Oral delivery of proteins and peptides: Challenges, status quo and future perspectives

Quangang Zhu\textsuperscript{a,\dagger}, Zhongjian Chen\textsuperscript{a,\dagger}, Pijush Kumar Paul\textsuperscript{a,c,\dagger}, Yi Lu\textsuperscript{a,b}, Wei Wu\textsuperscript{a,b,*}, Jianping Qi\textsuperscript{a,b,*}

\textsuperscript{a}Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai 200443, China
\textsuperscript{b}Key Laboratory of Smart Drug Delivery of MOE, School of Pharmacy, Fudan University, Shanghai 201203, China
\textsuperscript{c}Department of Pharmacy, Gono Bishwabidyalay (University), Mirzanagar Savar, Dhaka 1344, Bangladesh

Received 28 November 2020; received in revised form 29 January 2021; accepted 12 February 2021

Abstract Proteins and peptides (PPs) have gradually become more attractive therapeutic molecules than small molecular drugs due to their high selectivity and efficacy, but fewer side effects. Owing to the poor stability and limited permeability through gastrointestinal (GI) tract and epithelia, the therapeutic PPs are usually administered by parenteral route. Given the big demand for oral administration in clinical use, a variety of researches focused on developing new technologies to overcome GI barriers of PPs, such as enteric coating, enzyme inhibitors, permeation enhancers, nanoparticles, as well as intestinal micro-devices. Some new technologies have been developed under clinical trials and even on the market. This review summarizes the history, the physiological barriers and the overcoming approaches, current

**KEY WORDS**
Proteins; Peptides; Oral delivery; Permeation enhancer; Enzyme inhibitor; Stability; Clinical

---

*Corresponding authors.
E-mail addresses: wuwei@shmu.edu.cn (Wei Wu), qijianping@fudan.edu.cn (Jianping Qi).
\dagger These authors made equal contributions to this work.
Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.04.001
clinical and preclinical technologies, and future prospects of oral delivery of PPs.

© 2021 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

With rapid advancement of biotechnology, more and more proteins and peptides (PPs) have been developed for treatment of various diseases. The PPs have become one of alternatives of small molecular drugs because they are highly selective and effective, but low toxicity, which stimulates interests of pharmaceutical industry. The statistical data of Coherent Market Insights revealed the global biologics market was approximately US $255.19 billion in 2019 and was expected to be increasing over the forecast period (2019–2027) with a compound annual growth rate (CAGR) of 7.6%. Similarly, biologics, including nucleotides and PPs, account for nearly 30% of all drugs approved by the U.S. Food and Drug Administration (FDA) between 2015 and 2018. In addition, more than 90% of the recently approved biologics were monoclonal antibodies (mAbs) based drugs.

PPs are constituted of lots of amino acids linked by peptide bonds. Generally, the short chains between two and fifty amino acids are defined as peptides. There are oligopeptides which have less than ten or fifteen amino acids, and polypeptides which have more than fifteen amino acids. It is known as a protein when the chains longer than fifty amino acids. However, there is still controversy with respect to the use of proteins or peptides, for example, mature human insulin with 51 amino acid is confused to define as proteins or peptides. Some references have also regarded the peptides as the smaller proteins with molecular mass less than 9000 Da. Therefore, PPs have large variations in molecular size and structure (Fig. 1). Besides, PPs have big differences in physicochemical characteristics with chemical drugs. Most of PPs are highly hydrophilic, but some cyclic peptides exert hydrophobic properties, such as cyclosporine. Owing to the ionization of amino and carboxyl groups, PPs have isoelectric point (pI) which leads to different charges under different pHs. The largest difference with chemical drugs is that the conformation is able to affect the pharmacological activity of PPs absolutely. Hence, unlike conventional small molecular drugs, it is impossible to develop clinical use of PPs without some sort of sophisticated pharmaceutical technology.

Appropriate administration routes enable not only the therapeutic efficacy of drugs but also patient compliance. However, the administration route of PPs is usually parenteral injection due to their poor oral bioavailability. The long-term continuous injection could pose a big challenge of medication adherence, including pain, aversion to injections, concerns about needle size and local irritation. Consequently, many scientific groups attempted to develop the alternative routes to deliver the PPs, such as oral, nasal, ophthalmic, buccal and transdermal administration, among which oral route is the most attractive alternative due to the higher safety and compliance. Furthermore, the oral route is able to mimic the physiological fate of the endogenous insulin which could achieve better glucose homeostasis than subcutaneous injection. The oral route would enhance the health outcomes for the treatment of certain chronic conditions by improving the living conditions of patients. According to the recent report from allied market research, the global market of oral PPs is expected to grow from US$643 million in 2016 to 8.23 billion in 2028. In addition, academic efforts are also focused on developing some novel technologies to improve the oral absorption of PPs. Since 1995, the number of publication about oral delivery of PPs was increasing exponentially (Fig. 2). However, the commercial products of oral proteins and peptides are very limited to some special peptides, such as Neoral for cyclosporin A and Rybelsus for semaglutide. The main obstacles to develop the oral delivery systems of PPs include the harsh environments of gastrointestinal (GI) tract, large molecular size, high hydrophobicity, and poor transmembrane permeability.

To be honest, there are still numerous excellent reviews about oral delivery of PPs, which however have different viewpoints. For instance, there are lots of reviews focused on oral delivery strategies of peptides, while more reviews focused on how nanoparticles improve the oral delivery of PPs. Some big reviews were written from the biologics which include a large amount of irrelevant contents with PPs. This review aims to offer a comprehensive overview of the developing history of oral delivery of PPs, the major delivery challenges and the strategies of improving oral absorption, the current technologies in clinical and preclinical phases, as well as the future prospects.

2. The history

Although it is really tough to develop the oral delivery systems of PPs, various attempts have ever not ceased since the discovery of insulin. Insulin was discovered by Dr. Frederick Banting and Dr. Charles Best in Canada in 1921 and developed by collaborators in the United States and Europe. Only after one year, the first attempt of oral delivery of insulin was conducted in 1922, which opened prelude to develop oral formulations of PPs. Unfortunately, the results of the first attempt were negative, which makes the critical challenges of oral protein delivery become apparent. Therefore, it is necessary to employ novel delivery technologies to facilitate the oral absorption of PPs. The first paper about oral delivery of insulin was published in 1923, in which alcohol was used to improve the oral absorption of insulin. Edgar A. Ferguson firstly tried to mix anhydroformaldehyde-aniline with insulin as oral absorption enhancer and won the first patent of oral formulation of insulin in 1965. With the deep research of oral insulin, more and more scientists and companies kept eyes on other PPs drugs. In 1990, Sandimmune approved by FDA was the first oral formulation of cyclosporin A which is a cyclic peptide with molecular weight of 1202 and also recognized as the first oral dosage form of peptides though it is usually sorted into poorly soluble drugs. After 5 years, the improved formulation of cyclosporin A, Neoral, was developed by Novartis and approved. Henceforth, self-nanoemulsifying drug delivery systems (SNEDDS) were regarded as an important strategy
for improving oral absorption of drug molecules. However, SNEDDS was not able to increase the oral bioavailability of hydrophilic PPs to large extent. Lots of companies have claimed to develop new delivery technologies to overcome the barriers of oral PPs. Nevertheless, some companies have vanished or been not interested in this field currently, such as AutoImmune, Biosante, Coremed, Coretecs, Eligen, Nobex and Protein Delivery. Five companies are always working on oral insulin for many years and have established some platforms, including Emisphere in USA, Biocon in India, Diabetology in UK, Diasome in USA and Oramed in Israel.

The first oral insulin formulation by Emisphere was allowed to conduct phase I clinical trials by FDA in 2001. In 2006, a 90-day double-blind phase II clinical study in India performed by Emisphere showed no significant differences from placebo. But the pace of development of oral insulin does not stop. In 2014, ORMD-0801 developed by Oramed was approved to perform phase III clinical trials by FDA. Emisphere developed an enhancer, SNAC (sodium N-[8-(2-hydroxybenzoyl)amino]caprylate), for improving oral delivery of insulin, which has been finally used to improve the oral absorption of semaglutide. In 2019, Rybelsus, an oral formulation of semaglutide developed by Novo Nordisk, was approved by FDA for treatment of type II diabetes. Subsequently, the sustained release capsule of octreotide (Mycapssa) developed through transient permeability enhancer (TPE) technology was also approved by FDA in 2020. These two successful oral products of peptides would bring about revolutionary changes in clinical administration of PPs and accelerate the development of oral delivery systems of PPs.

3. The barriers to the oral absorption of PPs

Though the oral delivery of PPs has attracted enormous interests of the pharmaceutical companies and the funding agencies, there are lots of factors impeding the development of oral PPs, such as instability in GI tract, poor permeability across intestinal epithelia and difficulty in development of formulation. The physiological barriers are the major obstacles to hinder the oral absorption of PPs due to the innate nature of GI tract which is not only the major position of food digestion and nutrient uptake but also is the first line defense against toxins and pathogens. Thus, it is necessary to
fully understand the physiological and formulation factors for overcoming barriers of oral delivery of PPs.

3.1. The physiological barriers

After oral administration, drugs suffer from gastric fluids firstly in stomach and then move into small intestine where most of drugs are absorbed. However, it is absolutely different of environments between stomach and intestines, including pH, enzymes, mucus and even epithelial permeability (Fig. 4), all of which influence the stability and absorption of PPs.

3.1.1. pH gradient

The GI pH is absolutely different in each region of GI tract and influenced by various factors including presence of food, pathological conditions, even age and gender. Generally, in the healthy adult, the pH of gastric fluids is acidic (pH 1.5–3.5), and rises to around pH 5–6 in the duodenum due to neutralization of carbonate and bile juices, and then increases to pH 7–8 in the distal jejunum and ileum, while the colonic pH could be more than 8 or drop to pH 6 with high interindividual variability. The age growth is almost no effect on GI pH, which indicates the GI pH around 6 a large extent. For example, the gastric pH was detected to be 5.3 in patients with Crohn’s disease (CD), while the colonic pH was becoming more acidic. The average colonic pH was characterized with big individual variability, but the general trend significantly, such as elevation of gastric pH. In addition, the personal diet custom could be an important reason of individual variability.

3.1.2. Enzymes

PPs are highly susceptible to various proteolytic enzymes including luminal enzymes from gastrointestinal and pancreatic secretions, bacterial enzymes in the colon and mucosal enzymes. They are primarily degraded by luminal enzymes (Table 1) before penetration across mucus. The entry of the protein could stimulate the gastric mucosa to secret pepnys by the cells lining the stomach. Pepsin is able to degrade proteins into smaller fragments of peptides by hydrolyzing the peptide bonds. A great deal of proteolytic enzymes in the upper part of the small intestine are secreted by pancreas, such as trypsin, chymotrypsin, carboxypeptidase and elastase. Moreover, the remaining parts of the proteins are finally digested by various peptidases (e.g., aminopeptidase and dipeptidase) in brush border membrane into tripeptides, dipeptides and respective amino acids that are able to be absorbed into the blood capillaries from epithelium. However, most degradation data of PPs were obtained by in vitro simulated gastric or intestinal fluids with specific enzymes which are hard to be same activity as in vivo condition. For example, the pH 1.2 hydrochloride solution with 0.32% pepsin and the pH 6.8 phosphate buffer with 1% trypsin were often used to evaluate the stability of PPs in vitro. Most proteins can be degraded very fast in simulated gastric fluids (SGF), such as no insulin detected after 30 min incubation with SGF. Through comparison of human or pig GI fluids and simulated GI fluids, Wang et al. found there were good correlation between SGF and both human and pig gastric fluids for the stability of peptides, while the rate of peptide degradation in simulated intestinal fluids (SIF) was more rapid than that in human or pig intestinal fluids. What’s more, there is a very interesting result that only 3 of 17 peptides left in human in intestinal fluids after 30 min incubation. These 3 peptides are respectively cyclosporin (99%), desmopressin (25%) and octreotide (22%) which are all developed as oral therapeutic products named as Neoral, Minirin and Mypacasa. Therefore, it is the one of important prerequisites for successful development of oral PPs to protect the stability of PPs in GI tract.

