

ASSOCIATE EDITOR: CLIVE PAGE

Exploring Intestinal Surface Receptors in Oral Nanoinsulin Delivery

Carlynne Choy, Lee Yong Lim, Lai Wah Chan, Zhixiang Cui, Shirui Mao, and Tin Wui Wong

Department of Pharmacy, Faculty of Science, National University of Singapore, Republic of Singapore (C.C., L.W.C., T.W.W.); Pharmacy, School of Allied Health, The University of Western Australia, 35 Stirling Highway, Crawley WA, Australia (L.Y.L.); School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China (Z.C., S.M.); Non-Destructive Biomedical and Pharmaceutical Research Centre, Smart Manufacturing Research Institute, Universiti Teknologi MARA Selangor, Puncak Alam, Selangor, Malaysia (T.W.W.); and Particle Design Research Group, Faculty of Pharmacy, Universiti Teknologi MARA Selangor, Puncak Alam, Selangor, Malaysia (T.W.W.)

Abstract	962
Significance Statement	962
I. Diabetes Mellitus and Therapy	963
II. Intestinal Receptors Targeted for Nanoinsulin Delivery	964
A. Apical Sodium-Dependent Bile Acid Transporter	964
B. Folate Receptor	964
C. Heparan Sulfate Proteoglycan Receptor	970
D. Neonatal Fragment Crystallizable Receptor	971
E. Transferrin Receptor	971
F. Intrinsic Factor-Vitamin B ₁₂ Receptor	971
G. Proton-Coupled Amino Acid Transporter	972
H. Niemann-Pick C1-Like Protein 1	972
III. Limitations, Challenges, and Future Perspectives	972
IV. Final Remarks	981
Authorship Contributions	981
References	981

Abstract—Subcutaneous and inhaled insulins are associated with needle phobia, lipohypertrophy, lipodystrophy, and cough in diabetes treatment. Oral nanoinsulin has been developed, reaping the physiologic benefits of peroral administration. This review profiles intestinal receptors exploitable in targeted delivery of oral nanoinsulin. Intestinal receptor targeting improves oral insulin bioavailability and sustains blood glucose-lowering response. Nonetheless, these studies are conducted in small animal models with no optimization of insulin dose, targeting ligand type and content, and physicochemical and molecular biologic characteristics of nanoparticles against the *in vivo*/clinical diabetes responses as a function of the intestinal receptor population characteristics with diabetes progression. The interactive effects between nanoinsulin and antidiabetic drugs on intestinal receptors, including their up-/downregulation,

are uncertain. Sweet taste receptors upregulate SGLT-1, and both have an undefined role as new intestinal targets of nanoinsulin. Receptor targeting of oral nanoinsulin represents a viable approach that is relatively green, requiring an in-depth development of the relationship between receptors and their pathophysiological profiles with physicochemical attributes of the oral nanoinsulin.

Significance Statement—Intestinal receptor targeting of oral nanoinsulin improves its bioavailability with sustained blood glucose-lowering response. Exploring new intestinal receptor and tailoring the design of oral nanoinsulin to the pathophysiological state of diabetic patients is imperative to raise the insulin performance to a comparable level as the injection products.

Address correspondence to: Prof. Tin Wui Wong, Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, 117543, Republic of Singapore. E-mail: phawong@nus.edu.sg; or Non-Destructive Biomedical and Pharmaceutical Research Centre, Smart Manufacturing Research Institute, Universiti Teknologi MARA Selangor, Puncak Alam, 42300, Selangor, Malaysia. E-mail: wongtinwui@uitm.edu.my

No author has an actual or perceived conflict of interest with the contents of this article.

dx.doi.org/10.1124/pharmrev.122.000631

I. Diabetes Mellitus and Therapy

Diabetes mellitus in humans is characterized by an inability to metabolize blood glucose for energy (Xiao et al., 2020). In 2019, approximately 463 million adults worldwide were found to be living with diabetes (Pinchevsky et al., 2020). The International Federation of Diabetes predicted that by 2045, 700 million adults worldwide would be diabetic. Insulin is employed to manage specifically type 1 diabetes and late-stage type 2 diabetes where oral therapy alone is inadequate to attain euglycemia (Xiao et al., 2020). It is most commonly available as subcutaneous formulations. Despite the good bioavailability it confers, the subcutaneous route is associated with needle phobia, trauma, and inconvenient administration as well as lipodystrophy, local tissue necrosis, and infection (Gedawy et al., 2018; Xiao et al., 2020). These drawbacks lead to poor patient compliance, with around 60% of patients failing to attain long-term blood glucose control (Wong et al., 2016; Gedawy et al., 2018), and risks developing diabetic complications such as macrovascular and microvascular complications, retinopathy, nephropathy, peripheral neuropathy, and periodontal disease (Wong et al., 2016; Gedawy et al., 2018; Polak et al., 2000; Stöhr et al., 2021). These in turn provide the impetus for significant research into developing insulin formulations for administration by less invasive routes, such as the buccal, ocular, rectal, oral, pulmonary, and transdermal routes (Mutalik, 2011; Gedawy et al., 2018).

In 2006, Pfizer launched Exubera, an inhaled insulin (Wong et al., 2016). This product was withdrawn from the market a year later owing to the undesirable respiratory burden of inhaled insulin, along with a lack of cost effectiveness (Hollander et al., 2004; Wong et al., 2016). Undeterred by the failure of Exubera, MannKind Corp obtained Food and Drug Administration approval in June 2014 for Afrezza, another inhaled insulin. Afrezza managed to capture some market share, possibly due to improvements in design and formulation over Exubera. However, Afrezza is not routinely recommended by physicians due to side effects and cost concerns (Al-Tabakha, 2015). The failure of inhaled insulins diverted focus to other routes of administration, especially the oral and buccal routes, for insulin formulation research. Despite drawbacks of buccal administration, which include salivary clearance as well as differing epithelia in sublingual, cheek, and palate mucosae, Generex Biotechnology, Inc.

introduced Oralgen, an insulin spray targeting the buccal cavity using the Rapidmist technology (Heinemann and Jacques, 2009; Palermo et al., 2012). Oralgen has shown some success in achieving glycemic control among type 2 diabetic patients; however, clinical use of Oralgen is limited due to high cost and lack of strong evidence showing efficacy. The transdermal route has been explored but showed little success because poor skin permeability to macromolecular therapeutics leads to unacceptably low insulin bioavailability (Zhang et al., 2019). The applications of microneedles and microwave radiation to promote transdermal insulin delivery have recently been introduced, and these have met with varying degrees of success (Harjoh et al., 2020; Ahad et al., 2021; Zhang et al., 2021; Liu et al., 2022).

Peroral delivery of insulin is highly desirable from a patient's perspective, but this route has its fair share of barriers. Insulin is susceptible to degradation by enzymes in the gastrointestinal tract (GIT), such as pepsin, chymotrypsin, elastase, carboxypeptidases and, at the brush border membrane, aminopeptidase, and the extreme pH range along the GIT inducing oxidation and deamination of insulin (Gedawy et al., 2018; Xiao et al., 2020). The mucosa and mucus layers in the GIT present a physical barrier to insulin absorption (Wong et al., 2016; Gedawy et al., 2018; Xiao et al., 2020). Insulin has to avoid being trapped in the mucus and being lost to mucus turnover to maintain high bioavailability (Tan et al., 2020). Paracellular transit across the mucosal layer is not naturally accessible to insulin, and transcellular passage across the intestinal enterocytes, achievable via nanoformulations, poses risks of intracellular enzymatic degradation by endosomal lysosomes (Fan et al., 2018).

Nonetheless, the oral route remains an attractive option as it provides insulin availability that closely mimics the physiologic pathway. Insulin absorbed across the intestinal mucosa is transported to the liver via the portal vein, setting up a portal-peripheral gradient (Arbit and Kidron, 2009; Gedawy et al., 2018) that results in lower levels of systemic insulin and reducing the risk of hypoglycemia and weight gain (Gedawy et al., 2018). Consequently, substantial resources have been invested into formulating insulin to enable it to withstand degradation and to undergo transmucosal absorption in the GIT. For more than two decades, insulin nanoparticles (NPs), or interchangeably known as nanoinsulin, have been formulated, most

ABBREVIATIONS: Apo-Tf, apo-transferrin; ASBT, apical sodium-dependent bile acid transporter; CPP, cell-penetrating peptide; CRT, CRTIGPSVC; CUB, complement C1r/C1s, Uegf, BMP1; DC-LIP, deoxycholic acid-chitosan conjugate-decorated liposome; Dex, dextran; DNP, deoxycholic acid-decorated nanoparticle; DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine; Epi, epichlorohydrin; FA, folate; FA-CS, folate-chitosan; FA-CS NP, folate-chitosan conjugate nanoparticle; FcRn, human neonatal fragment crystallizable receptor; FR, folate receptor; GIT, gastrointestinal tract; GLP-2R, glucagon-like peptide 2 receptor; HAI, HAIYPRH; He@CP-Ca, NPC1L1-targeting amphiphilic nanoparticle; HSPG, heparan sulfate proteoglycan receptor; IF-VB₁₂, intrinsic factor-vitamin B₁₂ receptor; MSN, mesoporous silica nanoparticle; MSN@PLA-mPEG-CPP, mesoporous silica nanoparticles modified with poly(lactic acid)-methoxy poly(ethylene glycol) and decorated with cysteine-modified low molecular weight protamine cell penetrating peptide; NP, nanoparticle; NPC1L1, Niemann-Pick C1-like protein 1; PAT1, human proton-coupled amino acid transporter; PCB, polycarboxybetaine; PCFT, proton-coupled folic acid transporter; PEG, polyethylene glycol; PLGA, polylactide-co-glycolide; RFC, reduced folate carrier; SGLT-1, sodium-glucose cotransporter 1; T1R2, taste 1 receptor member 2; T1R3, taste 1 receptor member 3; Tf, transferrin; TfR, transferrin receptor; TMC, trimethyl chitosan.

of which incorporated with functional excipients, such as protease inhibitors and tight junction modulators to enhance insulin absorption (Cárdenas-Bailón et al., 2013; Wong et al., 2017; Gedawy et al., 2018; Xiao et al., 2020). The paracellular pathway is, however, not ideal as it makes up less than 1% of the intestinal mucosal surface, limiting the amount of insulin that can be transported. More recent studies explore the transcellular pathway, utilizing specific intestinal receptors, to mediate the delivery of nanoinsulin.

In diabetic patients, the microenvironment of the intestine, specifically receptors, undergoes pathophysiological changes in response to the progression of the disease (Chen et al., 2012; Young et al., 2013). Sha et al. (2016) demonstrated that receptors for advanced glycation end-products and transforming growth factor β 1 receptors are more abundant in the mucosa, submucosa, and muscular layer of the colonic wall of diabetic rats, whereas higher concentrations of receptors for advanced glycation end-products in the colon as well as small intestine of the diabetic rats are reported elsewhere (Chen et al., 2012; Zhao et al., 2017). This review aims to profile and critically review the potential of published intestinal receptors that have been exploited, as well as apical membrane receptors of intestinal enterocytes that hold potential to equip researchers with relevant information for nanoinsulin formulations that facilitate oral absorption in diabetic individuals.

