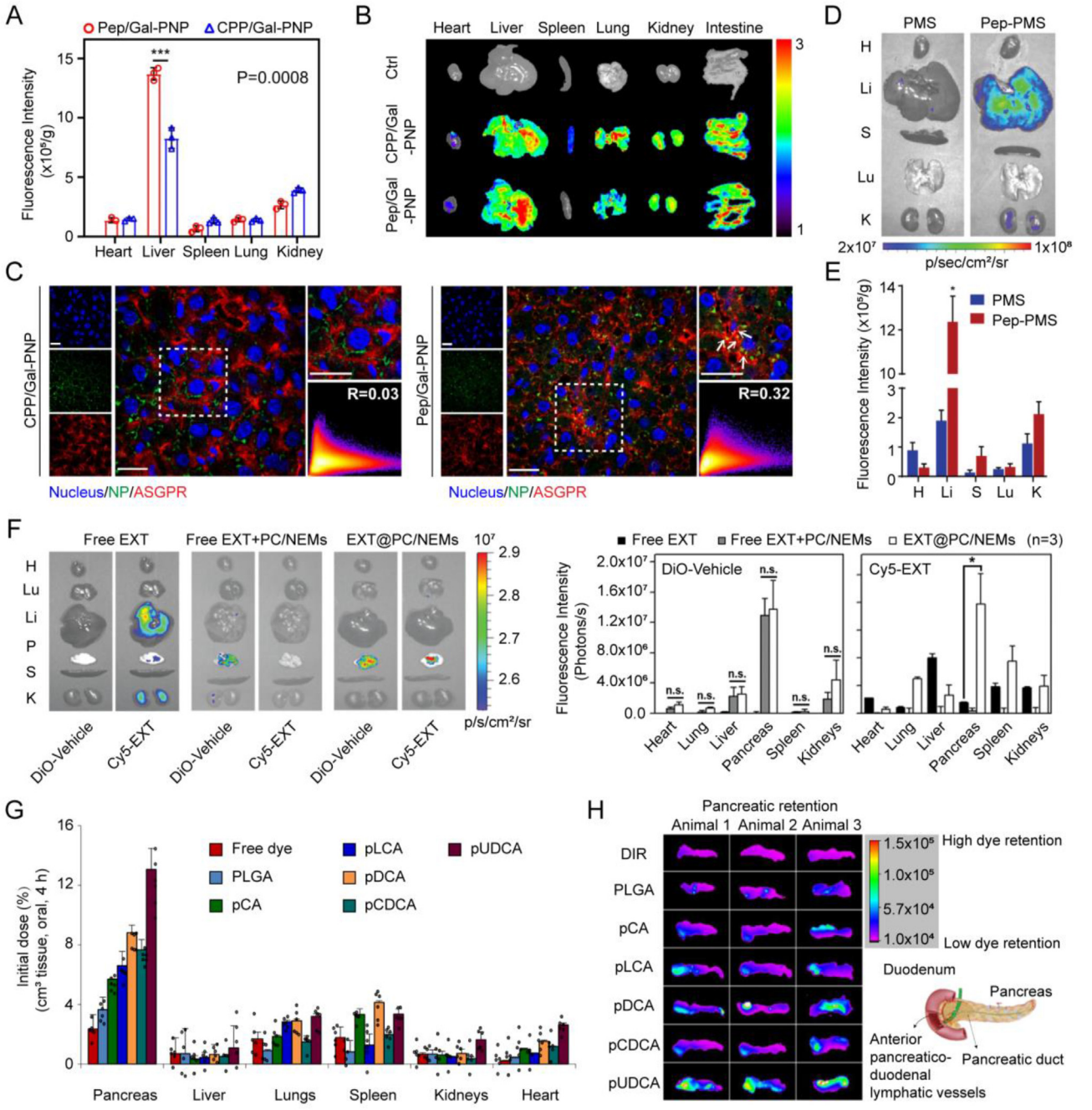

Figure 7 Delivery Platforms for major metabolic organs-targeted delivery. (A) Fluorescence intensity and (B) accumulation in major organs using the *in vivo* imaging system (IVIS) and (C) colocalization with ASGPRs in liver sections of rats 4 h after the oral administration of FITC-labeled Pep/Gal-PNP and CPP/Gal-PNP (mean ± SD, $n = 3$, $^{***}P = 0.0008$). The color scale represents radiant efficiency × 10^7 $p \cdot s^{-1} \cdot cm^{-2} \cdot sr^{-1}$. Scale bars: 20 μm. (D) Organ-level biodistribution measured by IVIS and (E) fluorescence intensity of FITC-labeled PMS and Pep-PMS after oral administration to healthy rats (mean ± SD, $n = 3$, $^{*}P < 0.05$). (F) Organ-level biodistribution measured by IVIS and corresponding fluorescence intensity within 1 h after SC administration of Cy5-EXT, oral administration of free Cy5-EXT + empty PC/NEMs, or Cy5-EXT@PC/NEMs of rats. (G) Organ-level biodistribution of orally ingested pBA NPs (mean ± SD, $n = 6$). (H) Accumulation in the pancreas at 4 h after oral gavage. Reprinted with the permission from Refs. 107, 183, and 185. Copyright © 2020 John Wiley and Sons, 2019 John Wiley and Sons, and 2021 Springer Nature.

metabolic organs, thereby leveraging their critical roles in blood glucose regulation. Such an expansion holds significant promise for the development of a more scientifically rigorous framework for the prevention and treatment of diabetes and its associated complications.

5. Conclusions and outlooks

The oral delivery of anti-diabetic peptides has long been recognized as a crucial goal within the field of diabetes treatment. This approach not only addresses the inconveniences associated with

injections but, more importantly, could replicate the biodistribution profile of anti-diabetic peptides under physiological conditions. This emulation holds significant promise in enhancing blood sugar control and reducing the risk of diabetes-related complications. The foundation for achieving stable blood sugar regulation lies in optimizing absorption and ensuring favorable pharmacokinetic parameters. In this review, we present a comprehensive exploration of several exemplary strategies, encompassing a diverse array of approaches aimed at improving absorption and targeting major metabolic organs. These strategies are meticulously designed to surmount absorption barriers and navigate the metabolic pathways of peptides, thus ensuring both drug efficacy and safety. A profound understanding of the underlying mechanisms governing these strategies is pivotal in facilitating their more efficient development and utilization for anti-diabetic peptides.