3.1.3. Mucus

Mucus is a sticky and viscoelastic gel layer covering the entire GI tract. It is secreted by the goblet cells. Mucus can capture the foreign moieties and protect epithelia from the attack of exogenous pathogens. The mucus in whole GI tract is composed of two layers including loosely and firmly adherent mucus layer from

![Figure 3](image-url)
lumen to epithelia (Fig. 5). The thickness of mucus layer varies significantly in different GI regions. Taking rat GI tract as an example, it ranges from 200 μm in upper part to 800 μm in lower part of GI tract. In humans, the thickest mucus layers are also located on the stomach (180 μm) and the colon (110–160 μm). The components of mucus are very complex. The mucin glycoprotein is the major functional constituent and the other components include carbohydrates, proteins, lipids, salts, immunoglobulins, bacteria and cellular remnants. Mucins, including secreted and cell-bound types, are at least twenty subtypes encoded by the MUC genes. There are three secreted mucin types found in the GI tract, such as MUC2, MUC5AC and MUC6. The interactions between mucins make mucus gel layer viscoelastic, however, the viscoelasticity could be influenced by water, lipid or ion content. What’s more, there is a pH gradient across whole mucus layer, especially gastric mucosa. The gastric mucus pH on the luminal surface is about 1–2 which is similar as gastric pH, but increases to neutral pH at the epithelial surface. This pH gradient could be main mechanism to protect gastric epithelial cell against digestion of pepsin.

The mucus exerts multiple barriers against the transport of drugs into the submucosal tissue. The high viscosity decreases the diffusivity of PPs through mucus, which directly affects the residence time of PPs in the small intestine. In the intestine, the average mucus turnover time is around 50–270 min resulting in the removal of trapped particles in the mucus layer thereby, limiting the adhesion and holding time of the particles or PPs.

The continuous secretion and replacement of mucus make it quite challenging for the PPs passing through the unstirred mucus layer by infiltration before reaching the surface of the epithelium. Greater interaction through electrostatic force may exist between mucin and drug particles which may be attributed to the fact that mucin is highly negatively charged due to the glycosylation of serine, and the presence of threonine and proline domain. Moreover, mucins may function as a size-exclusion filter lowering the mobility of large compounds like proteins due to their brush-like scaffold structure. What’s more, structural modification of proteins or entrapment of particles may occur due to the fact that mucin fibers interact non-covalently with proteins or particles via van der Waals and electrostatic forces, hydrogen bonding, and hydrophobic interactions, thus hindering their absorption.

3.1.4. Epithelial barriers

The epithelial cells lying beneath the mucus also act as another predominant restrictions towards oral protein drug delivery. The intestinal epithelia include various types of cells with specific functions, such as enterocytes for absorption, goblet cells for secretion of mucus, paneth cells for secretion of enzymes and M cells for transporting foreign particles. The enterocytes are the major absorptive cells and also comprise around 90% of intestinal epithelium. A continuous monolayer is formed by these polarized epithelial cells, separating the intestinal lumen from the underlying lamina propria. The tight junctions (TJs), found between two neighboring epithelial cells, make the intestinal epithelium impermeable and a gatekeeper for transporting a wide variety of particulates including intestinal antigens and large proteins, and thus recognized as immune cells of intestinal lumen.

The intestinal absorption of drugs is primarily dependent on transepithelial pathway, while paracellular pathway is the main route of some small hydrophilic molecules. According to Lipinski “Rule of 5”, PPs are predicted to be extremely low transepithelial permeability because LogP of PPs is likely to be below −1 that is far lower than 5, and PPs have a great number of hydrogen bond donors or acceptors, and molecular weight is far more than 500 Da. Thus, PPs are hard to be absorbed into portal vein by transepithelial pathway. Moreover, the paracellular route
Table 1  Main digestive enzymes that degrade PPs along with their sites of action.

| Secretion site | Enzyme               | Specificity                           |
|---------------|----------------------|---------------------------------------|
| Stomach       | Pepsin               | Asp, hydrophobic amino acids          |
|               | Trypsin              | Arg, Lys                              |
|               | Chymotrypsin         | Aliphatic amino acids (Phe, Tyr)      |
|               | Carboxypeptidase A   | Aromatic amino acids in C-terminal (Tyr, Phe, Ile, Thr, Glu, His, Ala) |
|               | Carboxypeptidase B   | Arg, Lys in C-terminal                |
|               | Elastase             | Ala, Gly, Ser                         |
| Pancreas      | Aminopeptidase A     | Asp, Glu in N-terminal                |
|               | Aminopeptidase N     | Ala, Leu in N-terminal                |
|               | Aminopeptidase P     | Pro in N-terminal                     |
|               | Aminopeptidase W     | Typ, Tyr, Phe in N-terminal           |
| Small intestine | γ-Glutamyl transpeptidase | γ-Glutamic acid in N-terminal            |
|               | Dipeptidyl peptidase IV | Pro, Ala                |
|               | Peptidylpeptidase A  | His–Leu                              |
|               | Carboxypeptidase M   | Lys, Arg in C-terminal                |
|               | Carboxypeptidase P   | Pro, Gly, Ala in C-terminal           |
|               | γ-Glutamyl carboxypeptidase | γ-Glutamic acid               |
|               | Endopeptidase-24.11  | Hydrophobic amino acids              |
|               | Endopeptidase-24.18  | Aromatic amino acids                 |
|               | Enteropeptidase      | (Asp)α-Lys                           |

refers to the passage of drugs through water-filled pores of TJIs, the pore sizes of which usually range between 3 and 10 Å. The molecules larger than 500 Da are generally not recognized to be able to move through these small pores. TJIs can be regulated by some permeation enhancers, which makes pores larger. However, the width is still less than 20 nm even in fully opened state and the total surface of water filled pores only account for 0.01% of entire intestinal epithelia. Therefore, the oral bioavailability of PPs is still extremely low even though the intestinal permeation enhancers have been added in formulation, such as transient permeability enhancer (TPE) and SNAC. Compared with normal epithelia, lumen antigens, macromolecules and pathogenic particles are transported effectively and rapidly by M cells from the lumen to the underlying gut-associated lymphoid tissue (GALT) via pinocytosis and phagocytosis, which looks a favorable route for oral delivery of PPs. However, the numbers of M cell are very limited in human intestines, accounting for less than 1%. In addition, some endogenous PPs transported by M cells may stimulate the immune responses.

3.1.5. Inter-individual variability

The tremendous inter-individual variability is also a barrier to limit the development of oral PPs. Inter-individual variability in the anatomical and physiological properties of humans and animals is a common sense. For oral delivery, the inter-individual variability in the physiology of GI tract has significantly affected the bioavailability of oral PPs, such as the condition of mucus, the secretion of enzymes and gut motility. Especially in some disease states, the inter-individual variability is more evident. For example, gastric emptying and oesophageal motility have shown large variability in type 2 diabetic patients with different stages. Moreover, the pH and the expression of digestive enzymes in GI tract vary with individuals significantly, which leads to the inter-individual variability of degradation of PPs in GI tract. The relevant transporters potentially contribute to the extent and rate of transmucosal absorption of PPs, but the expression of transporters in the intestinal epithelia is dependent on individual genes. In addition, most of PPs are endogenous substances for regulating the physiological factors, but some physiological factors can also be influenced by other endogenous PPs. For example, glucose level can be regulated by insulin and glucagon simultaneously. The inter-individual variability of glucagon secretion also cause the differential hypoglycemic effect of oral insulin in different patients or animals. Therefore, it is necessary to establish some models to evaluate the inter-individual variability of oral PPs for clinical development, such as physiologically based pharmacokinetics (PBPK) modelling.

3.2. Formulation factors

Except for physiological barriers, formulation is also a great challenge during the development of oral PPs commercial products. The chemical and physical stability of PPs are the most important considerations in formulation development, which aims to enable stability of PPs in manufacturing processes, transportation, storage and administration. There have been some excellent reviews to summarize the formulation factors influencing stability of PPs, especially for parenteral formulations. Unlike small molecular drugs, the major stability of PPs is generally referred to as their conformational integrity which is dominated by hydrophobic interaction, hydrogen bonding and electrostatic interaction. The formulation pH could change the protein’s surface charge, density and distribution, which could cause the alteration of conformation of PPs. Meanwhile, the pH can also influence the colloidal stability of PPs and then changes the rate of protein aggregation and degradation. Ionic strength also affects the physical stability of PPs in solution as pH. For example, the increase of salts could improve the aggregation of proteins due to enhanced hydrophobic interactions. Hence, buffer solutions are usually employed to stabilize PPs in solution formulation. In addition, some excipients are very necessary to be added in formulation to improve solubility or suppress aggregation of proteins, such as arginine, histidine, glycine and so on. Arginine can reduce aggregation of proteins to stabilize the proteins’ structure. The surfactant is also generally used to stabilize proteins through reducing molecular interactions in different
interfaces, such as polysorbate\textsuperscript{97}. Chemical instability of PPs involves in product development, manufacturing and even post-administration. Oxidation is the most common factor inducing instability of PPs with residues of methionine, tryptophan, histidine, cysteine, phenylalanine or tyrosine\textsuperscript{98}, which could be hindered by anti-oxidants including methionine and ascorbic acid. However, enzymatic degradation is the biggest challenge in protecting PPs in GI environments after oral administration as described in Section\textsuperscript{3.1.2}. Most oral formulations are primarily to protect stability of PPs in GI tract against various digestive enzymes, such as enteric coating, encapsulation and enzymatic inhibitors, which will be described in detail in Section\textsuperscript{4.1}. In order to enhance epithelial permeability, some permeation enhancers are added in oral formulations, such as SNAC, bile salts and non-ionic surfactants (Section \textsuperscript{4.3.2}).

Excipients can reduce the molecular interactions between PPs to avoid aggregations, but the interactions between PPs and excipients can also not be ignored. Understanding the protein–excipient interactions is indispensable to better design stable formulations of PPs. There is an excellent review to fully sum up the protein–excipient interactions in liquid formulations including mechanism and characterization\textsuperscript{99}. Owing to the complexity of PPs molecules, multiple interactions involved between PPs and excipients, such as electrostatic interactions, hydrogen bonding, preferential hydration and dispersive forces, which can be characterized by various technologies including Raman spectroscopy, circular dichroism, fluorescence, nuclear magnetic resonance, scanning probe microscopy and electron paramagnetic resonance (EPR) spectroscopy, and some advance numerical analysis methods including principal component analysis (PCA) and empirical phase diagrams (EPD). For example, most of amino acids are able to stabilize proteins in liquid formulation through preferential hydration or direct binding\textsuperscript{100}, while sugars and carbohydrates can stabilize protein in solid state with the combining effect of specific interactions and formation of highly viscous glassy matrices\textsuperscript{101}. In PPs formulations, some polymers and non-ionic surfactants are usually used to increase the stability. The non-ionic surfactants can compete the hydrophobic surface with protein molecules to avoid adsorbing-induced denaturation\textsuperscript{102}. Some polymers are employed to encapsulate PPs for improving the stability or controlling the release. But the hydrophobic domain and charges of polymers can influence the stability of PPs, such as aggregation or adsorption\textsuperscript{103,104}.

4. Current strategies towards enhancement of the oral absorption of PPs

Despite multiple strategies to increase the oral absorption of PPs, the primary principles are based on three aspects including stabilization, mucus penetration or adhesion, and permeation enhancer (Fig. \textsuperscript{6}). These approaches are commonly integrated into one delivery system together.