II. Intestinal Receptors Targeted for Nanoinsulin Delivery

Table 1 summarizes the physicochemical and physiologic characteristics of intestinal receptors used in oral nanoinsulin targeting. Apical sodium-dependent bile acid transporter (ASBT), folate receptor (FR), heparan sulfate proteoglycan receptor (HSPG), human neonatal fragment crystallizable receptor (FcRn), transferrin receptor (TfR), intrinsic factor-vitamin B₁₂ receptor (IF-VB₁₂) receptor, human proton-coupled amino acid transporter (PAT1), and Niemann-Pick C1-like protein 1 (NPC1L1) are available in the duodenum, jejunum, terminal ileum, or throughout the intestinal tract. They exhibit a high affinity for specific substrates such as bile acid, folic acid, cell-penetrating peptide, antibody, transferrin (Tf)-iron, vitamin B₁₂, amino acid, and cholesterol, respectively. Ligands with similar physicochemical attributes as these receptor substrates, as well as the substrates themselves, can be used as the targeting moiety of nanoparticles for oral insulin delivery. Broadly, the ligands promote endocytosis and cellular uptake of nanoinsulin, protect the nanoinsulin from lysosomal degradation, and/or facilitate its exocytosis from the enterocytes into the blood stream and target site of action (Fig. 1).

A. Apical Sodium-Dependent Bile Acid Transporter

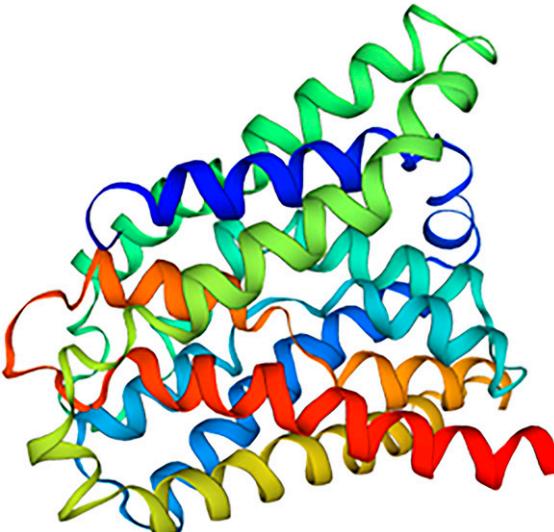
Orally administering the diabetic rats with deoxycholic acid-decorated nanoparticles (DNPs) and deoxycholic acid-chitosan conjugate-decorated liposomes (DC-LIPs) was characterized by an insulin bioavailability of about 16%, with reference to subcutaneous insulin (Fan et al., 2018; Wu et al., 2019). The significant *in vivo* hypoglycemic effects were attributed to ASBT targeting. DNPs were found to evade lysosomal degradation, whereas the undecorated nanoparticles were trapped in lysosomes, leading to enzymatic degradation of their insulin load (Fan et al., 2018). DC-LIPs similarly escaped from the lysosomal digestion; however, the lysosomal escape studies were not conducted with the nondecorated liposomes as control, making it difficult to conclusively attribute the lysosomal escape to deoxycholic acid (Wu et al., 2019).

B. Folate Receptor

The successful development of anticancer drugs through FR α targeting has spawned a widespread interest in reduced folate carrier (RFC) and proton-coupled folic acid transporter (PCFT) as potential targets for nanoparticle drug delivery system. In 2012, Jain et al. (2012) designed folate (FA)-coupled polyethylene glycol (PEG)ylated polylactide-co-glycolide (PLGA) nanoparticles as oral insulin carrier. More recently, El Leithy et al. (2019) encapsulated insulin for peroral delivery in folate-chitosan conjugate nanoparticles (FA-CS NPs), and Yazdi et al. (2020) formulated PEGylated liposomes with folate moieties. Liposomes are developed as they are elastic vesicles and deemed to be able to interact with the intestinal mucosa to a greater extent than the relatively rigid nanoparticles such as folate-coupled polyethylene glycolylated polylactide-co-glycolide nanoparticles. The introduction of PEG to liposomes helped circumvent liposomal instability in the harsh environment of the GIT (Iwanaga et al., 1997; Yazdi et al., 2020). PEGylation, however, may hinder mucosal permeation of liposomes and their uptake across the enterocytes (Suk et al., 2016; El Leithy et al., 2019). The impediment by PEGylation was found to be mitigated via FR targeting (Jain et al., 2012; Tan et al., 2020). Administering a fixed dose of 50 IU/kg insulin orally in the form of PLGA NPs to diabetic rats was characterized by a C_{max} of plasma insulin of 23.25 μ IU/ml. PEGylation of the nanoparticles reduced the C_{max} to 14.90 μ IU/ml. Decorating the PEGylated nanoparticles with FA raised the C_{max} to 26.78 μ IU/ml. FA promoted folate receptor-mediated endocytosis and its translation into nanocarrier has the potential to raise drug bioavailability (El Leithy et al., 2019).

FA has also been employed in the development of virus-mimicking oral insulin nanoparticles made of insulin-loaded poly(n-butylcyanoacrylate) core and hybrid coat consisting of positively charged chitosan-

TABLE 1
Physicochemical and physiologic characteristics of intestinal receptors used in oral nanoinsulin targeting.

Receptor Type	Characteristics	Conformation	Reference
ASBT	<p>ASBT is a 348-amino acid glycoprotein found on cell surface membranes of proximal renal convoluted tubule cells, large cholangiocytes, gall bladder epithelial cells, apical cells of the duodenum (in small amounts), and most abundantly, the apical surface of brush border enterocytes of the terminal ileum, with its carboxyl terminus within the cell cytoplasm and its glycosylated amino terminus located extracellularly.</p> <p>ASBT is responsible for the luminal uptake of bile acids from the intestine whereas other transporters carry out the subsequent intracellular translocation and export of the bile acid at the basolateral side of the cell back to the portal circulation.</p>		Dawson, 2011; Bienert et al., 2017

(continued)

TABLE 1—Continued

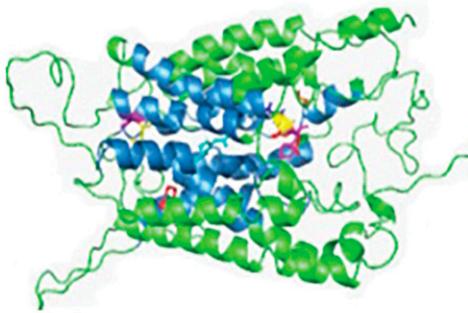
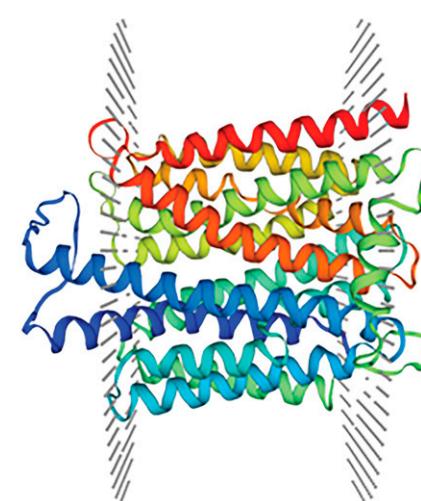
Receptor Type	Characteristics	Conformation	Reference
FR	FR present in the human small intestine constitutes of the RFC and the proton-coupled folic acid (FA) transporter among others. The RFC is made up of 591 amino acids and can be found on the apical brush border membrane of gastrointestinal cells, the basolateral membrane of the proximal renal tubule, and the apical membranes of the choroid plexus and retinal pigment epithelium, as well as on most tissues and cell lines.		Ashokkumar et al., 2007; Desnoulin et al., 2012; Hou and Matherly, 2014; Visentin et al., 2014; Bienert et al., 2017
RFC	The PCFT consists of 459 amino acids. Unlike the RFC, which prefers neutral pH environments and has a relatively low affinity for FA, the PCFT operates optimally at lower pH levels and has a much higher affinity for FA. High levels of PCFT are expressed in the human duodenum and jejunum. Lower levels of PCFT expression are found in the large intestine, although the level of folate transport activity across the proximal and distal human colonic apical brush border membranes (pH 5.5) can be high. Both RFC and PCFT have their amino and carboxyl termini located in the cytoplasm. These proteins play a crucial role in facilitating the uptake of folate, required for RNA and DNA synthesis, across the apical brush border membrane of enterocytes and express specificity to the monoglutamate form of their folate substrates.		Ashokkumar et al., 2007; Desnoulin et al., 2012; Hou and Matherly, 2014; Visentin et al., 2014; Bienert et al., 2017
PCFT			(continued)

TABLE 1—Continued

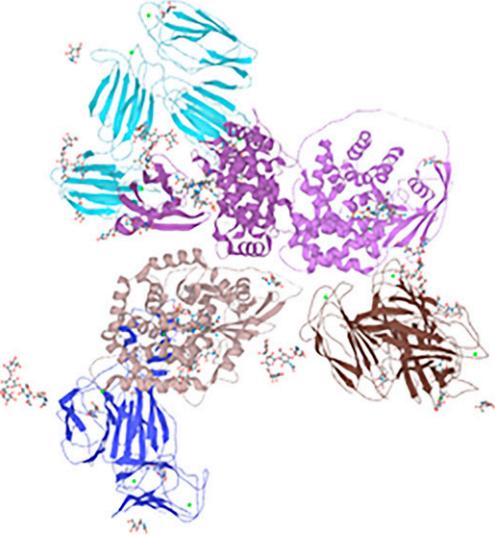
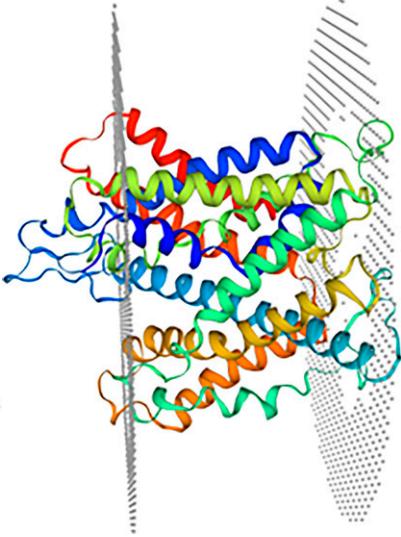
Receptor Type	Characteristics	Conformation	Reference
HSPG	HSPG refers to a group of glycoproteins, characterized by one or more covalently attached heparan sulfate chains. HSPG can be classified into three main classes according to their location of expression—membrane, extracellular matrix, and secretory vesicles; their structures and functions vary with the location of expression. HSPG found on secretory vesicles is expressed on mast cells and hematopoietic cells, whereas HSPG located in the extracellular matrix is usually found on the basement membrane of cells. The membrane-bound HSPG can be found on epithelial cells and/or fibroblasts and has 1–3 heparan sulfate-bound chains. These HSPG have multiple physiologic functions such as protease inhibitor receptors, growth factor coreceptors, and endocytic receptors for clearance of bound ligands in lipoprotein metabolism of liver.		Sarrazin et al., 2011; Christianson and Belting, 2014; UniProt Consortium, 2021
FcRn	FcRn is a 365-residue-long transmembrane protein, essential from birth and expressed throughout life. It aids in the transport of human IgG across the placenta during gestation, conferring passive immunity from mother to fetus. FcRn is expressed throughout the intestine across a large surface area. Found on the apical region, FcRn binds to the CH ₂ –CH ₃ portion of IgG's Fc domain and is responsible for its transcytosis, inclusive of protection of IgG through its intracellular journey, to the basolateral side of enterocytes.		Brambell, 1963; Brambell et al., 1964; Leach et al., 1996; Simister et al., 1996; Pridgen et al., 2013; Rath et al., 2015; Lawrence et al., 2021; UniProt Consortium, 2021