Regrettably, despite the implementation of these delivery strategies, the conversion rate of oral anti-diabetic peptides remains suboptimal. This issue can be attributed, in part, to technological inadequacies, including challenges such as notably low bioavailability, dietary interference, and significant inter-individual variability in absorption[6,186]. Furthermore, limitations within the existing delivery strategies contribute to their inability to meet regulatory standards. While some absorption enhancers have facilitated drug marketing, numerous challenges persist in their practical application, encompassing issues such as unknown toxicity, irritation, and drug−excipient interactions, requiring in-depth study. Moreover, the absence of a standardized evaluation system for the absorption-promoting effects of absorption enhancers results in unclear mechanisms of action[113]. ILs, a more recent entrant for oral delivery of anti-diabetic peptides, have shown improved biocompatibility by incorporating natural metabolites; however, additional clinical data are requisite to verify therapeutic efficacy and ascertain long-term toxic effects[129]. Nanotechnology, while a relatively mature technology, encounters challenges in large-scale NP preparation, quality assurance, consistency, and stability. Potential immunological responses to NPs, leading to clearance or inflammatory reactions, necessitate further investigation into their biocompatibility[187]. MNs, capable of traversing multiple absorption barriers with efficacy comparable to subcutaneous injections[58], pose challenges due to difficulty in removal, safety concerns related to gastrointestinal perforation, and inflammatory responses. The success rate of *in vivo* MN injection warrants additional scrutiny, considering potential hindrances posed by digestive juices and food debris. Beyond these challenges, the role of tissue distribution and association concerning anti-diabetic peptides has been somewhat overlooked. Different tissues exhibit varying sensitivities and functionalities in response to anti-diabetic peptides, often interconnected in intricate ways. For example, β cells demonstrate pulsatile insulin secretion, and the portal vein cyclically transports pancreatic insulin to the liver, occurring roughly every 5 min. Subsequently, insulin enters systemic circulation, leading to vasodilation and targeted distribution to vital organs such as the brain, adipose tissue, and kidneys. In this sequential process, the orchestration of tissue-specific effects plays a pivotal role in achieving glycemic homeostasis[188−190]. Similarly, GLP-1 receptors are predominantly expressed in pancreatic β cells but are also found in organs like the stomach, small intestine, heart, kidneys, lungs, and brain[191], each with varying sensitivities and functions concerning GLP-1. It is essential to maintain concentration gradients to avoid adverse reactions. Therefore, we advocate for the development of targeted formulations for metabolic organs through the oral route, extending beyond liver targeting. These organs, owing to their roles in glucose metabolism, significantly contribute to the broader improvement of systemic glycemic control. However, challenges such as off-target effects, individual variations, and large-scale vehicle production need resolution for the clinical translation of targeted formulations.