4.1. Stabilization

Based on physiological and formulation factors, the stability of PPs after oral administration is primarily affected by pH and enzymes in GI tract. In addition, the structure of peptides influences their stability significantly. This section explores the stabilization strategies for oral PPs which have been widely used in formulation development.
4.1.1. pH modulation
The GI enzymes are the main sources to degrade oral PPs, but they need optimal pH to exert their effect. For example, pepsin can cleave multiple proteins or peptides readily in the acidic environment, however, pepsin starts to lose their effect when the pH is over 3\(^{105}\). Therefore, if we can modify the pH of microenvironment to 5, PPs can be protected against degradation in stomach. Nevertheless, enteric coating is generally used to overcome the degradation of PPs in stomach rather than pH modulation due to simpler formulation\(^{106}\). Unfortunately, the proteolytic enzymes in the small intestine are also proficient at degradation of PPs. Similarly, these enzymes are also dependent on pH environment. Luminal proteases, such as trypsin and chymotrypsin, exhibit maximum activity at pH 6.5\(^{107}\). Therefore, adjusting the pH of the intestinal contents has become an efficient approach to protect PPs in intestine. Some organic acids, such as citric acid, have been generally used as pH-lowering agents to inhibit the activity of intestinal enzymes\(^{108}\). It has been proven that co-administration of citric acid and salmon calcitonin (sCT) is able to enhance the oral absorption of sCT in beagle dogs by reducing the activity of pancreatic serine protease trypsin\(^{109}\). In addition, Tarsa Therapeutics (Philadelphia, USA) has successfully completed a phase III trial for oral delivery of sCT (ORACAL\(^{22}\)) which comprises of an enteric coated capsule to bypass the stomach and citric acid to modulate the pH microenvironment in intestine\(^{110}\).

4.1.2. Enzymatic inhibitors
Except for pH modulation, the most important approach for inhibiting enzymes is using enzyme inhibitors. Enzyme inhibitors inactivate the target enzymes by binding to the specific site of the enzyme reversibly or irreversibly\(^{111}\). There are multiple categories of enzyme inhibitors including non-aminic acids, amino acids and modified amino acids, peptides and modified peptides. Many chemical molecules can inhibit the activity of enzymes, such as cholic acids and their derivatives, diisopropyl fluorophosphates\(^{2,112}\). However, these chemical molecules are rarely used due to their high toxicity. Besides, they could be absorbed faster than PPs itself due to low mass, leading to systemic side effects and loss of inhibition capacity. Amino acids and modified amino acids have the same problems as chemical inhibitors\(^{22}\). Hence, peptides and modified peptides derived enzymatic inhibitors have been extensively studied, such as aprinotin inhibiting trypsin and chymotrypsin and soybean trypsin inhibiting pancreatic endopeptidases. However, it is noteworthy that long duration administration of such enzymatic inhibitors could result in the deficiency of these enzymes in humans. The chicken and duck ovomucoids are recently developed and recognized as safer. They can efficiently inhibit the activity of α-chymotrypsin and trypsin and offer 100% protection for insulin\(^{113}\).

The enzymatic inhibitors have been extensively used in clinically developing products. For instance, soybean trypsin inhibitor and chelating agent which is a cofactor for many proteases have been used in ORMD-0801 (developed by Oramed) as a formulation component for oral delivery of insulin\(^{114}\). The Chronotropic™ platform technologies developed by Dexcel Pharma Technologies, Ltd. (Jerusalem, Israel) combine the protease inhibitor (camostat mesilate) and absorption enhancer (sodium glycocholate) to improve the oral bioavailability of insulin\(^{115}\). However, we have to consider their toxicities which are caused by high concentration and long duration.

4.1.3. Enteric coating and colon-specific delivery
The enteric coating can prevent the drug release in stomach but permit the drug release in the small intestine due to the dissolution of coating materials in higher pH\(^{116}\). Thus, the enteric coating is able to protect PPs against degradation by low pH and pepsin in stomach completely. Some pH-responsive polymers are usually used to coat the tablet, capsule or even micro-/nano-particles.

---

**Figure 6** Flow chart of the general considerations in enhancement of oral bioavailability of PPs. There are various technologies based on three rationales including stabilization, absorption enhancement and mucus-related technologies.
Polyacrylic polymers have been widely used for enteric coating and shown to release insulin at different rates and different pH, such as Eudragit S100 or L100. Most of oral PPs products have adopted enteric coating technology to bypass the stomach, such as enteric coating capsule for oral insulin by Oramed (ORMD 0801) and Diabetology (Capsulin™ OAD). However, the oral bioavailability of PPs can’t be improved significantly if only enteric coating is used because there are still a great amount of digestive enzymes in intestine. Therefore, enteric coating is usually employed to improve the oral absorption of PPs by combination with protease inhibitors or permeation technologies. Nanoparticles coated by enteric materials have shown significant enhanced effect for oral absorption of PPs. The relative bioavailability of insulin was found to be approximately 20% after oral administration of enteric coated capsules filled with chitosan/poly(γ-glutamic acid) nanoparticles. In addition, pH-responsive polymers can fabricate nanoparticles directly with other polymers which can enhance the permeability for oral delivery of PPs. Nanoparticles composed of hydroxypropyl cellulose phthalate (HPMCP) and chitosan increased the hypoglycemic effect of insulin by more than 9.8 and 2.8-fold as compared to oral insulin solution and chitosan nanoparticles without enteric materials.

The colon acts as a more suitable absorption site for PPs compared to stomach and small intestine due to its decrease of enzyme activity and neutral pH value. Moreover, longer residence time and higher responsiveness to absorption enhancers make colon as ideal administration site of oral PPs. There are a number of examples to develop colon targeted delivery systems for PPs, such as vasopressin, insulin, calcitonin, glucagon and so on. However, proper care must be taken to enable the release of the PPs at the target site. Among the various pH responsive polymers, Eudragit® enteric release polymers have been extensively exploited for oral delivery of PPs. It was reported that the oral bioavailability of insulin was enhanced by 1.73-fold via Eudragit S100®-coated chitosan nanoparticles loaded with insulin and trans-activating transcriptional peptide (TAT), compared to nanoparticles without enteric coating. Other carbohydrate polymers that are used to specifically deliver oral PPs to the colon include anionic carboxymethyl starch, cationic quaternary ammonium starch, gelan gum, retrograded starch and pectin etc. However, there are some challenges hindering the colon-specific delivery systems of PPs including lower surface area and tight junctions in colonic absorption site. What’s more, alteration of enzymatic activity induced by some certain colonic diseases could affect drug release or stability.

4.1.4. Micro/nano-encapsulation

Therapeutic drugs or PPs can be protected from hydrolytic and enzymatic degradation in the harsh gastric milieu of the GI tract via encapsulation, by which a drug or protein of interest is encased inside polymeric carriers, so as to improving their intestinal absorption. Particles can also enhance transport across epithelia except for protecting PPs against degradation. Therefore, the therapeutic PPs could be encapsulated in nanoparticles to improve the blood concentration after oral administration, while vaccines encapsulated in microparticles can be taken up by Peyer’s patches for enhancing mucosal immunity. The particle transport is also affected by the stability in GI tract, surface properties, morphology and specific ligands. Both natural and synthetic materials can be used to encapsulate PPs, such as natural materials including chitosan, dextran, alginate, hyaluronic acid, and lipidic materials, and synthetic polymers including poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), polycaprolacton (PCL), and so on. The generally recognized as safe (GRAS) ingredients are highly recommended to prepare edible micro/nano-particles. So far, nanoparticles have been extensively developed for oral macromolecular drug delivery, such as polymeric nanoparticles, lipid nanoparticles, liposomes, nano-emulsions and inorganic nanoparticles, which were described in detail in Section 4.3.8.

4.1.5. PEGylation and peptide cyclization

The stability of PPs can be also improved by chemical approaches, such as PEGylation and peptide cyclization. PEGylation is generally used to reduce plasma clearance rate by increase the stability of PPs in the systemic circulation. Several injectable PEGylated proteins have been launched to the market, such as growth hormone antagonist (Somavert®, Pfizer, USA), erythropoietin (Mircera®, Roche, Switzerland), and anti-TNF-α Fab (Cimzia® UCB, Belgium). Similarly, PEGylation can increase pH and thermal stability of PPs, and also resistance to intestinal proteolytic digestion. In addition, the branched chain PEGs demonstrate better than the linear PEGs. However, it is important to realize that PEGylation could lead to risk of different efficacy and side effect profiles with parent protein.

Cyclization makes peptide non-susceptible to enzymes by removing exposed N and C terminal from peptide molecules. It is inspired by many natural small cyclic proteins, such as cyclosporine and desmopressin. Desmopressin which is a cyclic analogue of vasopressin displays greater resistance to enzymatic degradation than vasopressin. Ring closure of a peptide can be attained by four different ways: head-to-tail (C-terminus to N-terminus), head-to-side chain, side-chain-to-tail or side chain-to-side chain, depending on its functional groups. The typical example is Arg-Gly-Asp (RGD) which is highly susceptible to chemical degradation due to presence of aspartic acid residue in its structure, while the rigidity can prevent the Asp side chain carboxylic acid from positioning itself in the right position for attack on the peptide backbone after cyclization. Furthermore, cyclization can also decrease the exposure of polar atoms to surroundings by folding peptides into bioactive conformations, leading to the increase of oral bioavailability.

4.2. Mucus penetrating and mucoadhesive systems

As mentioned before, mucus lining along the intestinal membrane of the GIT serves major hurdle for protein absorption by presenting multiple barriers. However, mucus is a double-edge sword in design of drug delivery systems. There are two opposing approaches to improve the delivery efficiency, including mucus-penetrating and muco-adhesive systems. Mucus penetrating systems are able to pass through the unstirred layer rapidly to reach intestinal epithelium for absorption. In contrast, muco-adhesive systems can prolong drug residence time for absorption at the intestinal tract by avoiding mucociliary clearance. There have been lots of excellent reviews to clarify the mucus-penetrating and mucoadhesive systems.

4.2.1. Mucus penetrating systems

For mucus-penetrating systems, the mucolytics have been firstly used to disrupt mucus barrier. The mucolytics are generally used to remove abnormal mucus in pulmonary disease, such as chronic
obstructive pulmonary disease (COPD), while able to diminish the mucus barrier transiently for healthy mucosa\textsuperscript{146}. For example, N-acetyl-L-cysteine (NAC) is a commonly used mucolytic and can cause a 6-fold increase in the absorption of 3.2 µm polystyrene particles in Peyer’s patches\textsuperscript{147}. Although mucolytics can facilitate the attachment of particles to intestinal absorptive cells by removing mucus covering surface of epithelium and further enhance the oral absorption, the depletion of mucus barrier could lead to the injury of intestinal epithelium due to direct contact with proteolytic enzymes and acid. Therefore, it is necessary to employ the particles with specific properties to penetrate through mucus for drug delivery.

Inspiring from viruses, scientists deduced some possible characteristics of mucus-penetrating particles, including small size, highly hydrophilic and net-neutral surfaces\textsuperscript{154,165}. A study has demonstrated that polymeric particle less than 230 nm could pass through mouse colorectal mucus rapidly\textsuperscript{149}, which is similar as the size of some viruses. In order to increase the hydrophilicity of particle surface, the particles are commonly modified by PEG to enhance mucus penetration\textsuperscript{150}. In addition to PEG, poly vinyl alcohol (PVA)\textsuperscript{151} and N-(2-hydroxypropyl)methacrylamide copolymer (pHPMA)\textsuperscript{152} can also engineer the mucus-inert particles to improve the oral absorption. The nanocomplex of insulin and cell penetrating peptide (CPP) demonstrated no evident hypoglycemic effect after oral administration to diabetic rats, while the blood glucose level can decline to around 50% by nano-complex coated by pHPMA. Meanwhile, nanocomplex coated by pHPMA exhibited 20-fold higher transport than free insulin on mucus-secreting epithelium cells\textsuperscript{152}. Recently, protein corona liposomes are also able to facilitate the penetration of mucus and transepithelial transport\textsuperscript{153}.

Another important factor influencing the mucus-penetrating is surface charge of nanoparticles. Both positive and negative charge are not good for mucus-penetrating, but nanoparticles with densely charges coated net-neutral surfaces which is like virus surface exhibited higher diffusion through mucus layer. A biomimetic virus-like or charge reversible nanoparticles are able to improve the oral insulin delivery by overcoming mucus barriers\textsuperscript{154}. In addition, particle geometry can affect the mucus-penetrating ability significantly by micromovement\textsuperscript{155}. It has been revealed that the nanorods diffused across mucus layer rapidly by rotation\textsuperscript{156}. Owing to the strong mucus-penetrating capacity, the rod shaped nanoparticles can penetrate into deep mucus and reside there to prolong the residence time in GI tract\textsuperscript{150}. What’s more, SNEDDS produces droplets ($\leq$50 nm) with hydrophilic surfaces and their shape deformability facilitates them suitable for diffusion through mucus\textsuperscript{157}. Better mucus diffusion was achieved by medium chain lipids (MC)-SNEDDS compared to lipids with short or long chains. For example, MC-SNEDDS produced 2-fold increase of oral bioavailability of enoxaparin\textsuperscript{158}.