(continued)

TABLE 1—Continued

Receptor Type	Characteristics	Conformation	Reference
TfR	<p>TfR presents in the human body in two forms: TfR1 and TfR2, of which TfR2 has two isoforms, TfR2-α and TfR2-β. TfR1 and TfR2-α have molecular masses ranging from 90 to 105 kDa. They are type 2 membrane proteins that can form homodimers, which function to mediate transepithelial iron transport.</p> <p>Tf stabilizes iron through binding to it. Thereafter, Tf-bound iron is taken up into cells by TfR1 (and TfR2-α) at a fraction of TfR1's binding affinity) via clathrin-mediated endocytosis.</p>		Bienert et al., 2017; Liu et al., 2018; Kawabata, 2019; UniProt Consortium, 2021
	<p>TfR1 and TfR2-α receptors can be found on most cells, especially primitive erythrocytes, hepatocytes, and cells with a high turnover rate. They are also present in sizeable amounts at the epithelium of the small intestine.</p>	<p>TfR1</p>	
		<p>TfR2-α</p>	(continued)

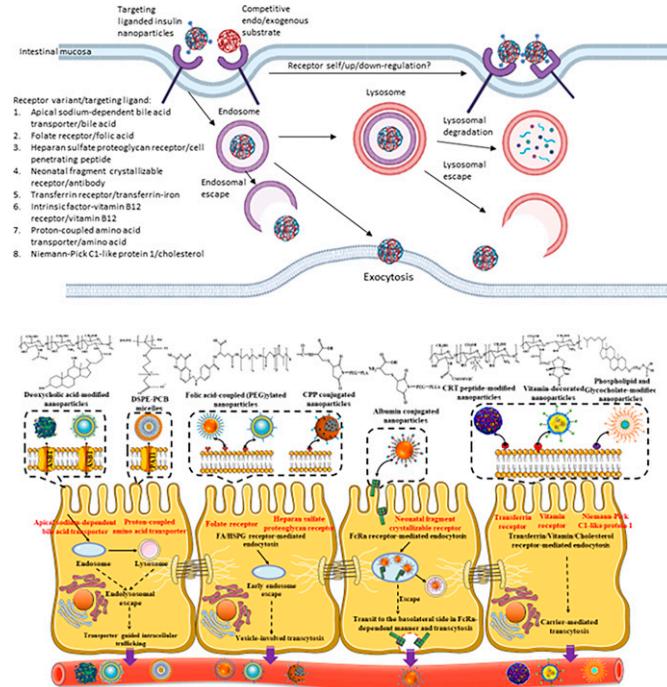
TABLE 1—Continued

Receptor Type	Characteristics	Conformation	Reference
IF-VB ₁₂	Cubilin, the IF-VB ₁₂ receptor, is a 460 kDa peripheral membrane protein. Human cubilin consists of 27 complement C1r/C1s, Uegf, BMP1 (CUB) domains; of the 27 CUB domains, four domains, CUB _{5–8} , bind to the IF-VB ₁₂ complex. Cubilin is mainly expressed in the kidney, where VB ₁₂ reabsorption occurs, and the ileum, where the IF-VB ₁₂ complex is absorbed.		Chalasani et al., 2007b; Andersen et al., 2010; Kozyraki and Cases, 2013; Ke et al., 2015; UniProt Consortium, 2021
PAT1	PAT1 is a 476-residue-long transmembrane protein. It is found on the cell membrane and is largely expressed on the small intestine, where it partners with protons to facilitate transepithelial amino acid uptake. It transports amino acids such as glycine, alanine, and proline and can bind to a variety of substrates, such as α -methylaminobutyric acid, α -aminoisobutyrate, γ -aminobutyrate, D-serine, and D-cysteine.		Chen et al., 2003; Bienert et al., 2017

(continued)

TABLE 1—Continued

Receptor Type	Characteristics	Conformation	Reference
NPC1L1	Human NPC1L1 is a 1359-amino acid transmembrane protein, well known clinically as the target of the cholesterol-lowering drug ezetimibe. It plays a key role in the intestinal cholesterol absorption. There is some disagreement over NPC1L1 expression; some papers claimed ubiquitous expression, whereas others claimed limited expression in the liver and the apical surface of the duodenum and proximal jejunum. Ample intestinal expression of NPC1L1 has been identified.		Davies et al., 2000; Altmann et al., 2004; Yamanashi et al., 2007; UniProt Consortium, 2021

**Fig. 1.** Fate of intestinal receptor-targeted nanoinsulin delivery.

folate and negatively charged hyaluronic acid (Cheng et al., 2021). The coat electroneutrality enables the nanoparticles to penetrate through the intestinal mucus without excessive mucus retention or repulsion, and FA enhances the intestinal mucosal binding affinity of the nanoparticles. In vivo study in diabetic rats indicated that a FA graft content of 12.51% is met with a pharmacological bioavailability of 9.8%, with a given oral insulin dose at 50 IU/kg.

C. Heparan Sulfate Proteoglycan Receptor

On the basis of its hepatic association, the HSPG was investigated by Tan et al. (2020) for its role in the uptake of nanoinsulin from the intestine to the portal circulation. Tan et al. (2020) designed mesoporous silica nanoparticles (MSNs) modified with poly(lactic acid)-methoxy poly(ethylene glycol) and decorated with cysteine-modified low molecular weight protamine as the cell-penetrating peptide (CPP) (MSN@PLA-mPEG-CPP). Low molecular weight protamine is relatively safe and able to deliver insulin, and other peptide drugs, in the form of nanoparticles across the intestine without damaging cell membranes or causing immune responses (Sheng et al., 2016; Zhang et al., 2018a). MSN@PLA-mPEG-CPP was designed with CPP modulation to achieve net particle electroneutrality and mucopenetrating behavior to improve accessibility to receptor (Fig. 2), and its oral insulin bioavailability was about 2.7 times higher than that of MSN@PLA-mPEG (Tan et al., 2020).

A reduction in MSN@PLA-mPEG-CPP uptake by human colorectal adenocarcinoma HT29 cells in vitro was observed in the presence of sodium heparin, a competitive

HSPG inhibitor, suggesting that MSN@PLA-mPEG-CPP cellular uptake was mediated by HSPG. PEG is hydrophilic and generally known to be mucus inert and this aids nanoparticles in mucus penetration (Fig. 2) despite the claim of Yazdi et al. (2020) that it “impedes mucus penetration and weakens cellular uptake.” Reduced hydrophobic and electrostatic interactions of MSN@PLA-mPEG-CPP with mucus decreased its mucus trapping by 36% and promoted its mucus penetration capacity (Fig. 2). The HSPG- and caveolae-mediated endocytosis, and the favorable electrostatic interactions, enhanced the intestinal cellular uptake of the nanoparticles by two- to threefold.

D. Neonatal Fragment Crystallizable Receptor

To target the FcRn, Pridgen et al. (2013) conjugated PLA-PEG nanoparticles with the Fc fragment of IgG, whereas Azevedo et al. (2020) opted for albumin, for its nontoxic, nonimmunogenic and biodegradable nature, to be conjugated to PLGA-PEG nanoparticles. Oral administration of the Fc-conjugated PLA-PEG nanoparticles to wild-type mice elicited a prolonged hypoglycemic response at a notably low dose of insulin (1.1 U/kg), compared with doses typically employed in other nanoparticles (10–100 U/kg) (Pridgen et al., 2013). The same observation was not found when the nanoparticles were administered to FcRn knockout mice, which ascertained the role of FcRn in mediating the improved cellular uptake and insulin delivery of the Fc-conjugated PLA-PEG nanoparticles.

FcRn was shown to be localized at the duodenum of the wild-type mice; however, it is expressed throughout the human intestine at an expression level above 30 pmol/g of tissue (Pridgen et al., 2013; Fan et al., 2019). Given the differences between rodents and humans, Azevedo et al. (2020) developed a mouse model to express human FcRn to enhance the receptor-binding propensity for albumin. Oral administration of PLGA-PEG nanoparticles, decorated with human albumin genetically engineered to improve binding to human FcRn, gave rise to the steepest reduction of blood glucose level at 1 hour and the lowest blood glucose level at 4 hours against controls, including the nanoparticles decorated with an albumin variant, which rejected FcRn binding. However, and unexpectedly, the minimum blood glucose concentration was achieved with free insulin orally administered rather than any of the insulin-loaded nanoparticles.

E. Transferrin Receptor

TfR has been exploited by many researchers for the transcytosis of insulin and other peptide drugs, such as exenatide, across the intestinal mucosa (Zhu et al., 2016; Liu et al., 2018; Zhang et al., 2018b). A limiting factor is the presence of the endogenous ligands ferritin and Tf (Liu et al., 2018). In humans, serum concentration of Tf is approximately 200–300 mg/dL (Kawabata, 2019). Tf has a high binding affinity with TfR and can competitively inhibit the binding of Tf-decorated nanoparticles to TfR and hinder their absorption. Liu et al.

(2018) circumvented this hurdle by developing nanoparticles that targeted the Tf-TfR complex instead of TfR. Trimethyl chitosan (TMC) nanoparticles were conjugated with CRTIGPSVC (CRT), a ligand that binds to the Tf-TfR complex. A control sample was similarly prepared using HAIYPRH (HAI)—a ligand that binds to TfR. Tf-TfR complex targeting via TMC-CRT NPs provided the best hypoglycemic effect in vivo (41.5% blood glucose level reduction at 4 hours of administration) compared with the other orally administered insulin formulations in the study. TMC-CRT NPs displayed the highest bioavailability relative to subcutaneous insulin at 6.84%, whereas TMC-HAI NPs provided a bioavailability of only 3.59%, similar to the undecorated nanoparticles at 3.31%.