The successful examples in this field offer valuable insights. For instance, the case of oral semaglutide tablets, marketed as Rybelsus®, effectively employs Eligen® technology, incorporating SNAC as an absorption enhancer to augment bioavailability[21]. Notably, while SNAC enhances absorption potential multifacetedly, the oral bioavailability of semaglutide remains within the range of 0.4%−1%. However, Rybelsus® derives its efficacy not only from SNAC's absorption-enhancing properties but also from semaglutide's extended half-life of 165 h. In the context of a daily dosing regimen, the establishment of a steady-state drug concentration with ten-fold accumulation underscores its impact. Equally important, the pharmacokinetic coefficient of variation (CV) experiences a substantial reduction from an initial 150% to a more controlled range of 70−80% upon reaching a steady state[192]. This shift implies that sporadic instances of reduced absorption by patients once or twice a week do not result in significant fluctuations in blood sugar levels. Consequently, the diminished CV values, facilitated through careful dietary management and consistent semaglutide administration, effectively compensate for individual variabilities in semaglutide absorption. This approach serves as a notable model for emulation.

Additionally, the case of the oral insulin capsule, ORMD-0801, developed by Oramed through its proprietary POD™ technology, highlights the importance of personalized administration to mitigate the influence of individual differences. Although the phase III clinical trial of ORMD-0801 did not meet primary and secondary endpoints[193], it revealed a favorable response within a subgroup of patients stratified based on specific parameters such as BMI. This observation aligns with positive data from the Phase III study conducted in China. The baseline BMI of the subgroup in the United States closely resembled that of subjects in the Chinese clinical trial. In April 2023, the National Medical Products Administration (NMPA) approved the application for listing ORMD-0801 in China, suggesting its anticipated future listing in the country[28]. Drawing inspiration from the ORMD-0801 experience, it is reasonable to infer that the future prospects of oral insulin may involve a more targeted approach, emphasizing personalized administration to mitigate the influence of individual differences.

In conclusion, oral anti-diabetic peptides represent a rational choice for diabetes treatment, with this approach gaining prominence due to advancements in fundamental research. However, the bioavailability of oral anti-diabetic peptides remains lower than that achieved through injections, highlighting the need for further optimization. The functions and interconnections of metabolic organs in response to anti-diabetic peptides warrant investigation, and the development of targeted formulations for these organs holds promise. Scientifically designed clinical trials, distinct from those for injectable formulations, should be established, guided by the unique pharmacological attributes of oral anti-diabetic peptides. This alignment will enable a robust realization of the clinical translation of oral anti-diabetic peptides, providing diabetic patients with enhanced and convenient therapeutic options.

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Author contributions

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Conflicts of interest

The authors have no conflicts of interest to declare.

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