### 4.2.2. Mucoadhesive systems

Mucoadhesion is a common phenomenon for particles, which was found by Florey in 1962 from India ink particles adhering intestinal mucus\textsuperscript{159}. Most of microparticles or nanoparticles exerted non-specific mucoadhesion with intestinal mucus. However, mucoadhesive polymers have to be used for improving residence time significantly. For example, mucoadhesive microspheres with a diameter of 680–850 µm fabricated by copolymers of fumaric acid and sebacic acid were able to significantly prolong retention time in the rat GI tract compared to that of non-adhesive polymers\textsuperscript{160}. The hydrophobicity, surface charge and chemistry influence the mucoadhesive properties of polymers significantly. The hydrophobic particles were absorbed 100-fold more than particles composed of hydrophilic cellulose\textsuperscript{161}, which indicated that hydrophobic interactions was also an important aspect for mucoadhesion. Due to the negative charge of mucus layer, the positively charged particles are strongly mucoadhesive. Chitosan, especially N-trimethyl chitosan (TMC), was commonly used to engineer or coat nanoparticles for improving the drug absorption through electrostatic mucoadhesive with mucins. TMC nanoparticles produced much higher antibody titers of IgG and secretory IgA after oral delivery of urease than the solution by increasing mucoadhesion and epithelial permeability\textsuperscript{162}. Thiolation on the surface of polymer is a common strategy to increase the mucoadhesive ability owing to formation of disulfide between thiol group of polymer or cysteine-rich subdomains of mucus glycoproteins\textsuperscript{163}. The mucoadhesive properties of polymers could be enhanced by up to 100-fold after thiolation\textsuperscript{164}. TMC nanoparticles modified by cysteine increased insulin transport by 1.7–2.6-fold compared to TMC nanoparticles\textsuperscript{155}. Compared with the mucoadhesive polymers, the another category of molecules exert the bioadhesion on epithelial cells rather than mucus gel layer, such as lectins. They are able to specifically recognize receptor-like structures of the cell membrane and therefore bind directly to epithelium and hence called as the second generation of bioadhesives\textsuperscript{146}. The lectin modified nanoparticles are able to not only bind to intestinal epithelium for prolonging the residence time but also probably triggering the active transport by receptor mediated uptake. Many studies employed lectin modified nanoparticles to target M cell for enhancing transport of large molecules\textsuperscript{167,168}. The absorption enhancement of lectin was described in Section 4.3.3, in detail.

In addition to mucoadhesive micro-/nano-particles, intestinal patches have also been attempted to improve the oral delivery of PPs\textsuperscript{169,170}. Intestinal patches are like transdermal patches and millimeter sized patches composed of a pH sensitive layer, mucoadhesive drug reservoir layer and a backing layer\textsuperscript{171}, which are suitable to deliver PPs orally because they can release PPs locally near the mucosa and protect it from proteolytic degradation\textsuperscript{172,173}. Insulin-loaded intestinal patches can significantly reduce the blood glucose level at the dose of 10 IU/kg after jejunal administration. The author attributed it that the intestinal micro-patches can be put into enteric capsules and strongly adhesive to the intestinal mucosa after entering small intestine (Fig. 7), which also facilitate oral absorption of insulin significantly\textsuperscript{171}. Similar as transdermal patches, intestinal patches can load various formulations to modify the drug loading, release or absorption, such as solid-in-oil formulation as a drug reservoir in intestinal patch for oral delivery of insulin\textsuperscript{175}.

Hydrogels have also been extensively explored for enhancing oral absorption of PPs\textsuperscript{177}. Hydrogels can enable PPs reside within specific gut regions for a prolonged residence time due to their mucoadhesive properties and resist enzymatic degradation simultaneously. Complexation hydrogels are the optimal choice for oral delivery of PPs due to their environment responsiveness. For example, complexation hydrogels composed of polymethacrylic acid) grafted with poly(ethylene glycol) do not swell in acidic environment due to strong hydrogen bonds and hence prevent insulin release in stomach, while dissociate in small intestine, resulting in rapid swelling and release\textsuperscript{178}. The complexation hydrogels led to a drastic reduction of plasma calcium concentration by improving intestinal absorption of sCT. Moreover, the oral bioavailability of insulin-loaded complexation
hydrogels reached up to 7.2%.\textsuperscript{179} In addition, superporous hydrogels have also been used for enhancing intestinal absorption of PPs\textsuperscript{180}. They can swell to several hundred times within a few minutes and exhibit enhanced mucoadhesive force\textsuperscript{181}. Oral administration of insulin-loaded superporous hydrogels leads to notable insulin absorption and hypoglycemic effect, which could attribute the prolonged residence time in high concentration of insulin within specific intestinal region and reversible opening of tight junctions\textsuperscript{182}.

4.3. Absorption enhancement

In addition to instability in GI tract, another important factor limiting oral bioavailability of PPs is their extremely poor permeability across epithelial membrane due to large molecular weight and high hydrophilicity. It is indispensable to enhance the intestinal permeability of PPs by chemical or pharmaceutical approaches for development of oral products. So far, there are various strategies to enhance oral absorption of PPs, among which absorption enhancers could be the most commonly used in clinical or preclinical products.

4.3.1. Prodrugs

The prodrugs strategy is the most common approach to modulate physicochemical properties of drugs via chemical derivatization, such as improving stability, solubility or permeability. The prodrugs molecules are able to overcome barriers and then converted to be active form by \textit{in vivo} degradation reactions\textsuperscript{11}. The bio-reversible cyclization of peptide backbone has been recognized as a promising prodrug methodology for PPs, which increases the intramolecular hydrogen bonding interactions, but decreases the intermolecular hydrogen bonding interactions with aqueous solvent\textsuperscript{183}. Borchardt et al.\textsuperscript{184} used this method to develop the phylpropionic acid based cyclic prodrugs of (Leu\textsubscript{5})-enkephaline which have shown around 1680 folds higher permeability across Caco-2 cell monolayer than parent peptide. In the same study, coumarinic acid-based prodrugs of (Leu\textsubscript{5})-enkephaline exhibited both high permeability and good stability. Lipidization is another promising approach to create prodrugs of PPs. Lipidization can increase hydrophobicity of peptides, leading to improved permeability. For example, two palmitoylated insulins lipidized by B1-monopalmitoyl and B29-dipalmityl showed higher lipophilicity and greater stability, which leads to increased intestinal absorption\textsuperscript{185}. However, lipidization could reduce the biological activity of a peptide, which have been overcome by a reversible lipidization technique\textsuperscript{186}. This method can be carried out in an aqueous solution for conjugation of fatty acid and polypeptide, and can regenerate the original active polypeptides after oral absorption\textsuperscript{187}. The oral absorption of reversible lipidic prodrugs of salmon calcitonin was improved by at least 19 times compared to parent peptide\textsuperscript{188}. In addition, the prodrug design combining with lipid raft can generate site specific delivery by conjugation with targeting moiety. The combination of lipid and receptor targeting exhibited synergistic effect, leading to rapid transport through the cell membrane, which could be an alternative technology for enhancing absorption of hydrophilic biomacromolecules including PPs\textsuperscript{189}. However, the prodrug strategy is currently limited in modification of peptides. Proteins are hard to optimize their characteristics by chemical modification due to huge molecule, and conformational instability during chemical reaction.

4.3.2. Absorption enhancers

The largest obstruct for oral delivery of PPs is poor permeability across intestinal epithelium. The absorption enhancers are recognized to improve the intestinal permeability by altering the epithelial structure transiently, therefore extensively used in oral formulations of PPs. The possible mechanisms involved in current absorption enhancers are shown as Fig. 8. There are over 250 substances that have been used in preclinical studies as absorption enhancers for oral delivery of PPs according to an excellent review about intestinal permeation enhancers\textsuperscript{21}, some of typical which have been listed in Table 2. Absorption enhancers have attracted more attention from pharmaceutical and biomaterial scientists since 1961 when a study found that sodium ethylene diamine tetraacetic acid (EDTA) was able to improve the oral absorption of heparin at dose of 50 mg/kg in dogs\textsuperscript{190}. Some semi-synthetic and synthetic substances have been developed as absorption enhancers including chelating agents, surfactants, polymers and bacterial toxins. These absorption enhancers can facilitate oral absorption of PPs either paracellularly \textit{via} the opening of tight junctions or transcellularly through increasing membrane permeability, or a combination of both. Chelating agents, like EDTA and citric acid, can generally enhance paracellular absorption by opening tight junctions which

\textbf{Figure 7}  Schematic illustration of structure of intestinal patch and administration device, and \textit{in vivo} mechanism of adhesion, drug release and absorption across intestinal epithelium. Reprinted with the permission from Ref. 176. Copyright \textcopyright 2016 Springer.
is caused by reduction of intracellular calcium due to chelating properties\textsuperscript{196}. Diethylamine triamine pentaacetic acid (DTPA), a novel chelator, has been approved to improve the oral insulin absorption with relative bioavailability of 20\% by integrating into chitosan nanoparticles\textsuperscript{134,111}.

Surfactants are main absorption enhancers in clinical studies, such as sodium caprylate/caprate and their derivatives, and endogenous bile salts. Endogenous bile salts and their derivatives have been investigated to increase oral relative bioavailability of insulin by protecting stability of PPs and enhancing intestinal permeability\textsuperscript{201,192,193}. The advantages of endogenous bile salts and their derivatives include good biocompatibility and high drug loading for PPs when they are used in fabrication of liposomes\textsuperscript{194}. Sodium caprylate/caprate and their derivatives are the most promising absorption enhancers and have been marketed for oral delivery of PPs, such as sodium caprylate in oral octreotide (Mycappssa\textsuperscript{165}, Chsia Pharma, USA/Israel) and SNAC in oral semaglutide (Rybelsus\textsuperscript{166}, Novo Nordisk, Denmark). The SNAC was firstly approved using in Eliigen\textsuperscript{167} carrier for improving oral delivery of vitamin B\textsubscript{12} developed by Emisphere (USA). It is a derivative of sodium caprylate whose structure and mechanism of action are presented in Fig. 9. It was reported that SNAC were capable of enhancing permeation of heparin, sCT and insulin significantly\textsuperscript{195-197}. Most of studies regarded hydrophobic SNAC non-covalently associated with peptides improves their absorption across the intestinal epithelium. After transported, the peptide disassociated from the SNAC carrier and passed into the circulation freely\textsuperscript{198}. The success of Rybelsus\textsuperscript{166} is related to its strong association with semaglutide\textsuperscript{199}. However, some studies also thought the SNAC enhanced transport of peptides through opening tight junctions because they caused significant decline of TEER and a 36-fold increase in mannitol permeability across Caco-2 monolayers\textsuperscript{200}.

Chitosan and its derivatives are the most common polymers for enhancing oral delivery of PPs depending on the positive charge density and bioadhesive ability. They are generally fabricated as nano/micro particles to encapsulate PPs for improving oral absorption, which described in Section 4.4 in detail.