In Caco-2 cells where apo-Tf was absent, TMC-HAI NPs exhibited the highest cellular uptake, 1.5 times that of TMC-CRT NPs and the undecorated nanoparticles (Liu et al., 2018). With the addition of apo-Tf and the use of a coculture model of Caco-2/E12 to mimic mucus impediment, the transepithelial transport of TMC-HAI NPs was greatly reduced. Conversely, the cellular internalization of TMC-CRT NPs was 4.3 times higher when apo-Tf was present. The Tf was required to generate the Tf-TfR complex, the target of CRT, and therefore, the presence of Tf increased the effectiveness of TMC-CRT NPs to facilitate cellular insulin uptake. The transport of nanoparticles via TfR is mediated in apical-to-basolateral direction (Lim et al., 2007; Norouziyan et al., 2008; Liu et al., 2018). This study demonstrates that a judiciously selected ligand can reverse the antagonistic effect of endogenous ligands in receptor-mediated cellular uptake of exogenous therapeutic compounds. It is a more commendable approach in achieving therapeutic insulin levels than simply increasing the insulin dose, which translates to increased manufacturing costs and medication expenditure (Heinemann and Jacques, 2009).

F. Intrinsic Factor-Vitamin B₁₂ Receptor

Chalasani et al. (2007b) conjugated VB₁₂ to dextrans (Dex) and epichlorohydrin (Epi) in the form of nanoparticles in an effort to target the IF-VB₁₂ receptor, also known as cubilin. Targeting cubilin produced an enhanced hypoglycemic effect. An insulin dose of 20 IU/kg administered through the VB₁₂-Dex-Epi-NPs (3% w/w insulin loading) produced a bioavailability of 26.5% relative to subcutaneous insulin in diabetic rats, which was significantly greater than the 10.3% achieved using undecorated nanoparticles. The positive outcomes of cubilin targeting were observable in both streptozotocin-induced diabetic rats, used in most studies, as well as in non-obese diabetic mice, a congenital rodent diabetic model (Chalasani et al., 2007a). Cubilin targeting also allowed for relatively low doses of insulin to be administered. VB₁₂-Dex-Epi-NPs were able to produce a relative bioavailability of 26.5% at a dose of 20 IU/kg.

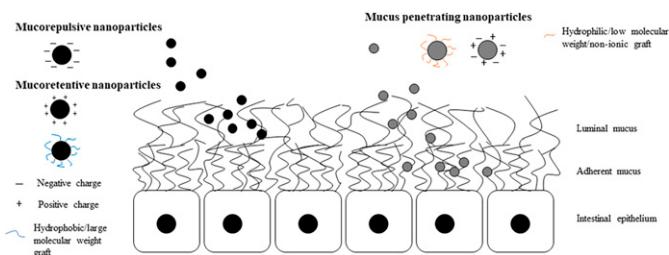


Fig. 2. Design of mucus penetrating, mucoretentive, and mucorepulsive oral insulin nanoparticles.

With the success of VB₁₂ receptor targeting, a recent study explored the vitamin B₇ (biotin) receptor for oral nanoinsulin delivery (Cui et al., 2021). The biotinylated chitosan was synthesized and complexed with insulin followed by hyaluronic acid coat decoration into a virus-mimicking polyelectrolyte nanocomplex. Its relative bioavailability was nonetheless low (<5%) at an oral dose of 50 IU/kg.

G. Proton-Coupled Amino Acid Transporter

Han et al. (2020) encapsulated insulin in a micellar system (DSPE-PCB), which conjugated the zwitterionic betaine polymer polycarboxybetaine (PCB), a PAT1 targeting ligand, with the 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) lipid. The zwitterionic carrier effectively navigated through the mucus and epithelial barriers to improve insulin uptake without inducing significant tight junction opening, which may lead to autoimmune disease, bacterial infection, and inflammatory bowel diseases over long-term applications (Lerner and Matthias, 2015; Han et al., 2020). The DSPE-PCB possessed an extremely low critical micelle concentration and was therefore unlikely to have dissociated into its constituents that can potentially modulate intestinal cell membranes and tight junctions.

The lyophilized insulin-containing DSPE-PCB was packed into a size M porcine gelatin capsule and enteric-coated to protect against the acidic environment of the stomach for oral administration to streptozotocin-induced diabetic mice at an insulin dose of 20 IU/kg (Han et al., 2020). A greater bioavailability was obtained with the DSPE-PCB capsule (42.6%) than betaine-free formulation (8.35%). The DSPE-PCB capsule lowered the rodent blood glucose level by more than fivefold of the control capsule.

H. Niemann-Pick C1-Like Protein 1

Ding et al. (2020) designed NPC1L1-targeting amphiphilic nanoparticles (Hc@CP-Ca), where cholesterol as hydrophobic targeting moiety was conjugated to phosphate groups, chosen for their hydrophilicity and biocompatibility, via hexane linkers and stabilized by calcium ion chelation. In vitro studies using the Caco-2 cell model revealed a significant 31.4-fold increase in insulin uptake with Hc@CP-Ca compared with free

insulin at 1 hour of incubation, indicating the involvement of NPC1L1 and cholesterol-mediated endocytosis. This was further confirmed by a reduction in the insulin uptake efficiency of Hc@CP-Ca when ezetimibe, which blocks NPC1L1, was added. The benefit of surface modification with amphiphilic phospholipid and glycocholate was evident from in vivo studies where a dose of 50 IU/kg of insulin administered to diabetic rats via Hc@CP-Ca achieved a C_{max} of 49.1 μIU/ml at 2 hours of administration, whereas nonsurface decorated nanoparticles displayed a lower C_{max} (32.4 μIU/ml) at 3 hours.

III. Limitations, Challenges, and Future Perspectives

Overall, the in vitro cell culture and in vivo small animal studies suggested that intestinal receptor targeting promotes cellular uptake of oral nanoinsulin and enhances insulin bioavailability and its antihyperglycemic activity. Nonetheless, only a limited number of studies have been conducted for each of the intestinal receptors targeted in oral nanoinsulin delivery research. The physiologic functionality of the target intestinal receptors, the optimal physicochemical designs of the nanoparticles, and the level of specificity of targeting ligands have not been comprehensively understood. All in vivo studies reported to date are conducted in the small animal models, which may have different intestinal receptor profiles from that of the human. An example is the FcRn, which is expressed throughout the human intestine but is only detected in the duodenum of the wild-type mice (Pridgen et al., 2013). The research outcomes derived from the small animal may not be applicable in the clinical setting for humans. In vitro gut models, as a prescreening tool, may have underestimated the level of drug transport in vivo due to lack of peristalsis, fluid flow, microbial flora, and receptor-active cell line (Kim et al., 2012).

With reference to drug release study, the targeting ligand-decorated nanoparticles are found to be characterized with a lower insulin release propensity than undecorated nanoparticles and/or free insulin (Jain et al., 2012; Ding et al., 2020; Tan et al., 2020; Yazdi et al., 2020). A large number of investigations, however, reflect that 20%–40% of the insulin load is released in the gastric phase (Chalasani et al., 2007a; Jain et al., 2012; Wu et al., 2019; Azevedo et al., 2020; Han et al., 2020; Yazdi et al., 2020). The nanoparticles reaching the intestinal region are characterized by lower insulin loads. Even when the intestinal targeting formulation strategies are used, the effective dose of insulin transported across the mucosa may be far from satisfactory. Such complication is further exacerbated by oral nanoinsulin development studies that presented no drug release investigation in the gastric

medium (Pridgen et al., 2013; Fan et al., 2018; Liu et al., 2018).

Given that the oral nanoinsulin has its drug release profiles optimized, the pathophysiological changes of intestinal receptors as a function of diabetes progression may complicate its insulin delivery performance. Annaba et al. (2010) reported that ASBT expression and function were increased following streptozotocin-induced diabetes in rats. The expression of NPC1L1 in diabetic rats was also found to double that of healthy rats (Ding et al., 2020). Thus far, there is no known report investigating the genotype-phenotype relationship of ASBT, FR, HSPG, FcRn, TfR, IF-VB₁₂ receptor, PAT1, and NPC1L1 with reference to the progression of diabetes in humans and the interplay of diabetes type, age, gender, race/ethnic group, and geographical distribution of these receptors. The relationship between oral nanoinsulin delivery and pathophysiological changes of intestinal receptors has yet to be investigated *in vivo* using small animal models, and their clinical implications are not known.

Another risk factor is the long-term effects of oral nanoinsulin administration. Insulin is a growth factor (Chen et al., 2011; Gedawy et al., 2018). Its long-term oral administration at high doses raises concerns of mitogenic changes in the gastrointestinal epithelium. The associated risks and benefits of nanoinsulin, with their inherent requirement for higher oral doses to be administered, will need to be weighed against the current mode of management—that is, subcutaneous insulin administration.

A reduction in oral insulin dose relative to the doses reported in the current literature is generally preferred. To realize this goal, it is imperative to develop an efficient nanoinsulin delivery system capable of achieving high oral insulin bioavailability and minimizing the availability of nonabsorbed or degraded insulin in the gastrointestinal tract. The capability is associated with the physicochemical properties of the nanoinsulin formulations. Table 2 shows the primary physicochemical attributes of oral nanoinsulin that have been developed for intestinal receptor-specific delivery. The uptake of nanoparticles by mammalian cells is broadly governed by their size, shape, surface roughness, crystallinity, and surface chemistry (Oh and Park, 2014; Musalli et al., 2020). The aggregative behavior of nanoparticles may affect their biologic performances at the cellular interface via changes in the primary particulate physicochemical properties in response to physiologic milieu (Shaedi et al., 2021). Currently, there is little consensus on an optimized design for nanoinsulin. The oral nanoinsulin formulations reported in the literature consisted of a wide range of matrix materials of differing polarities (Table 2). In most of these studies, parameters such as particulate surface roughness and shape are not quantified, and the matrix

crystallinity and particle aggregation tendency are not examined. In addition, the oral nanoinsulin bioavailability has not been determined in some studies. Both mice and rats, with expected physiologic and pharmacokinetic variations, are used as the animal model. The summative observations, within a small number of studies, do not allow one to generalize the desired physicochemical attributes of oral nanoinsulin that are possibly favorable in diabetes treatment. Moreover, different intestinal receptors may have affinity for nanoparticles of different size, shape, surface roughness, and crystallinity characteristics, though this has yet to be determined.

Optimization of nanoinsulin design should therefore be target-specific, requiring intestinal receptor-specific studies instead of generalization of findings obtained from investigations involving different intestinal receptors. This complication is further exacerbated by endogenous and exogenous substrates that compete with the targeting ligand of nanoinsulin for the same receptor (Liu et al., 2018). Metformin, a drug that is widely prescribed to treat type 2 diabetes, has been shown to inhibit calcium-dependent vitamin B₁₂ absorption by interfering with intestinal calcium metabolism and uptake (Kibirige and Mwebaze, 2013; Wong et al., 2018). Metformin-induced vitamin B₁₂ deficiency is relatively common in type 2 patients with diabetes (Aroda et al., 2016; Kim et al., 2019). As these patients may also require insulin, the impact of metformin on the intestinal uptake of vitamin B₁₂-conjugated nanoparticles and their blood glucose-lowering performance warrants further investigation.

Thus far, only the DSPE-PLB provides a substantial improvement in oral insulin bioavailability (>40%) (Table 2). In terms of blood glucose reduction, a reduction of more than 70% blood glucose is only attained with DSPE-PLB, VB₁₂-Dex-Epi NP and Hc@CP-Ca NP. The underlying reasons for why some formulations are more successful than others may be complex and possibly due to a lack of nanoinsulin formulation optimization and in-depth knowledge about the population density of intestinal receptors and their physiologic behaviors in association with nanoinsulin delivery.

Alongside efficient binding to the target receptor, the intracellular translocation of nanoinsulin also affects its bioavailability and efficacy. The intracellular lysosomal insulin degradation can be minimized by enabling rapid endosomal escape and exocytosis of the nanodelivery system. Influenza virus-derived hemagglutinin-2 is a pH-responsive membrane fusogenic peptide (Xu et al., 2018). In endosomes, the protonation of the influenza virus-derived hemagglutinin-2 induces a conformational change to α -helices. This enables the peptide to promote endosome-cellular membrane fusion and subsequent exocytosis, a mechanism that was inferred from a recent study on insulin-loaded solid lipid nanoparticles.

TABLE 2
Physicochemical, pharmacokinetics, and pharmacodynamics profiles of intestinal receptor-specific oral nanoinsulin.

Receptor Type	Nanoparticle Design and Preparation Method	Physicochemical Properties						Insulin Loading and Release	Reference
		Size (nm)	Polydispersity Index	ζ Potential (mV)	Shape	Surface Roughness (nm)	Crystallinity (%)		
ASBT	Enteric-coated DNP _s (Coacervation and self-assembly)	217.6 ± 7.9	0.195 ± 0.029	+8.5 ± 1.7	Spherical	ND	ND	Encapsulation efficiency: 72.4 ± 1.6% Loading content: 32.8 ± 0.2% Only a small amount of insulin is released in media of pH 5, 6.8, and 7.4	Fan et al., 2018
	DC-LIP _s (Reverse-phase evaporation)	145.0	0.273	+21.6	Spherical	ND	ND	Encapsulation efficiency: 65% 20% of insulin is released within 2 h of incubation in pH 1.2 medium; 40% of insulin is released in pH 6.8 medium	Wu et al., 2019
FR	Lyophilised FA-PEG-PLGA NPs (Double emulsion solvent evaporation)	258.15 ± 6.56	0.11 ± 0.04	-16.6 ± 1.5	Spherical	ND	ND	Encapsulation efficiency: 87.7 ± 1.9% Loading content: 6.57 ± 0.15% 40% of insulin is released in pH 1.2 medium, with the remaining insulin being released over 22 h thereafter	Jain et al., 2012
	FA-CS NPs (Ionic gelation)	288 ± 5.11	0.211 ± 0.014	+21.90 ± 1.69	Spherical	ND	ND	Encapsulation efficiency: 93.24 ± 2.35% Less than 10% of insulin is released in pH 1.2 medium; less than 40% of insulin is released along with gastrointestinal transit from pH 1.2 for 2 h to pH 6.8 for 2 h and pH 7.4 for 4 h	El Leithy et al., 2019
	PEGylated liposomes with folate (Film hydration and extrusion)	208 ± 2	0.1 ± 0.1	-6.8 ± 7.2	Spherical	ND	ND	Encapsulation efficiency: 66.5 ± 1.3% Loading content: 1% Close to 40% of insulin is released within 2 h in pH 1.2 medium;	Yazdi et al., 2020

(continued)

TABLE 2—Continued

Receptor Type	Nanoparticle Design and Preparation Method	Physicochemical Properties						Reference
		Size (nm)	Polydispersity Index	ζ Potential (mV)	Shape	Surface Roughness (nm)	Crystallinity (%)	
								more than 50% of insulin is released within an hour, and close to 75% of insulin is released beyond 24 h of incubation in pH 6.8 medium
		167 ± 1	0.2 ± 0.3	-4.9 ± 4.3	Spherical	ND	ND	Encapsulation efficiency: 63 ± 2%
								Loading content: 2%
								Close to 40% of insulin is released within 2 h in pH 1.2 medium; more than 50% of insulin is released within an hour, and close to 75% of insulin is released beyond 24 h of incubation in pH 6.8 medium
								Cheng et al., 2021
								Efficiency: 99.9 ± 0% Less than 5% of insulin is released in pH 1.2 medium, and less than 50% of insulin is released along the gastrointestinal transit of 12 h.
								Tan et al., 2020
								Encapsulation efficiency: 81.0 ± 1.9%
								Less than 10% of insulin is envisaged to be released in pH 1.2 medium within 2 h of incubation
								Pridgen et al., 2013
								Efficiency: 87.0 ± 1.9% Loading content: 0.5%
								More than 80% of insulin is released within 5 h of incubation in pH 7.4 medium; no release study is conducted in simulated gastric medium
								(continued)

TABLE 2—Continued

Receptor Type	Nanoparticle Design and Preparation Method	Physicochemical Properties								Reference
		Size (nm)	Polydispersity Index	ζ Potential (mV)	Shape	Surface Roughness (nm)	Crystallinity (%)	Insulin Loading and Release		
PLGA-PEG-albumin NPs (Solvent emulsification-evaporation)	Wild-type albumin	155 ± 7	0.13 ± 0.03	-9.6 ± 0.3	Spherical	Smooth	ND	Encapsulation efficiency: 83.8 ± 6.5% Loading content: 10.9 ± 0.8%	Azevedo et al., 2020	
K573P albumin		154 ± 7	0.14 ± 0.02	-9.3 ± 0.2	Spherical	Smooth	ND	Generally, less than 3% of insulin is released along the gastrointestinal transit Encapsulation efficiency: 83.8 ± 6.5% Loading content: 10.9 ± 0.8%		
TIR	TMC-CRT NPs (Crosslinking)	204.3 ± 12.2	Nil	+23.1 ± 0.3	Spherical	ND	ND	Generally, less than 3% of insulin is released along the gastrointestinal transit Encapsulation efficiency: 83.8 ± 6.5% Loading content: 10.9 ± 0.8%	Liu et al., 2018	
IF-VB ₁₂ receptor	Lyophilised VB ₁₂ -Dex-Epi NPs (Solvent emulsification-evaporation and spray drying)	192	0.81	ND	ND	ND	ND	No insulin-release study is conducted Encapsulation efficiency: 63.49 ± 3.12%	Chalasani et al., 2007b	
PAT1	DSPE-PCB enteric capsule (Insulin/zinc chloride ratio, 2.5:1) (Crosslinking and ultrafiltration)	256	0.83	ND	ND	ND	ND	20%–30% of insulin is released in pH 7.4 medium, with 75%–95% release following 48 h of incubation Encapsulation efficiency: 67.5 ± 4.1% Loading content: 3% 20%–30% of insulin is released in pH 7.4 medium, with 75%–95% release following 48 h of incubation Encapsulation efficiency: > 98% Loading content: 6.10%	Chalasani et al., 2007a	
NPC1L1	Lyophilised Hc@CP-Ca NPs (Solvent emulsification-evaporation and lyophilization)	145.70 ± 3.23	0.14 ± 0.07	-38.17 ± 0.27	Spherical (drug-free nanoparticles)	ND	ND	Close to 30% of insulin is released in pH 1.2 medium; Close to 30% of insulin is released in pH 6.8 medium Encapsulation efficiency: 96.70 ± 0.24% Loading content: 4.44 ± 0.31%	Ding et al., 2020	
								Less than 1% of insulin was released in pH 2 medium; less than 10% of insulin is released in pH 6.8 medium		(continued)

TABLE 2—Continued

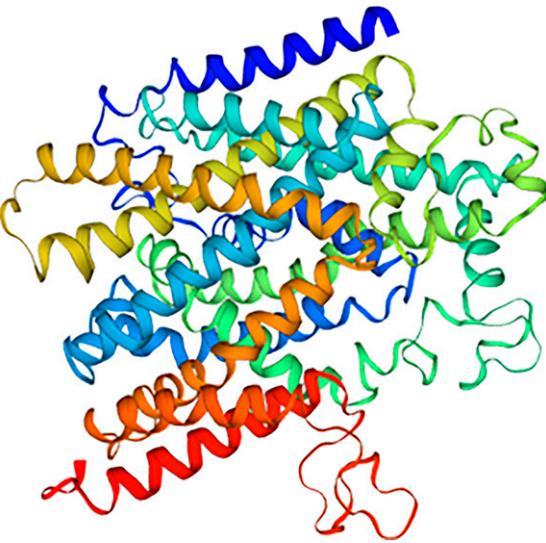
Pharmacokinetics and Pharmacodynamics Profiles

Receptor Type	Nanoparticle Design	Animal Model/Administration Mode	Dose (IU/kg)	Cmax (μIU/mL)	T _{max} (h)	(μIU*h/mL)	Bioavailability (%) ^a	Maximum Blood Glucose-Lowering Response	Reference
ASBT	Enteric-coated DNNPs	Streptozocin-induced diabetic male Sprague Dawley rats Oral administration	30	36.9 ± 11.2	4.0	185.2 ± 32.3	15.9	Reduced by ~55%	Fan et al., 2018
	DC-LIPs	Streptozocin-induced diabetic male Sprague Dawley rats Oral administration	50	48.3 ± 6.7	4	270.7 ± 26.9	16.1	Reduced by ~60%	Wu et al., 2019
FR	FA-PEG-PLGA NPs	Streptozocin-induced diabetic male Sprague Dawley rats Oral administration	50	26.78 ± 3.66	4	296.32 ± 10.75	19.62 ± 1.68	Reduced by 56.52 ± 5.33%	Jain et al., 2012
	FA-CS NPs	Streptozocin-induced diabetic male Albino rats Oral administration	50	31.04 ± 1.11	6	502.67 ± 15.74	17.04 ± 1.34	Reduced by ~55%	El Leithy et al., 2019
	PEGylated liposomes with folate	Diabetic Wistar rats 1% insulin Oral administration loading content 2% insulin loading content	50	60% of subcutaneous insulin 50% of subcutaneous insulin	~4	ND	19.08	Reduced by ~50%	Yazdi et al., 2020
HSPG	Poly(n-butylcyanoacrylate) NPs with hybrid coat of chitosan-FA and hyaluronic acid MSN@PLA-mPEG-CPP	Alloxan-induced diabetic Wistar rats Oral administration	50	ND	ND	ND	ND	Reduced by ~25%	Cheng et al., 2021
FcRn	PLA-PEG-Fc NPs	Streptozocin-induced diabetic male Sprague Dawley rats Intragastric administration	80	ND	ND	ND	ND	Reduced by 51.22 ± 14.70%	Tan et al., 2020
	PLGA-PEG-albumin NPs	Wild-type Balb/c mice Oral administration	1.1	ND	ND	ND	ND	Reduced by ~30%–42.5%	Pridgen et al., 2013
	K573P albumin	Streptozocin-induced diabetic male homozygous human FcRn Tg32 mice Oral administration	50	ND	ND	ND	ND	Reduced by ~38%	Azevedo et al., 2020
TfR	TMC-CRT NPs	Streptozocin-induced diabetic male Sprague Dawley rats Oral administration	50	~30	4	219.2 ± 19.1	6.84	Reduced by ~40%	Liu et al., 2018
IF-VB ₁₂ receptor	Lyophilised VB ₁₂ -DexEpi-NPs	Streptozocin-induced diabetic female Wistar rats Oral administration	20	ND	ND	ND	ND	Reduced by ~41.5%	
		Streptozocin-induced diabetic female Wistar rats Oral administration	20	231	6	ND	ND	Reduced by 70%–75%	Chalasani et al., 2007b
		Female nonobese diabetic mice (NOD/J) Oral administration	20	197	4	ND	ND	Reduced by ~70%	Chalasani et al., 2007a
		Streptozotocin-induced C57BL/6 diabetic mice Oral administration	20	80	~4	ND	42.6	Reduced by ~70%	Han et al., 2020
PAT1	DSPE-PCB-insulin enteric capsule (Insulin:zinc chloride ratio, 2.5:1)	Streptozotocin-induced male Sprague Dawley rats Oral administration	50	49.1 ± 9.7	2.0	193.3 ± 30.0	12.2	Reduced by 75%	Ding et al., 2020
NPCL1	Hc@CP-Ca NPs								