Some absorption enhancers emerging from toxins have gradually been used in improving oral delivery of PPs by altering paracellular or transcellular permeability\textsuperscript{102}. Due to safety consideration of native toxin, the common approach is that the short peptide sequence is developed by structure activity relationships (SAR) studies. For example, native ZoT (45 kDa) can enhance small intestine permeability via PKC-dependent cytoskeletal contraction which is exerted by its first six amino acids\textsuperscript{203,204}. Alba Therapeutics (USA) developed a short peptide sequence AT1002 (H-FICGRL-OH) which can lead to 40-fold increment of lucifer yellow in Caco-2 monolayer\textsuperscript{205}. The larazotide acetate, an 8-mer peptide that promotes tight junctions assembly, has been used in clinical development by Alba therapeutics (USA)\textsuperscript{206}. In addition, some short peptides emerging from toxins could target tight junctions related proteins, such as claudins or occludins, to increase paracellular permeability\textsuperscript{207,208}. CPP are a sort of peptides derived from the transactivator of transcription (HIV-1 TAT) protein of the human immunodeficiency virus and can increase the membrane permeability of PPs\textsuperscript{209}. The first CPP, Penetratin\textsuperscript{210} ((RQIKIWFQNRRMKWKK) consisting of 16 amino acids, was discovered in 1994\textsuperscript{210}. The therapeutic PPs are linked with the CPPs by chemical conjugation or complexed with the CPPs by non-covalent bonds\textsuperscript{211}. The possible mechanism of CPPs on enhancing cellular uptake is that they can increase paracellular and transcellular transport through endocytic pathway\textsuperscript{212}. The low molecular weight protamine (LMWP) with a sequence of V5RRRRRROGRRRRC is the one of CPPs, which can increase the intestinal cell membrane permeability and oral relative bioavailability of exenatide-Zn\textsuperscript{2+} by 29-fold\textsuperscript{113}. Both Penetratin\textsuperscript{210} and its analog PenetraMax\textsuperscript{216} exerted absorption enhanced ability for oral insulin in D-form by non-covalent approach; but there is no synergistic effect observed when using the combination of these two CPPs\textsuperscript{214}. Although CPPs have exerted excellent capacity in improving membrane permeability, they have not yet been validated in the clinic studies of oral delivery of peptides due to complex GI environment.

Absorption enhancers have been evidenced in improving oral absorption of PPs, even some enhancers have been used in marketed products, such as SNAC and EDTA. However, safety and regulation are the main concerns in the application of absorption enhancers in oral delivery. Toxicity has been considered as a potential drawback impeding the application of enhancers\textsuperscript{215}. Fortunately, there have no significant adverse events reported for any absorption enhancers tested in clinical trials to date.

### 4.3.3. Active targeting

Increasing active transport has also become a promising approach to facilitate the oral absorption of PPs by targeting receptors, transporters and specialized cells in intestinal epithelia\textsuperscript{216}. The colloidal carriers decorated with a specific ligand (Table \textsuperscript{314,216-226}) have emerged as a promising technology to increase interaction with the epithelium by active targeting followed by higher transport.

Receptor-mediated endocytosis can take up extracellular substances efficiently by internalization triggered by binding ligand molecules with receptors, which is a critical pathway to acquire sufficient essential nutrients for human body, such as vitamins, transferrin and hormones\textsuperscript{229}. Therefore, some nutritional components including vitamins, saccharides and fatty acids have been extensively explored to decorate drug carriers for enhancing active transport. Some nutritional vitamins have to be transported by receptor mediated mechanisms from diet and other exogenous sources, such as vitamin B\textsubscript{12} (VB\textsubscript{12}) and folate. VB\textsubscript{12} was used as a ligand to modify dextran-g-poly-ethylenoxide cetyl ether micelles for oral delivery of cyclosporine A, which demonstrated increased permeability of cyclosporine A on Caco-2 monolayer. Moreover, VB\textsubscript{12} modified nanoparticles loading insulin produced 70\%–75\% blood glucose reductions\textsuperscript{218}. However, the limited absorption site of VB\textsubscript{12} in the distal ileum leads to slow uptake\textsuperscript{219}, compromising its potential application. Folate and biotin are also the aqueous vitamin B family members and there are a large number of receptors in whole intestinal tract. A folic acid (FA)-pluronic 85-poly(lactide-co-glycolide) polymersome exhibited higher cellular uptake than unmodified polymersome and showed better enhanced absorption effect of insulin. The folate receptor mediated endocytosis pathway was also validated by cellular uptake mechanisms study\textsuperscript{211}. Insulin loaded liposomes modified by biotin showed significantly higher hypoglycemic effect with almost 2-fold relative bioavailability compared to the conventional liposomes\textsuperscript{217}. Like vitamins, there are a variety of saccharide receptors located on the intestinal epithelia for active transport, such as mannose, galactose and hyaluronic acid receptors\textsuperscript{222}. For example, the galactose-modified nanoparticles exhibited higher cellular uptake and \textit{ex vivo} intestinal epithelial permeability compared with galactose free nanoparticles\textsuperscript{223}. However, most saccharide receptors locate in M cells, by which oral delivery of vaccines can be enhanced\textsuperscript{224}. Transferrin receptors (TfR)
has recently become a promising target for oral delivery of PPs because they are distributed throughout the small intestinal epithelium\textsuperscript{235}. In a recent study, Yong et al.\textsuperscript{236} found that nanoparticles modified by transferrin (Tf) were taken up by Caco-2 cells via TfR-mediated transcytosis more than the unmodified counterparts significantly. However, the increased number of endogenous Tf reduces the specificity of Tf-functionalized nanocarriers\textsuperscript{237}. To address this issue, Liu et al. developed a nanosystem modified by cycle peptide CRTIGPSVC (CRT) to target the Tf–TfR complex for circumventing the competitive inhibition, which significantly increased the Caco-2 cellular uptake via a non-canonical allosteric directed mechanism\textsuperscript{238}. The Fc receptor (FcRn) is the most promising ligand candidate to actively transport biomacromolecules into circulation from small intestines due to its high efficiency in

| Enhancer               | Mechanism         | Application                          |
|------------------------|-------------------|--------------------------------------|
| EDTA                   | Chelating agents; paracellular | ORMD-0801; ORMD-0901 (Oramed Pharma, USA) |
| Citric acid            | Chelating agents; paracellular | Peptelligence™ (Tarsa, USA)             |
| Bile salts             | Multimodal        | IN-105 (Biocon, India)                |
| Sodium caprate (C\textsubscript{10}) | Multimodal   | GIPET\textsuperscript{®} (Merrion Pharma, Ireland) |
| Sodium caprylate (C\textsubscript{8}) | Multimodal | TPE\textsuperscript{®} (Chiasma, Israel) |
| SNAC/5-CNAC            | Transcellular     | Eligen\textsuperscript{®} (Emisphere, USA) |
| Chitosan               | Multimodal        | Oral insulin (NanoMega, USA)          |
| Penetratin/PenetraMax  | Transcellular     | Reported for various peptides         |
transporting immunoglobulin G antibodies across epithelial barriers. FcRn targeted nanoparticles were able to increase a mean absorption efficiency up to approximately 13-fold and oral insulin-loaded FcRn targeted nanoparticles could lead to similar hypoglycemic effect as s.c. insulin at the same dose. More recently, Martins et al. developed porous silica nanoparticles conjugated with Fc fragment of immunoglobulin G by microfluidics technology which exerted higher cytocompatibility and greater interaction with the intestinal cells, as well as ensued better absorption of glucagon-like peptide 1 (GLP-1).

Transporters located on the epithelia surface can selectively transport some specific molecules into the cytoplasm but rarely cause cell membrane active deformation to engulf the particles unlike receptor mediated endocytosis. Therefore, most transporters are used for improving oral bioavailability of small molecular drugs through development of prodrugs, but rarely for biomacromolecules. For example, the prodrug of zanamivir with amino acid groups increased intestinal jejunal permeation by transportation of amino acid transporter. However, some studies also demonstrated ligand modified nanoparticles were also transported by transporters. For example, the nanoparticles functionalized with deoxycholic acid are able to overcome multiple obstacles and enhance oral absorption of insulin by targeting the apical sodium-dependent bile acid transporter (ASBT). Moreover, the insulin-loaded butyrate-PEG nanoparticles produced approximately 3.0-fold improvement of relative pharmacological bioavailability of oral insulin by targeting monocarboxylate transporter 1 (MCT-1) compared to the unmodified nanoparticles.

Recently, targeting specialized cells in intestinal epithelia attracted lots of interests as an approach of improving oral delivery, such as M cells in peyer’s patches, goblet cells and some immune cells. M cells are the most common target cell for oral drug delivery of antigens or proteins due to their special physiological functions. Particles in intestinal gut can be transported by M cells very fast from apical side to basolateral side and then captured by immune cells in “dome trap” or further enter into lymphatic vessels. There are a variety of receptors expressed on the surface of M cells for targeting, such as intercellular adhesion molecule (ICAM)-1, L-fucose, β3 integrin and glycoprotein 2 (GP2). Lectins are the most common used ligand binding reversibly to receptors of M cells, such as wheat germ agglutinin (WGA) and ulex europaeus agglutinin 1 (UEA1).
conjugated microparticles and lipid nanoparticles loading insulin resulted in larger glucose level reduction and increment of residence time at intestinal membrane\(^{106,248}\). The tripeptide RGD are extensively used for enhancing the transport of nanoparticles across M cells through targeting \(\beta 1\) integrin\(^{235}\). Besides, some new ligands for targeting M cells were obtained by the phage display technique, such as CKS9\(^{249}\). Goblet cells, a mucus secretion cells, are rarely used to be as target sites for oral delivery. However, recent studies started to pay attention to drug delivery system based on targeting goblet cells. Nanoparticles modified with a peptide of CSKSSDYQC (CSK) which can target to goblet cells can facilitate the uptake in villi and higher internalization via calthin and caveolae mediated endocytosis on HT29-MTX cells (goblet cell like model)\(^{250}\). Moreover, this nanoparticles loading insulin showed 1.5-fold improvement of relative bioavailability compared to the unmodified ones\(^{228}\). Besides, targeting dendritic cells (DCs) located on apical side of intestinal epithelia has been attempted to improve delivery of vaccines. Several DCs targeting peptides have been validated to increase oral delivery efficiency of antigen and enhance immunization, such as DC-pep that was screened out by phage display\(^{251,252}\).

Although active targeting is able to increase the uptake in specific intestinal cell group, the insufficient absorption area limits the absorption extent of PPs, which is difficult to increase the oral bioavailability to a large extent. More targets which distribute more extensively in intestinal epithelia need to be explored for oral delivery.

### 4.3.4. Lymphatic transport

Lymphatic route is also an important way for oral absorption. There are various accesses to lymph depending on characteristic of drugs (Fig. 10). After transported across intestinal epithelia, small hydrophilic drug molecules or macromolecules that are smaller than 10 nm (or 16–20 kDa for proteins) are transported primarily into the blood capillaries\(^{253,254}\). Nevertheless, the highly lipophilic drugs could be assembled into chylomicron with lipoproteins and subsequently transported into lymph. Particles or macromolecules (antigens or proteins) that are larger than 10 nm are hard to enter into blood capillaries due to small interstitial space of blood capillaries, but able to drain into lymph vessels. However, particles larger than 100 nm are poorly transported into lymph due to reduced diffusion and convection through the interstitium\(^{255,256}\). Both lymphoid (Peyer’s patches) and non-lymphoid tissue (villous) in intestinal lumen contribute to the lymphatic transport. Transferring into lymph vessels via non-lymphoid tissue depends upon the lipid pathway, vehicle effects, sieving mechanisms of the blood vessels and the application site. The proximal small intestine is the best lymphatic transport site, while the presence of lymphatic transport has also been proven in rectal administration. M cell in Peyer’s patches can take up intestinal particles by phagocytosis and complete transcytosis very fast, which is the main route for highly potent compounds such as lymphokines and antigens. Some excellent reviews have showed various approaches for improving oral lymphatic delivery\(^{257,258}\).