ND, not determined.

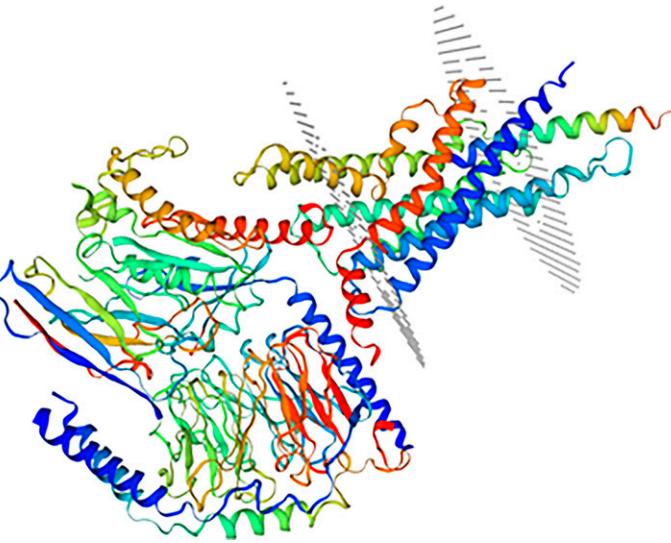
^aSubcutaneous route as control.

TABLE 3
Physicochemical and physiologic characteristics of potential intestinal receptors for oral nanoinsulin targeting

Receptor Type	Characteristics	Conformation	Reference
SGLT-1	<p>SGLT-1 protein consists of 482–718 amino acid residues and is made up of 14 transmembrane α-helices. It is largely located at the brush border membrane of enterocytes, where it absorbs glucose and galactose.</p> <p>Although there have been no reports of SGLT-1-targeted nanoparticle delivery, researchers have successfully formulated glucose-decorated nanoparticles, albeit the studies are aimed at examining the nanoparticles' interactions with different types of receptors.</p> <p>The potential of SGLT-1 as a receptor for oral nanoparticle uptake remains debatable. SGLT-1 works by transporting glucose across an aqueous pore. The lack of an endocytic mechanism may restrict nanoparticle uptake due to constraint of their physical size, ζ potential, and/or morphology with respect to pore diffusion.</p> 		<p>Loike et al., 1996; Wright et al., 1997; Valiela et al., 2014; Bienert et al., 2017; Abohassani et al., 2019</p>

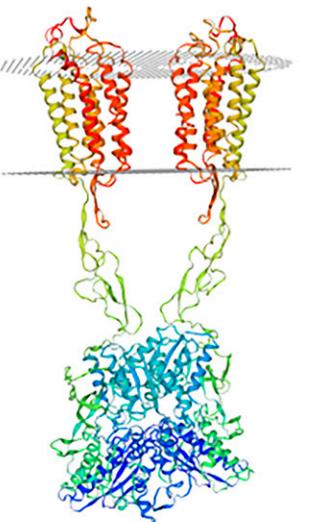
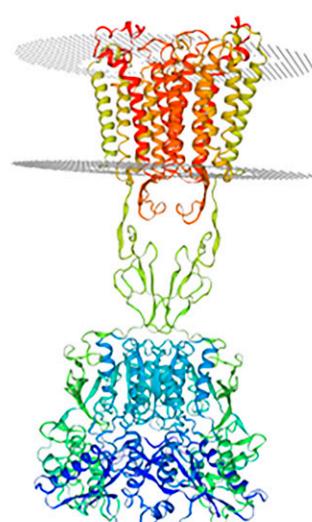
(continued)

TABLE 3—Continued

Receptor Type	Characteristics	Conformation	Reference
Glucagon-like peptide 2 receptor (GLP-2R)	<p>GLP-2R is a 553-amino acid transmembrane protein located in the epithelial region of the human intestine. Together with its natural ligand GLP-2, it regulates intestinal growth and homeostasis.</p> <p>GLP-2, which consists of 33 amino acids, is a peptide derived from proglucagon, which shares high sequence similarity (69.7%) with GLP-1. Clinically, GLP-2R serves as the target for teduglutide, a subcutaneously administered GLP-2 analog, in the treatment of Crohn Disease and short bowel syndrome. There are no reports of GLP-2 agonists administered orally for receptor targeting reported thus far.</p>		<p>Munroe et al., 1999; Lovshin et al., 2000; Bienert et al., 2017; Sun et al., 2020</p>

(continued)

TABLE 3—Continued

Receptor Type	Characteristics	Conformation	Reference
Sweet taste receptors	<p>Sweet taste receptors are heterodimers made up of taste 1 receptor member 2 (T1R2) and taste 1 receptor member 3 (T1R3). They have been found to be expressed in most cells of the body, including the enteroendocrine cells of the GIT such as the enterochromaffin cells, I cells, K cells, and L cells of the small intestine.</p> <p>T1R2 and T1R3 are expressed abundantly in the intestinal mucosa. Sweet taste receptors interact with a multitude of ligands: sugars, such as glucose, fructose, sucrose, and maltose; artificial sweeteners, like saccharin, aspartame, and cyclamate; sweet amino acids, for instance, D-tryptophan, D-phenylalanine, and D-serine; and sweet proteins, for example, monellin, brazzein, and thaumatin. Upon ligand binding, a phosphorylation cascade is activated, resulting in the release of adenosine triphosphate and the activation of adjacent neurons that will forward the taste signals to the brain.</p> <p>Sweet taste receptor was reported as a potential novel therapeutic target for diabetes treatment through regulation of satiety and hormonal release. T1R2 and T1R3 are expressed in enteroendocrine cells throughout the small intestine, where satiety hormones, namely glucagon-like peptide 1 and glucose-dependent insulinotropic peptide, are secreted. Knockout or inhibition of sweet taste receptors in mice was found to block the satiety hormone secretion.</p>	 	Bezencon et al., 2007; Laffitte et al., 2014; Low et al., 2014; Bienert et al., 2017; Lee and Owyang, 2017; Lee et al., 2019

Similar observations were obtained with bile acids and engrailed secretion peptide (Fan et al., 2018; Cohen et al., 2020). Apart from these few reports, the intracellular destiny of the majority of oral nanoinsulin systems as a function of particle composition remains unknown. Advancing delivery science at the subcellular level is therefore necessary to further optimize particle design for nanoinsulin.

Recently, apple-derived nanoparticles containing the fruit microRNA were developed and found to downregulate, via the matrix materials, the mRNA expression of the human intestinal transporter OATP2B1/SLCO2B1, the apical sodium dependent bile acid transporter ASBT/SLC10A2, and the carnitine transporter OCTN2/SLC22A5 in the Caco-2 cells (Arai et al., 2021). This suggests that the composition of nanoparticles, through regulating intestinal transporter expression, may facilitate or hinder cellular uptake of oral nanoinsulin. It is imperative to examine the molecular biology aspects of the oral nanoinsulin systems to enable one to establish the complex relationship between nanoparticle design and cellular trafficking.

Table 3 highlights intestinal receptors with potential for oral nanoinsulin targeting. Among these, the sweet taste receptors hold particularly strong promise. The expression of sweet taste receptors was enhanced in patients with type 2 diabetes (Greenfield and Chisholm, 2013; Young et al., 2013). Although there have been no studies conducted among humans thus far, sweet taste receptors have been reported to upregulate sodium-glucose cotransporter 1 (SGLT-1) in species such as rats and mice (Margolskee et al., 2007; Stearns et al., 2010). Sweet taste receptors appear to exert opposing influences in diabetes control. They promote intestinal glucose absorption (Greenfield and Chisholm, 2013; Young et al., 2013); on the other hand, they induce the satiety hormone release to control the state of hyperglycemia. With reference to oral nanoinsulin delivery, the targeting ligand for sweet taste receptors as such should preferentially be an agonist for satiety hormone release only, apart from being the homing device for oral insulin nanoparticles. Should SGLT-1 be a feasible transport pathway for oral nanoinsulin, dual targeting of sweet taste receptors and SGLT-1 can be an alternative strategy to promote oral nanoinsulin delivery using targeting ligands that stimulate the expression of sweet taste receptor, which in turn leads to the upregulation of SGLT-1 and have binding affinity for both the sweet taste receptor and SGLT-1. A synergistic action between the sweet taste receptor and SGLT-1 in nanoparticle binding is envisaged to lead to enhanced insulin absorption and its bioavailability.

IV. Final Remarks

Receptor targeting has been exploited for oral nanoinsulin delivery to improve oral insulin bioavailability and

blood glucose-lowering response. Despite lower bioavailability when compared with subcutaneous insulin, a more sustained glucose reduction was observed for most of the orally administered nanoinsulin systems examined in this review. Receptor targeting in oral nanoinsulin delivery has not been extensively explored. The relationships of insulin dose, targeting ligand type and content, physicochemical, and molecular biologic characteristics of nanoparticles with in vivo or clinical diabetes responses require further investigation. The designs of future oral nanoinsulin delivery systems should consider the population characteristics of intestinal receptors with the progression of diabetes, albeit the latter is still poorly defined. Additionally, the oral nanoinsulin delivery must effectively overcome both mucus and mucosa barriers. Of the intestinal receptors reviewed in this paper, PAT1 appears to be a promising intestinal target receptor for the transmucosal transport of nanoinsulin, with a bioavailability greater than 40% reported.