In order to increase absorption of lipid pathway, some lipid formulations were used to mimic the absorption process of dietary fats for improving oral lymphatic transport of PPs. For example, insulin-loaded solid lipid nanoparticles (SLNs) demonstrated significant drug accumulation within intestinal lymphatic system\(^{259}\). In addition, lipidization of peptides via chemical modification with fatty acids is an important approach to increase the lymphatic transport by improving association with chylomicrons, which have been applied in oral delivery of several peptides, such as sCT, encephalin, tetragastrin and insulin\(^{260}\). However, the flow rate of lymph through the intestinal lymphatic system is approximately 500-fold slower than that of blood through intestinal blood capillaries and portal vein, which leads to not sufficient quantity absorbed in systemic circulation for therapeutics\(^{262}\). Therefore, it is very limited to elevate oral bioavailability of therapeutic PPs by targeting lymphatic systems. So far, there are no clinical and commercial products developed by lymphatic targeting technology. However, M cells route has been regarded as an effective pathway to oral deliver vaccine and protein therapeutics. The glucan microparticles incorporating with thermosensitive poloxamer 407 gel improved the oral absorption of insulin, hence producing mild reduction in blood glucose level for over 20 h in diabetic rats\(^{263}\). Meanwhile, the lymphatic transportation is highly correlated with pharmacological bioavailability, which indicates the lymphatic route plays critical role in oral absorption of insulin\(^{264}\). The M cell transport is likely related to the physicochemical characteristics of the particles, such as physical and chemical stability, size, surface charge, shape and elasticity. For example, the polystyrene particles with a range of 50 nm and 3 \(\mu\)m have 6%–34% absorption ratios after oral administration\(^{266}\). But particles larger than 10 \(\mu\)m are rarely able to be transported by M cell\(^{266}\). Meanwhile, the targeting ligands may influence the adhesive to M cell or uptake of M cell significantly, such as lectins and RGD peptides, which has been described in section 4.3.3 in detail. However, the GALT comprises less than 10% of whole intestinal epithelial surface, which limits the absorption extent of therapeutic PPs. In addition, the particles could be captured in dome trap after transcytosis by M cells to inhibit the entry of therapeutic PPs into systemic circulation via lymph vessels\(^{63}\). Due to high potency of vaccines, M cell uptake of particulate oral vaccines demonstrated promising potential in clinical trials. The PLGA microspheres containing the Escherichia coli colonization factor antigen II as potential vaccine for enterotoxigenic E. coli can generate antibody responses in 5 out of 10 human subjects\(^{267}\). The PLGA microspheres encapsulating CS6 antigen also demonstrated effective vaccination in phase I clinical trials\(^{268}\). Nevertheless, these clinical trials have not been continued because of variability in immune response generation. Except for the immunology issues, the formulation design may be important hurdles for antigen-loaded particles, including stability of antigen, and release in intestinal lumen or Peyer’s patches.

### 4.3.5. Ionic liquids

Ionic liquids (ILs) are a category of ionic compounds with melting point below 100 °C, while are called as room temperature ILs (RTILs) if the melting point declines to room temperature\(^{269}\). ILs have been extensively used in chemical engineering as solvents, catalysts, reagents and so on. ILs have good capacity for solubilizing poorly soluble drugs\(^{270}\) and strong permeation enhanced ability for biomacromolecules\(^{271,272}\). However, most of ILs in chemistry are not biocompatible for drug delivery or other biological use\(^{271}\). Recently, a class of RTILs based on natural components were developed for improving drug delivery, such as choline and organic acids\(^{274}\). They demonstrated good biocompatibility and permeation enhanced capacity. They are firstly employed to improve transdermal delivery of PPs, such as insulin
and bovine serum albumin (BSA)\textsuperscript{275}. Recent study indicated that choline and geranate (CAGE) ILs were able to enhance oral absorption of insulin and insulin-loaded CAGE ILs (3–10 IU/kg) produced a significant hypoglycemic effect after intrajejunal administration or oral intake of enteric capsules\textsuperscript{276}. Meanwhile, insulin can maintain conformational and chemical stability in CAGE ILs. ILs could form a self-assembled nanostructure with gastrointestinal fluids spontaneously, which probably contribute to oral absorption or biodistribution in vivo\textsuperscript{277}. Angsantikul et al.\textsuperscript{278} found that choline and glycolate ILs were also able to reduce the viscosity of the intestinal mucus and enhance the paracellular transport. Therefore, they can effectively deliver TNFα antibodies into the intestinal mucosa as well as systemic circulation. ILs also act as permeation enhancer for biomacromolecules in other mucosal barrier, such as nasal delivery\textsuperscript{279}. ILs can also combine with other formulations as a permeation enhancer. For example, Peng et al.\textsuperscript{280} fabricated a mucoadhesive ionic liquid gel patches that can improve the oral transport of insulin. However, the interaction between ILs and water has to be highly valued because water is the most common

Figure 10  The schematic illustration of the intestinal lymphatic transport of chemical drugs or antigens (proteins) after oral administration. (A) Dietary lipids and some highly lipophilic drugs are taken up by enterocytes and then assembled as chylomicron with lipoproteins to be drained into mesenteric lymph. (B) Soluble antigens (proteins) access the mesenteric lymphatics directly or via phagocytosis by dendritic cells after transport by various routes including paracellular diffusion (①), uptake into endosome and then exocytosis by exosomes (②), transcytosis by enterocytes (③), transport by M cells (④) or dendritic cells (⑤). (C) Particulate antigens (proteins) are primarily transported by M cells and then processed by a large amount of immune cells under subepithelial dome. Reprinted with the permission from Ref. \textsuperscript{259}. Copyright © 2016 Nature.
substances in biological body. It is not clear whether water can attenuate the effect of ILs or not.381

4.3.6. Intestinal microneedles
Microneedle-based technology has been broadly used in transdermal delivery in pharmaceutical and cosmetics products. Microneedles are able to overcome the main barriers hindering drug absorption, such as stratum corneum in transdermal delivery.382 In addition, microneedles can penetrate the physical barrier to improve drug penetration but bring about no damage to the tissue or nerves by tuning the needle length to appropriate size. Therefore, microneedle is a pain-free administration technology.383 Recently, microneedles have gradually used in other mucosal delivery routes, such as ocular, oral and vaginal.384

Mucosal and epithelial barriers are the main factors influencing the oral absorption of PPs. Traverso et al.385 firstly demonstrated proof-of-concept experiments in swine that microneedles were capable to completely overcome the gastrointestinal mucosa and epithelia, and promote oral bioavailability of a biologically active molecule. (Fig. 11A-C). This device was 2 cm in length and 1 cm in diameter, and the microneedles were made of metals. The drug can be loaded in hollow or solid microneedles for release (Fig. 11D). In spite of good safety and tolerability of this device indicating in experimental period, the biocompatibility is still a significant concern if it would be developed to be a clinical product. In order to avoid the toxicity caused by metals, biodegradable or dissolvable microneedles are fabricated for biomedical use. Abramson et al.386 employed polymers to fabricate a unfolding microneedle injector (LUMI) (Fig. 11E). This injector is composed of three flexible arms, each of which has a 0.5 cm² microneedle patch on the far end. The arms are initially bundled together and then the injector is filled in a capsule. The LUMI arms unfold outward after the injector is pushed out of capsule when the capsule reaches the intestine via intragastric administration. Then the arms press the microneedle patches against the intestinal wall to penetrate the epithelia barriers. A company (Rani Therapeutics, San Jose, CA, USA) is developing a related technology to deliver oral biologics, which has been studied in clinical trials.387 However, future studies have to be determined whether the microneedle cause distension of small intestine. In addition, the small damage of intestine caused by microneedle could bring about large risk of systemic infection due to presence of a large amount of microorganisms in intestines.

4.3.7. Self-orienting millimeter-scale applicator (SOMA)
In order to efficiently deliver the biomacromolecules by oral routes, various administration devices were attempted. A self-orienting millimeter scale applicator (SOMA) was inspired by the leopard tortoise’s ability to passively reorient (Fig. 12) and could deliver biologic drugs by penetrating gastric mucosa rather than intestinal gut.388 The thickness of stomach’s wall is approximately 4- to 6-mm which provides more space to design the length of needles for safety and efficacy. The hypoglycemic effect of insulin delivered by SOMA is similar as that of sc insulin. However, this oral device is intrinsically an injectable administration and the difference from the common injection is only injection site. Moreover, the injection in GI tract could cause larger risk than sc or intramuscular injection. Recently, a microjet vaccination system was developed to avoid the use of needles. It is a three-dimensional microelectromechanical systems-based drug delivery technology and can produce a high-pressure liquid jet of vaccine to penetrate the buccal mucosal layer.389

4.3.8. Nanocarrier-facilitated oral delivery of PPs
The largest barriers in oral delivery of PPs are mainly enzymatic degradation and poor permeability across intestinal epithelium. Almost most of strategies are based on overcoming these two barriers to improve oral bioavailability of PPs. Nanocarriers can entrap the active biomacromolecules into the matrix or the core to protect against enzyme degradation through GI lumen. In addition, nanocarriers can be taken up by epithelial cells or M cells in Peyer’s patches. Nanocarriers are also able to entrap penetration enhancers together with PPs or be decorated by ligands to further enhance the oral absorption. Hence, various nanocarrier systems have been extensively investigated in oral delivery of biologics including PPs, vaccines and nucleic acids. According to materials of nanocarriers, there are primarily three categories of nanocarriers, such as polymeric, lipid-based and inorganic nanoparticles, some of which have demonstrated good delivery effect for PPs by oral routes (Table 4).386,120,217,219,225,290-311.

4.3.8.1. Polymeric nanoparticles
Polymeric nanoparticles have been widely explored to circumvent multiple barriers hindering oral delivery of PPs. Except for protecting PPs from degradation in harsh GI environments, polymeric nanoparticles can increase the epithelial transport by improving cellular uptake and inhibiting efflux of P-gp.312 In addition, some polymers can reversibly open tight junctions, allowing transport of PPs through paracellular pathway, such as chitosan and its derivatives.313 The active transport through enterocytes, goblet and M cells can be enhanced by decorating ligands on the surface of nanoparticles.314 Polymeric nanoparticles are generally composed of synthetic, semi-synthetic or natural polymers with diameter ranging from 10 to 1000 nm. Natural polymers are abundantly present in nature and have good compatibility, hence, have gained extensive interests in oral delivery of PPs, such as chitosan, gelatin, alginate and hyaluronic acid. Chitosan is the most common materials as nanocarrier delivery system for PPs due to its mucoadhesive and permeation enhancement characteristics. In addition, chitosan is also easily modified to achieve various aims, such as pH-responsive release, increasing hydrophilicity and positive charges or improving mucoadhesive ability.314-316 A pH-responsive nanoparticles developed by conjugating chitosan and poly-γ-glutamic acid (PGA) can enhance the paracellular transport of insulin by opening the tight junctions between adjacent cells.317 Chitosan/gelatine nanoparticles can increase pharmacological availability of insulin to 6.8% and 3.4% for the 50 and 100 IU/kg dose respectively.318 The N-trimethyl chitosan (TMC) can significantly enhance the permeability of peptides or proteins in comparison of normal chitosan,319 hence, has been used as permeation enhancer to coat other nanocarriers or engineer nanocarriers together with other materials.320 Recently, chitosan oligomers were found to reduce the coulombic repulsion between anionic calcitonin and negatively charged intestinal epithelial cells, which facilitates the oral absorption of calcitonin.321 In terms of synthetic polymers, the PLGA, PLLA, PLA and polyacrylic acid (PAA) are frequently studied for PPs delivery due to good biocompatibility. These polymeric nanoparticles exhibited better capacity to protect against GI digestion, but it is necessary to combine with other permeation enhancers for improving intestinal permeability of PPs.323 However, lack of self-regulating release of insulin is still a big issue for oral delivery. Recently, Paul et al.324,325 developed a biomimetic imprinted nanoparticles to increase self-regulating adhesion and release of insulin by molecular imprinting technique. The imprinted
nanoparticles significantly prolonged hypoglycemic effect for up to 24 h and increased insulin transport via transcellular pathway. Yet, the oral bioavailability of PPs delivered by polymeric nanoparticles is still very limited and not sufficient for clinical therapy. Meanwhile, long-term exposure of these nanoparticles in GI tract is still a concern.

4.3.8.2. Lipid-based nanocarriers. Lipid-based nanocarriers (LBNs) are composed of natural lipids or phospholipids to form emulsion, solid particles or vesicles. Due to high biocompatibility, LBNs have received much attention as oral delivery systems. However, LBNs are difficult to entrap hydrophilic macromolecules with high efficiency, which is a primary hurdle to be developed for oral delivery of PPs. In addition, LBNs can be degraded by lipolysis in GI tract, leading to poor protecting ability for entrapped PPs. Therefore, SLNs appear to be better than emulsions or liposomes. Nevertheless, nanoemulsions and liposomes have also exerted certain superiority for oral delivery of some PPs by regulating formulation.

The nanoemulsions are generally used to incorporate lipophilic drugs into the oil droplets for improving oral absorption. Although the incorporation of hydrophilic PPs in the internal phase of o/w nanoemulsions is difficult to achieve, some poorly water soluble peptides have been successfully developed to be oral products by SNEDDS, such as Sandimmum Neoral for cyclosporine A. In order to effectively trap hydrophilic PPs in nanoemulsions, the w/o nanoemulsion was employed for oral delivery of PPs. The PPs can be completely encapsulated in the core of w/o nanoemulsions.

Figure 11  Schematic illustration of the rationale of orally administered microneedles. (A) Computer-aided design of the radial prototype; (B) The produced microdevice with metal endcap and pin; (C) Radiography of the microneedle; (D) Therapeutic use concept of both hollow and solid microneedles; (E) The concept of oral delivery of PPs via microneedle patch. Reprinted with the permission from Refs. 286 and 288. Copyright © 2015 Elsevier and 2019 Nature.
to protect against degradation in the harsh GI environments. A insulin-loaded w/o nanoemulsions with the diameter of 161.7 ± 24.7 nm enhanced oral bioavailability of insulin by 10-fold compared with plain insulin solution. Moreover, the w/o microemulsion exhibited more significant effect in oral delivery of other proteins, such as earthworm fibrinolytic enzyme which are increased to 208-fold higher bioavailability than that of control solution by intraduodenal administration. However, the w/o nanoemulsions definitely occur phase conversion due to a large amount of water in GI tract, which could cause the leakage of encapsulated PPs. Therefore, Li et al. employed chitosan and alginate to coat the nanoemulsion to avoid fast leakage of insulin from the core of nanoemulsion.

Liposomes are simulated natural cell membrane structure and have been successfully used in delivery of anticancer drugs to decrease the toxicity, such as Doxil for doxorubicin. Likewise, multiple remarkable advantages of liposomes received much interests in oral drug delivery, such as good biocompatibility, flexible encapsulation and tunable characteristics. Liposomes have been attempted to be investigated in oral drug delivery of insulin as early as the late 1970s. Unfortunately, the results are always indistinct. For example, a study indicated that oral insulin-loaded liposomes were able to exhibit hypoglycemic effect in only 54% of the normal rats and 67% of the diabetic rabbits, which could be ascribed to poor stability of conventional liposomes in GI tract. Recent modification technologies facilitate the development of liposomes in oral delivery by addition of polymer coating and modulating liposomal compositions. Liposomes containing bile salts have revealed better stability in GI tract than conventional liposomes by avoiding the destructive effect of physiological bile salts. In addition, bile salts, such as sodium glycocholate (SGC), sodium taurocholate (STC) and sodium deoxycholate (SDC), are capable of inhibiting the activity of GI enzymes including pepsin, trypsin and α-chymotrypsin. Moreover, liposomes containing bile salts can improve the trans-enterocytic internalization compared with conventional liposomes, which could be an important mechanism for enhanced oral absorption of PPs. Besides, cholesterols in conventional liposomes replaced by other sterols (i.e., ergosterol) is also a promising strategy for improving stability and transmembrane ability of liposomes in GI tract. Liposomes containing bile salts, also named as bilosomes, have been widely used in the fields of vaccine delivery. The general sense of bilosomes refers to bilayer vesicles fabricated by surfactants with the incorporation of bile salts which is to stabilize the vesicles in GI tract by preventing membrane destabilization. For example, bilosomes constructed from monopalmitoylglycerol, cholesterol, dicetyl phosphate and SDC showed promoted effect in reduction of viral cell load in an influenza challenge study by increasing uptake within the Peyer’s patches.

Lipid nanoparticles are composed of solid lipids or mixtures of solid and liquid lipids with a diameter size smaller than 1000 nm, including SLNs and nanostructured lipid carriers (NLCs). Due to highly hydrophilic nature of PPs, they are poorly encapsulated in the matrix of SLNs and could distributed in the water phase or interface between oil and water owing to the presence of surfactants. In addition, the drug expulsion usually occurs in SLNs after polymorphic transition during storage. NLCs can improve the loading capacity and avoid drug expulsion due to the involvement of liquid lipids compared with SLNs. Since the mid 1990’s, many groups have explored the encapsulation of various PPs in lipid nanoparticles through optimization of formulation and preparation, such as BSA, insulin, sCT, LHRH and protein antigens. Although entrapment efficiency can be improved significantly, the loading capacity for most of PPs is still not more than 10%. Sarmento et al. engineered an insulin-loaded SLNs with entrapment efficiency of over 43% demonstrated a considerable hypoglycemic effect during 24 h after oral administration to diabetic rats. Various surface modification technologies were employed to further increase the delivery efficiency of SLNs, such as chitosan coating, lectin and octaarginine modification. In addition, surface charge of lipid nanoparticles is a crucial factor determining the in vivo behaviors after oral administration. Anionic charges can slow down the lipolysis of SLNs, while cationic charge can accelerate this process. The absorption of intact SLNs into blood circulation with the fastest and largest absorption was observed for net neutral
| Nanotechnology          | Material                         | Modification | Size (nm) | PPs            | Absorption                                                                                      | Ref. |
|-------------------------|----------------------------------|--------------|-----------|----------------|---------------------------------------------------------------------------------------------|------|
| Polymeric nanoparticles | PLGA                             | TMC          | 247.6     | Insulin        | 2-Fold higher relative bioavailability for insulin                                          | 290  |
|                         |                                  | WGA          | 231.9     | Thymopentin    | Enhancing the interaction with M cells by 1.8–4.2-fold and increasing the values of CD4⁺/CD8⁺ ratios | 291  |
|                         |                                  | RGD-PEG      | 200       | Ovalbumin      | Concentrating in M cells particularly                                                        | 292  |
|                         |                                  | No           | 302–328   | β-LGDP         | Increasing mucosal immunity and milk allergy prevention                                      | 293  |
|                         |                                  | FC fragments | 55        | Insulin        | Producing a prolonged hypoglycemic effect in wild-type mice at a clinically relevant insulin dose of 1.1 U/kg | 225  |
| Chitosan                |                                  | PEG          | 232.9     | Insulin        | Approximately 20% of relative oral bioavailability                                         | 120  |
|                         |                                  | Dextran/TMC  | 200–250   | PTH1-34        | Improving oral bioavailability of PTH remarkably                                             | 294  |
|                         |                                  | Eudragit L100/mannosylated | 558.2 | BSA            | Increasing the humoral immunity and the level of antibody                                     | 295  |
| Lipid-based nanoparticles | Liposomes                        | WGA-carbopol | 100       | sCT            | Eliciting strong systemic IgG antibody and mucosal IgA responses                            | 106  |
|                         |                                  | Biotinylated | 150       | Insulin        | Controlling the blood glucose levels                                                        | 296  |
|                         |                                  | Sodium caprate | 200–250  | hGH            | Leading to a relative bioavailability of hGH of 3.4%                                        | 298  |
|                         |                                  | PLFE         | 500       | OVA            | Improving the immune response of OVA                                                         | 299  |
|                         |                                  | MPC          | 300       | BSA            | Higher levels of IgG in the sera and IgA in the mucosa                                      | 300  |
|                         |                                  | UEA-1        | 400–500   | BSM            | Higher sIgA level and cytokines level                                                        | 301  |
| SLN                    | Cationic lipids                  | 300         | Insulin    |                | Protecting insulin from the enzymatic degradation                                             | 302  |
|                         | CSK/IRQ                          | 244/410     | sCT        |                | Leading to the absolute bioavailability of 12.41% and 10.05%, respectively                 | 303  |
| Nanoemulsions           | Alginate/chitosan                | 500         | Insulin    |                | The oral relative bioavailability was 8.42%                                                  | 304  |
|                         | Oil-structured nanoemulsion      | 250.8       | Exenatide  |                | Improving the bioavailability of exenatide via intestinal lymphatic transport                | 305  |
| Inorganic nanoparticles | Silica                           | Chitosan     | 226       | Exenatide      | Enhancing transport across Caco-2 monolayer by 1.7-fold                                      | 306  |
|                         |                                  | CPP-PEG      | 173.2     | hGH            | Leading to 4.91-fold increase in pharmacodynamics                                             | 307  |
|                         |                                  | No           | 200       | Insulin        | Showing sustaining hypoglycemic effect                                                       | 308  |
| Calcium phosphate       | VB1₂                             | <250        | Insulin    |                | Enhancing oral bioavailability by 4-fold and showing sustained hypoglycemic effects up to 12 h | 219  |
| Gold                   | Polysaccharide                   | 14–35       | OVA        |                | Enhancing the mucosal IgA and serum IgG responses                                             | 310  |
|                         | Chondroitin sulfate              | 123         | Insulin    |                | The oral absorption was enhanced with 6.61-fold                                              | 311  |

Abbreviations: PLGA, poly(ε-caprolactone-co-glycolide); TMC, N-trimethyl chitosan chloride; WGA, wheat germ agglutinin; RGD, arginine-glycylaspartic acid; PLA-PEG, poly(lactic acid)-poly(ethylene oxide); PGA, poly(γ-glutamic acid); PTH1–34, parathyroid hormone 1–34; β-LGDP, beta-lactoglobulin-derived peptides; BSA, bovine serum albumin; sCT, salmon calcitonin; hGH, human growth hormone; PLFE, polar lipid fraction E; UEA-1, ulex europaeus agglutinin 1; BSM, bovine submaxillary mucin; MPC, mannose-PEG-cholesterol conjugate; CPP, cell-penetrating peptide; OVA, ovalbumin; VB1₂, vitamin B₁₂.
SLNs\textsuperscript{346}. The absorption amount of intact SLNs is highly significant for therapeutic PPs.

4.3.8.3. Inorganic nanoparticles. Inorganic nanoparticles have attracted increasing attention in drug delivery systems and also been used in improving oral delivery of PPs, such as silica nanoparticles\textsuperscript{26}, gold nanoparticles\textsuperscript{311}, calcium carbonate or phosphate nanoparticles\textsuperscript{327}.

Silica nanoparticles are the most commonly used drug carrier in inorganic nanoparticles due to good biocompatibility and tunability in size or morphology. Mesoporous silica nanoparticles (MSNs) have been widely used in improving oral bioavailability of poorly water soluble drugs\textsuperscript{348}. PPs can be loaded in MSNs by physical absorption and covalent conjugation. Physical absorption is difficult to achieve higher loading capacity which is generally lower than 15\%. Whereas, covalent conjugation can bring about environmental sensitive release for PPs\textsuperscript{351}. MSNs can protect PPs against degradation by harsh GI environments. In addition, the surface of MSNs can also be coated or modified for improving stability or intestinal transport. PEGylated MSNs are able to protect the conformation of insulin under simulated gastric and intestinal condition\textsuperscript{349} and polymeric methacrylates coating enables pH responsive release of insulin from MSNs\textsuperscript{350}. The SBA-15, a kind of MSNs, were used to deliver hepatitis B orally, indicating that they were able to protect and release the hepatitis B surface antigen (HBsAg) and offer better antibody response than intravenous delivery\textsuperscript{351}. Calcium based nanoparticles have attracted more interests in nanocarrier for PPs, such as calcium phosphate (CaP) and calcium carbonate (CaCO\textsubscript{3})\textsuperscript{352}. Similarly as MSNs, calcium based nanoparticles were generally coated using some polymers to improve the oral absorption and biocompatibility, for instance, hyaluronic acid coated CaCO\textsubscript{3} nanoparticles exerted satisfied hypoglycemic effect for oral insulin delivery\textsuperscript{227} and PEGylated CaP nanoparticles were found to be able to protect the insulin and sustain the release at the physiological pH\textsuperscript{228}. Further, vitamin B\textsubscript{12} was grafted to chitosan as a ligand to coat CaP nanoparticles for improving oral insulin absorption by 4.3-fold compared with naked nanoparticles\textsuperscript{19}. Gold nanoparticles capped with chondroitin sulfate or apple polysaccharide are able to increase plasma insulin concentration and anti-diabetic capability\textsuperscript{311,354}. The composites of insulin/zirconium phosphate (ZrP) coated with titanium dioxide (TiO\textsubscript{2}) were found to be non-toxic to biological environment and used for oral insulin delivery, which indicates promising in vitro drug release for a long time\textsuperscript{355}.