Acknowledgment

The authors would like to express their heart-felt thanks to National University of Singapore for facility support.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Choy, Lim, Chan, Cui, Wong.

References

- Abolhasani A, Biria D, Abolhasani H, Zarrabi A, and Komeili T (2019) Investigation of the role of glucose decorated chitosan and PLGA nanoparticles as blocking agents to glucose transporters of tumor cells. *Int J Nanomedicine* **14**:9535–9546.
- Ahad A, Raish M, Bin Jardan YA, Al-Mohizaea AM, and Al-Jenoubi FI (2021) Delivery of insulin via skin route for the management of diabetes mellitus: Approaches for breaching the obstacles. *Pharmaceutics* **13**:100.
- Al-Tabakha MM (2015) Future prospect of insulin inhalation for diabetic patients: The case of Afrezza versus Exubera. *J Control Release* **215**:25–38.
- Altmann SW, Davis JR HR, Zhu LJ, Yao X, Hoos LM, Tetzloff G, Iyer SP, Maguire M, Golovko A, Zeng M, et al. (2004) Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science* **303**:1201–1204.
- Andersen CB, Madsen M, Storm T, Moestrup SK, and Andersen GR (2010) Structural basis for receptor recognition of vitamin-B(12)-intrinsic factor complexes. *Nature* **464**:445–448.
- Annaba F, Ma K, Kumar P, Dudeja AK, Kineman RD, Shneider BL, Saksena S, Gill RK, and Alrefai WA (2010) Ileal apical Na⁺-dependent bile acid transporter ASBT is upregulated in rats with diabetes mellitus induced by low doses of streptozotocin. *Am J Physiol Gastrointest Liver Physiol* **299**:G898–G906.
- Arai M, Komori H, Fujita D, and Tamai I (2021) Uptake pathway of apple-derived nanoparticle by intestinal cells to deliver its cargo. *Pharm Res* **38**:523–530.
- Arbit E and Kidron M (2009) Oral insulin: the rationale for this approach and current developments. *J Diabetes Sci Technol* **3**:562–567.
- Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, Bray GA, Schade DS, Temprosa MG, White NH, et al.; Diabetes Prevention Program Research Group (2016) Long-term metformin use and vitamin B12 deficiency in the diabetes prevention program outcomes study. *J Clin Endocrinol Metab* **101**:1754–1761.
- Ashokkumar B, Mohammed ZM, Vaziri ND, and Said HM (2007) Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells. *Am J Clin Nutr* **86**:159–166.
- Azevedo C, Nilsen J, Grevys A, Nunes R, Andersen JT, and Sarmento B (2020) Engineered albumin-functionalized nanoparticles for improved FcRn binding enhance oral delivery of insulin. *J Control Release* **327**:161–173.
- Bezençon C, le Coutre J, and Damak S (2007) Taste-signaling proteins are coexpressed in solitary intestinal epithelial cells. *Chem Senses* **32**:41–49.
- Bienert S, Waterhouse A, de Beer TA, Tauriello G, Studer G, Bordoli L, and Schwede T (2017) The SWISS-MODEL Repository-new features and functionality. *Nucleic Acids Res* **45** (D1):D313–D319.
- Brambell FW (1963) Resemblances between passive anaphylactic sensitization and transmission of passive immunity. *Nature* **199**:1164–1166.
- Brambell FW, Hemmings WA, and Morris IG (1964) A theoretical model of gamma-globulin catabolism. *Nature* **203**:1352–1354.

- Cárdenas-Bailón F, Osorio-Revilla G, and Gallardo-Velázquez T (2013) Microencapsulation techniques to develop formulations of insulin for oral delivery: a review. *J Microencapsul* **30**:409–424.
- Chalasani KB, Russell-Jones GJ, Jain AK, Diwan PV, and Jain SK (2007a) Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles. *J Control Release* **122**:141–150.
- Chalasani KB, Russell-Jones GJ, Yandrapu SK, Diwan PV, and Jain SK (2007b) A novel vitamin B12-nanosphere conjugate carrier system for peroral delivery of insulin. *J Control Release* **117**:421–429.
- Chen Z, Fei YJ, Anderson CM, Wake KA, Miyauchi S, Huang W, Thwaites DT, and Ganapathy V (2003) Structure, function and immunolocalization of a proton-coupled amino acid transporter (hPAT1) in the human intestinal cell line Caco-2. *J Physiol* **546**:349–361.
- Chen MC, Sonaje K, Chen KJ, and Sung HW (2011) A review of the prospects for polymeric nanoparticle platforms in oral insulin delivery. *Biomaterials* **32**:9826–9838.
- Chen P, Zhao J, and Gregersen H (2012) Up-regulated expression of advanced glycation end-products and their receptor in the small intestine and colon of diabetic rats. *Dig Dis Sci* **57**:48–57.
- Cheng H, Guo S, Cui Z, Zhang X, Huo Y, Guan J, and Mao S (2021) Design of folic acid decorated virus-mimicking nanoparticles for enhanced oral insulin delivery. *Int J Pharm* **596**:120297.
- Christianson HC and Belting M (2014) Heparan sulfate proteoglycan as a cell-surface endocytosis receptor. *Matrix Biol* **35**:51–55.
- Cohen MJ, Chirico WJ, and Lipke PN (2020) Through the back door: Unconventional protein secretion. *Cell Surf* **6**:100045.
- Cui Z, Qin L, Guo S, Cheng H, Zhang X, Guan J, and Mao S (2021) Design of biotin decorated enterocyte targeting muco-inert nanocomplexes for enhanced oral insulin delivery. *Carbohydr Polym* **261**:117873.
- Davies JP, Levy B, and Ioannou YA (2000) Evidence for a Niemann-pick C (NPC) gene family: identification and characterization of NPC1L1. *Genomics* **65**:137–145.
- Dawson PA (2011) Role of the intestinal bile acid transporters in bile acid and drug disposition. *Handb Exp Pharmacol* **201**:169–203.
- Desmoulin SK, Hou Z, Gangjee A, and Matherly LH (2012) The human proton-coupled folate transporter: Biology and therapeutic applications to cancer. *Cancer Biol Ther* **13**:1355–1373.
- Ding Y, Wang Q, Liu G, Feng Y, and Zhou W (2020) Cholesterol moieties as building blocks for assembling nanoparticles to achieve effective oral delivery of insulin. *Biomater Sci* **8**:3979–3993.
- Fan Y-Y, Farrokhi V, Caiazzo T, Wang M, O'Hara DM, and Neubert H (2019) Human FcRn tissue expression profile and half-life in PBMCs. *Biomolecules* **9**:373.
- Fan W, Xia D, Zhu Q, Li X, He S, Zhu C, Guo S, Hovgaard L, Yang M, and Gan Y (2018) Functional nanoparticles exploit the bile acid pathway to overcome multiple barriers of the intestinal epithelium for oral insulin delivery. *Biomaterials* **151**:13–23.
- Gedawy A, Martinez J, Al-Salamy H, and Dass CR (2018) Oral insulin delivery: existing barriers and current counter-strategies. *J Pharm Pharmacol* **70**:197–213.
- Greenfield JR and Chisholm DJ (2013) How sweet it is: intestinal sweet taste receptors in type 2 diabetes. *Diabetes* **62**:3336–3337.
- Han X, Lu Y, Xie J, Zhang E, Zhu H, Du H, Wang K, Song B, Yang C, Shi Y, et al. (2020) Zwitterionic micelles efficiently deliver oral insulin without opening tight junctions. *Nat Nanotechnol* **15**:605–614.
- Harjoh N, Wong TW, and Carmella C (2020) Transdermal insulin delivery with microwave and fatty acids as permeation enhancers. *Int J Pharm* **584**:119416.
- Heinemann L and Jacques Y (2009) Oral insulin and buccal insulin: a critical reappraisal. *J Diabetes Sci Technol* **3**:568–584.
- Hollander PA, Blonde L, Rowe R, Mehta AE, Milburn JL, Hershon KS, Chiasson JL, and Levin SR (2004) Efficacy and safety of inhaled insulin (exubera) compared with subcutaneous insulin therapy in patients with type 2 diabetes: results of a 6-month, randomized, comparative trial. *Diabetes Care* **27**:2356–2362.
- Hou Z and Matherly LH (2014) Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. *Curr Top Membr* **73**:175–204.
- Iwanaga K, Ono S, Narioka K, and Morimoto K (1997) Oral delivery of insulin by using surface coating liposomes: Improvement of stability of insulin in GI tract. *Int J Pharm* **157**:73–80.
- Jain S, Rathi VV, Jain AK, Das M, and Godugu C (2012) Folate-decorated PLGA nanoparticles as a rationally designed vehicle for the oral delivery of insulin. *Nanomedicine (Lond)* **7**:1311–1337.
- Kawabata H (2019) Transferrin and transferrin receptors update. *Free Radic Biol Med* **133**:46–54.
- Ke Z, Guo H, Zhu X, Jin Y, and Huang Y (2015) Efficient Peroral Delivery of Insulin via Vitamin B12 Modified Trimethyl Chitosan Nanoparticles. *J Pharm Pharm Sci* **18**:155–170.
- Kibirige D and Mwebaze R (2013) Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? *J Diabetes Metab Disord* **12**:17.
- Kim J, Ahn CW, Fang S, Lee HS, and Park JS (2019) Association between metformin dose and vitamin B12 deficiency in patients with type 2 diabetes. *Medicine (Baltimore)* **98**:e17918.
- Kim HJ, Huh D, Hamilton G, and Ingber DE (2012) Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* **12**:2165–2174.
- Kozyraki R and Cases O (2013) Vitamin B12 absorption: mammalian physiology and acquired and inherited disorders. *Biochim Biophys Acta* **95**:1002–1007.
- Laffitte A, Neiers F, and Briand L (2014) Functional roles of the sweet taste receptor in oral and extraoral tissues. *Curr Opin Clin Nutr Metab Care* **17**:379–385.
- Lawrence SA, Blankenship R, Brown R, Estwick S, Ellis B, Thangaraju A, and Datta-Mannan A (2021) Influence of FcRn binding properties on the gastrointestinal absorption and exposure profile of Fc molecules. *Bioorg Med Chem* **32**:115942.
- Leach JL, Sedmak DD, Osborne JM, Rahill B, Lairmore MD, and Anderson CL (1996) Isolation from human placenta of the IgG transporter, FcRn, and localization to the syncytiotrophoblast: implications for maternal-fetal antibody transport. *J Immunol* **157**:3317–3322.
- Lee SJ, Depoortere I, and Hatt H (2019) Therapeutic potential of ectopic olfactory and taste receptors. *Nat Rev Drug Discov* **18**:116–138.
- Lee AA and Owyang C (2017) Sugars, sweet taste receptors, and brain responses. *Nutrients* **9**:653.
- El Leithy ES, Abdel-Bar HM, and Ali RA (2019) Folate-chitosan nanoparticles triggered insulin cellular uptake and improved in vivo hypoglycemic activity. *Int J Pharm* **571**:118708.
- Lerner A and Matthias T (2015) Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. *Autoimmun Rev* **14**:479–489.
- Lim C-J, Norouziyan F, and Shen W-C (2007) Accumulation of transferrin in Caco-2 cells: a possible mechanism of intestinal transferrin absorption. *J Control Release* **122**:393–398.
- Liu W, Guo W, Yang M, and Zhang X (2022) Grafted poly (vinyl alcohol) functionalized by folic acid and its transdermal microneedles. *Polym Bull* **79**:867–882.
- Liu M, Wu L, Shan W, Cui Y, and Huang Y (2018) Iron-mimic peptide converts transferrin from foe to friend for orally targeting insulin delivery. *J Mater Chem B Mater Biol Med* **6**:593–601.
- Loike JD, Hickman S, Kuang K, Xu M, Cao L, Vera JC, Silverstein SC, and Fischbarg J (1996) Sodium-glucose cotransporters display sodium- and phlorizin-dependent water permeability. *Am J Physiol* **271**:C1774–C1779.
- Lovshin J, Yusta B, Iliopoulos I, Migirdityan A, Dableh L, Brubaker PL, and Drucker DJ (2000) Ontogeny of the glucagon-like peptide-2 receptor axis in the developing rat intestine. *Endocrinology* **141**:4194–4201.
- Low YQ, Lacy K, and Keast R (2014) The role of sweet taste in satiation and satiety. *Nutrients* **6**:3431–3450.
- Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Illegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, and Shirazi-Beechey SP (2007) TIR3 and gustducin in gut sense sugars to regulate expression of Na⁺-glucose cotransporter 1. *Proc Natl Acad Sci USA* **104**:15075–15080.
- Munroe DG, Gupta AK, Kooshesh F, Vyas TB, Rizkalla G, Wang H, Demchyshyn L, Yang ZJ, Kamboj RK, Chen H, et al. (1999) Prototypic G protein-coupled receptor for the intestinotrophic factor glucagon-like peptide 2. *Proc Natl Acad Sci USA* **96**:1569–1573.
- Musalli AH, Talukdar PD, Roy P, Kumar P, and Wong TW (2020) Folate-induced nanostructural changes of oligochitosan nanoparticles and their fate of cellular internalization by melanoma. *Carbohydr Polym* **244**:116488.
- Mutalik M (2011) Long-awaited dream of oral insulin: Where did we reach? *Asian J Pharm Clin Res* **4**:15–20.
- Norouziyan F, Shen W-C, and Hamm-Alvarez SF (2008) Tyrphostin A8 stimulates a novel trafficking pathway of apically endocytosed transferrin through Rab11-enriched compartments in Caco-2 cells. *Am J Physiol Cell Physiol* **294**:C7–C21.
- Oh N and Park JH (2014) Endocytosis and exocytosis of nanoparticles in mammalian cells. *Int J Nanomedicine* **9** (Suppl 1):51–63.
- Palermo A, Maddalone E, and Pozzilli P (2012) Buccal spray insulin (Oralgen) for type 2 diabetes: what evidence? *Expert Opin Biol Ther* **12**:767–772.
- Pinchevsky Y, Butkov N, Raal FJ, Chirva T, and Rothberg A (2020) Demographic and clinical factors associated with development of type 2 diabetes: A review of the literature. *Int J Gen Med* **13**:121–129.
- Polak D, Sanui T, Nishimura F, and Shapira L (2020) Diabetes as a risk factor for periodontal disease-plausible mechanisms. *Periodontol 2000* **83**:46–58.
- Pridgen EM, Alexis F, Kuo TT, Levy-Nissenbaum E, Karnik R, Blumberg RS, Langer R, and Farokhzad OC (2013) Transepithelial transport of Fc-targeted nanoparticles by the neonatal f_c receptor for oral delivery. *Sci Transl Med* **5**:213ra67.
- Rath T, Baker K, Dumont JA, Peters RT, Jiang H, Qiao SW, Lencer WI, Pierce GF, and Blumberg RS (2015) Fc-fusion proteins and FcRn: structural insights for longer-lasting and more effective therapeutics. *Crit Rev Biotechnol* **35**:235–254.
- Sarrazin S, Lamanna WC, and Esko JD (2011) Heparan sulfate proteoglycans. *Cold Spring Harb Perspect Biol* **3**:a004952.
- Shah H, Zhao D, Tong X, Gregersen H, and Zhao J (2016) Mechanism investigation of the improvement of Chang Run Tong on the colonic remodeling in streptozotocin-induced diabetic rats. *J Diabetes Res* **2016**:1826281.
- Shaedi N, Naharudin I, Choo CY, and Wong TW (2021) Design of oral intestinal-specific alginate-vitexin nanoparticulate system to modulate blood glucose level of diabetic rats. *Carbohydr Polym* **254**:117312.
- Sheng J, He H, Han L, Qin J, Chen S, Ru G, Li R, Yang P, Wang J, and Yang VC (2016) Enhancing insulin oral absorption by using mucoadhesive nanoparticles loaded with LMWP-linked insulin conjugates. *J Control Release* **233**:181–190.
- Simister NE, Story CM, Chen HL, and Hunt JS (1996) An IgG-transporting Fc receptor expressed in the syncytiotrophoblast of human placenta. *Eur J Immunol* **26**:1527–1531.
- Stearns AT, Balakrishnan A, Rhoads DB, and Tavakkolizadeh A (2010) Rapid upregulation of sodium-glucose transporter SGLT1 in response to intestinal sweet taste stimulation. *Ann Surg* **251**:865–871.
- Stöhr J, Barbaresko J, Neuenenschwander M, and Schlesinger S (2021) Bidirectional association between periodontal disease and diabetes mellitus: a systematic review and meta-analysis of cohort studies. *Sci Rep* **11**:13686.
- Suk JS, Xu Q, Kim N, Hanes J, and Ensign LM (2016) PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv Drug Deliv Rev* **99** (Pt A):28–51.
- Sun W, Chen LN, Zhou Q, Zhao LH, Yang D, Zhang H, Cong Z, Shen DD, Zhao F, Zhou F, et al. (2020) A unique hormonal recognition feature of the human glucagon-like peptide-2 receptor. *Cell Res* **30**:1098–1108.

- Tan X, Yin N, Liu Z, Sun R, Gou J, Yin T, Zhang Y, He H, and Tang X (2020) Hydrophilic and electroneutral nanoparticles to overcome mucus trapping and enhance oral delivery of insulin. *Mol Pharm* **17**:3177–3191.
- UniProt Consortium (2021) UniProt: the universal protein knowledgebase in 2021. *Nucleic Acids Res* **49** (D1):D480–D489.
- Valtola L, Rahikkala A, and Raula J (2014) Synthesis and lectin recognition of glycosylated amphiphilic nanoparticles. *Eur Polym J* **59**:282–289.
- Visentini M, Diop-Bove N, Zhao R, and Goldman ID (2014) The intestinal absorption of folates. *Annu Rev Physiol* **76**:251–274.
- Wong CY, Al-Salami H, and Dass CR (2017) Potential of insulin nanoparticle formulations for oral delivery and diabetes treatment. *J Control Release* **264**:247–275.
- Wong CW, Leung CS, Leung CP, and Cheng JN (2018) Association of metformin use with vitamin B₁₂ deficiency in the institutionalized elderly. *Arch Gerontol Geriatr* **79**:57–62.
- Wong CY, Martinez J, and Dass CR (2016) Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities. *J Pharm Pharmacol* **68**:1093–1108.
- Wright EM, Hirsch JR, Loo DD, and Zampighi GA (1997) Regulation of Na+/glucose cotransporters. *J Exp Biol* **200**:287–293.
- Wu S, Bin W, Tu B, Li X, Wang W, Liao S, and Sun C (2019) A delivery system for oral administration of proteins/peptides through bile acid transport channels. *J Pharm Sci* **108**:2143–2152.
- Xiao Y, Tang Z, Wang J, Liu C, Kong N, Farokhzad OC, and Tao W (2020) Oral insulin delivery platforms: strategies to address the biological barriers. *Angew Chem Int Ed Engl* **59**:19787–19795.
- Xu Y, Zheng Y, Wu L, Zhu X, Zhang Z, and Huang Y (2018) Novel solid lipid nanoparticle with endosomal escape function for oral delivery of insulin. *ACS Appl Mater Interfaces* **10**:9315–9324.
- Yamanashi Y, Takada T, and Suzuki H (2007) Niemann-Pick C1-like 1 overexpression facilitates ezetimibe-sensitive cholesterol and beta-sitosterol uptake in CaCo-2 cells. *J Pharmacol Exp Ther* **320**:559–564.
- Yazdi JR, Tafaghodi M, Sadri K, Mashreghi M, Nikpoor AR, Nikoofal-Sahlabadi S, Chamani J, Vakili R, Moosavian SA, and Jaafari MR (2020) Folate targeted PEGylated liposomes for the oral delivery of insulin: In vitro and in vivo studies. *Colloids Surf B Biointerfaces* **194**:111203.
- Young RL, Chia B, Isaacs NJ, Ma J, Khoo J, Wu T, Horowitz M, and Rayner CK (2013) Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes. *Diabetes* **62**:3532–3541.
- Zhang L, Shi Y, Song Y, Sun X, Zhang X, Sun K, and Li Y (2018a) The use of low molecular weight protamine to enhance oral absorption of exenatide. *Int J Pharm* **547**:265–273.
- Zhang L, Shi Y, Song Y, Duan D, Zhang X, Sun K, and Li Y (2018b) Tf ligand-receptor-mediated exenatide-Zn²⁺ complex oral-delivery system for penetration enhancement of exenatide. *J Drug Target* **26**:931–940.
- Zhang Y, Wu M, Tan D, Lin Q, Xia R, Chen M, Liu Y, Xue L, and Lei Y (2021) A dissolving and glucose-responsive insulin-releasing microneedle patch for type 1 diabetes therapy. *J Mater Chem B Mater Biol Med* **9**:648–657.
- Zhang Y, Yu J, Kahkoska AR, Wang J, Buse JB, and Gu Z (2019) Advances in transdermal insulin delivery. *Adv Drug Deliv Rev* **139**:51–70.
- Zhao M, Liao D, and Zhao J (2017) Diabetes-induced mechanophysiological changes in the small intestine and colon. *World J Diabetes* **8**:249–269.
- Zhu X, Wu J, Shan W, Tao W, Zhao L, Lim JM, D'Ortenzio M, Karnik R, Huang Y, Shi J, et al. (2016) Polymeric Nanoparticles amenable to simultaneous installation of exterior targeting and interior therapeutic proteins. *Angew Chem Int Ed Engl* **55**:3309–3312.