However, biosafety is a big concern for inorganic nanoparticles in drug delivery since they are non-degradable in biological environments\textsuperscript{356}. Most of PPs are needed to be administered for a long time due to short half-life, which could lead to accumulation of inorganic nanoparticles in human body\textsuperscript{357}. Thus, inorganic nanoparticles could be not ideal carriers for oral delivery of PPs.

5. Oral delivery systems of PPs commercially available and under clinical and preclinical studies

5.1. Current oral PPs on the market

Since the approval of first recombinant insulin in 1981, a great number of PPs have been developed in the recent years as new therapeutics. However, few PPs are administered via oral route due to great challenges in oral delivery\textsuperscript{358}. Moreover, current oral PPs on the market include both systemic delivery and local retention in the GI tract. For instance, cyclosporine A is one of the marketed oral peptides and formulated as SNEDDS\textsuperscript{359}. While, numerous oral enzyme products are delivered to local GI tract to treat metabolic disorders. Some oral PPs commercially available are listed in Table 5.

Cyclosporin A is the one of the most successful oral peptide products in market. The combination of the cyclic lipophilic undecapeptide with SNEDDS technologies enables the oral bioavailability achieve 19\%–40\%\textsuperscript{360}. Meanwhile, SNEDDS formulation (Neoral\textsuperscript{360}) overcome the high intra- and inter-patients pharmacokinetics variability in a high percentage of patients by better control of droplet size, increasing intestinal permeability and inhibiting P-glycoprotein efflux and P450 metabolism\textsuperscript{364}. Desmopressin acetate (DDVAP) is also a cyclic peptide and an analog of arginine vasopressin. Its stability is improved by chemical modifications including deamination of the first amino acid and substitution of the eighth amino acid L-arginine by D-arginine\textsuperscript{361}. It was developed as an oral tablet by Ferring Pharmaceuticals (Denmark) as early as 1995 and subsequently a variety of generic products were also approved by FDA. However, the oral bioavailability of DDVAP is only around 0.1\% because there is no any permeation enhanced technology used in these oral tablet\textsuperscript{362}. Oxeotide is another cyclic peptide which has been commercially available oral products on the market. It is a synthetic analog of the endogenous hormone somatostatin and exerts higher stability than somatostatin in SGF with pepsin due to cyclic structure\textsuperscript{49,363}. The oral enteric capsule of oxeotide developed by an oily suspension containing the permeation enhancer sodium caprylate was approved in June 2020 by FDA. However, the phase I and II clinical pharmacokinetics showed the oral bioavailability was only 0.5\%\textsuperscript{364}. As a peptide drug treating diabetes, semaglutide was successfully developed as oral formulation by Novo Nordisk. Semaglutide is a GLP-1 analog consisting of 31 amino acid residues and far larger than DDVAP and oxeotide. The oral semaglutide (Rybelsus\textsuperscript{365}) was formulated as a tablet combining with permeation enhancer SNAC developed by Emisphere technologies\textsuperscript{75}. The clinical trials demonstrated that 40 mg oral dose was comparable with the 1 mg sc dose\textsuperscript{365}. In addition, taltirelin and reduced L-glutamihne can also be administered orally to exert therapeutic efficacy.

Some proteins are not needed to be delivered systemically, but needed to retain in GI tract to treat some local diseases, such as linaclootide for irritable bowel syndrome and oral enzyme products for GI metabolic disorders. Linaclootide acts as a guanylyl cyclase C agonist locally in the small intestine and was developed as an oral hard capsules to treat chronic idiopathic constipation and irritable bowel syndrome with constipation\textsuperscript{366}. The oral linaclootide is almost not absorbed into systemic circulation. Vancomycin is a glycosylated tricyclic heptapeptide antibiotics and poorly absorbed due to high hydrophilicity and large cyclic structure\textsuperscript{367}. Thus, oral vancomycin capsule was approved for treatment of pseudomembranous colitis by FDA. Some enzymes related with GI function prefer to be administered by oral route. There are a variety of oral enzyme products on the market for GI metabolic disorders such as Creon\textsuperscript{368}, Lacteeze\textsuperscript{369}, Sucraid\textsuperscript{369} and DAOSIn\textsuperscript{368}. However, most of them are approved as dietary supplements and only pancreatic was approved as drug by FDA.
5.2. Overview of the current clinical studies

In the past decades, the non-invasive routes have attracted more attention for delivery of PPs. A number of oral PPs developed by various new technologies are being clinically evaluated (Table 6) for both systemic and local delivery. The majority of the technologies for systemic delivery of PPs under clinical trials are for oral insulin. There are two typical oral insulin products under phase I clinical trials which are developed by Novo Nordisk (Denmark) and NOD Pharmaceuticals Inc. (China). Novo Nordisk employed gastrointestinal permeation enhancement technology (GIPET) to formulate an oral insulin tablet with Merrion Pharmaceuticals (Ireland). The formulation is comprised of micelles with absorption enhancers (sodium caprate). Moreover, NOD encapsulated insulin into enteric coated bioadhesive calcium phosphate nanoparticles which were filled into a capsule (Nodlin™). Nodlin™ has completed phase I clinical trials (ChiCTR-TRC-12001872) on healthy volunteers, which exhibited similar hypoglycemic effect compared with sc insulin.

An oral insulin enteric capsule containing protease inhibitors and permeation enhancers developed by Oramed Pharmaceuticals Inc. (Israel), which is under phase II clinical trial. The formulation is developed by inert silica nanoparticles (1–100 nm) loading oily suspended insulin together with the branched polysaccharides. Biocion limited (India) developed an oral insulin named Tregopil based on chemical modification. Tregopil employed chemical modification to conjugate the β29-Lys-amino group of human insulin with a single methoxy triethylene glycol propionyl unit through amide linkage. Tregopil has completed phase II clinical trials for two doses (45 mg and 30 mg). Tregopil demonstrated well toleration with the patients, and rapid and prolonged hypoglycemic effect at different dosing interval. Besides, the composition of the observed meal has no influences on pharmacodynamic effect of the insulin. In addition, there are some other oral peptides under phase II clinical trials, such as PTH for osteoporosis and leuprolide for endometriosis.

It is very interesting that a hepatocyte targeting liposomes of oral insulin developed by Diasome Pharmaceuticals (USA) has completed phase II clinical trials and is preparing for phase III clinical trials. This product is formulated by nanoparticle technology and comprised of liposomes smaller than 150 nm containing insulin conjugated with hepatocyte-targeting moieties (biotin-phosphatidyl-ethanolamine) (5-CNAC). These liposomes are transported by intestinal epithelia into portal vein and then captured by hepatocytes to mimic the physiological insulin delivery. Although recent results of clinical trials have not yet been disclosed, the preliminary results revealed that it showed well toleration with the patients and better control of blood glucose level even after oral administration of a low dose of insulin (5 IU). SCT is also one of the most developed peptides for oral delivery except for insulin. Emisphere technologies Inc (NJ, USA) employed their Eligen™ technology to develop oral sCT (SMC021) which is under phase III clinical trials. This formulation used permeation enhancer 8-(N-2-hydroxy-5-chlorobenzozyl)-amino-caprylic acid (5-CNAC) to improve the oral absorption of sCT, which is similar rationale with oral semaglutide.

For local delivery, most of products primarily employed new technologies to make them resistant against enzymatic degradation in GI tract. For instance, the Vectrix™ platform developed by Protagonist Therapeutics Inc. designed highly constrained and stable peptides by molecular design tools and libraries of scaffolds. Currently, there have been two products in clinical trials based on Vectrix™ platform, including PX-10-943 for UC and PTG-200 for the treatment of CD. Following a similar rationale, Avaxia Biologics, Inc developed a milk-derived antibody (AVX-470) to treat UC, which is now in phase I. In addition, biomimetic drug delivery systems are broadly used in local delivery of PPs. The cellulose wall of plant cell is able to protect the recombinant proteins from degradation along the GI tract. The company Protalix Biotherapeutics have developed several candidates in clinical trials based on the ProCellEx delivery platform, such as a recombinant human tumor necrosis factor receptor II fused to an IgG1 Fc domain (TNFRII-Fc) for UC in phase II and the glycosylated glucocerebrosidase enzyme (prGCD) for the treatment of Gaucher disease also in phase II. Furthermore, more and more new technologies are emerging for oral delivery of PPs to treat local diseases in GI tract, such as Vorabodies™ or ActoBio Therapeutics™.

5.3. Recent status of preclinical strategies

As mentioned above, there have been a variety of strategies to improve oral delivery of PPs, some of which have been widely used in marketed products and clinical trials, such as enteric coating, enzyme inhibitors, permeation enhancers, nanotechnology, colonic targeting, as well as chemical modification. There are hundreds of papers published about new oral delivery systems for PPs every year. Table 7 lists partial projects of oral delivery systems for PPs in preclinical phase.

Currently, nanotechnology have become as the main research hotspot to improve the oral bioavailability of PPs. The nanoparticle system of NanoMega (CA, USA) is capable of decreasing the blood glucose over 8 h in diabetic rats. What’s more, an oral suspension of nanoparticles demonstrates a relative bioavailability of 15%, while an enteric capsule containing freeze-dried nanoparticles shows a relative bioavailability of 20%. This nanoparticles is composed of chitosan and PGA. Chitosan could act as a permeation enhancer by transient opening of tight junctions. Meanwhile, this technology has also been used for oral delivery of exendin-4. Nanoparticles are further conjugated with ligands on surface to facilitate intestinal absorption by targeting intestinal receptors or transporters. Transgene Biotek Ltd. (India) conjugated VB12 or Tf on SLNs to establish a TrabiOral™ platform for oral delivery of PPs. A oral insulin project (TBL-1002OI) led by Transgene has revealed prolonged hypoglycemia in rats for 10 h after oral administration.

Additionally, the intestinal microdevices attract more and more attention in oral delivery of PPs, such as microneedles. A robotic pill was developed by Rani Therapeutics (USA) for oral protein delivery. It is actually a balloon-like structure of sugar microneedles encapsulated by PLGA which is a degradable polymer.
### Table 5  Oral products of PPs on markets.

| PPs          | Trade name | Technology                                      | Indication                                                   | Company                               |
|--------------|------------|-------------------------------------------------|--------------------------------------------------------------|---------------------------------------|
| Cyclosporin A| Neoral™/Sandimmune®, C226/Calox®, Sandimmune®   | SNEDDS                                         | Immunosuppression; systemic delivery                         | Novatis AG (Switzerland)             |
| Desmopressin acetate | DDAVP®/DDVP®/D2VP®/VP®/DDVP®/Vasopressin® | Chemical modification                           | Central diabetes insipidus; systemic delivery                 | Ferring Pharmaceuticals (Switzerland) |
| Octreotide   | Mycapssa®  | Enteric coating; permeation enhancer            | Long-term maintenance treatment in acromegaly patients; systemic delivery | Chiasma (USA)                        |
| Semaglutide  | Rybelsus®/Rybelsus® | Permeation enhancer | Type 2 diabetes mellitus; systemic delivery                   | Novo Nordisk (Denmark)               |
| Taltirelin hydrate | Cerest®/Cerest OD®/Cerest ODT®/Cerest ODT® | Chemical modification                           | Spinocebellar degeneration; systemic delivery                 | Mitsubishi Tanabe Pharma Co. (Japan) |
| Linacotide   | Linzess®   | Acts locally                                     | Irritable bowel syndrome, chronic idiopathic constipation; local delivery | Actavis, Inc. (USA)                  |
| Vancomycin   | Vancocin®  | Acts locally                                     | Infection                                                    | ANI Pharmaceuticals, Inc (USA)       |
| Colistin sulfate | Koolistin® | Acts locally                                     | Infection                                                    | Biocon Ltd. (India)                  |
| Tyrothricin  | Lozenges®  | Acts locally on the throat                       | Pharyngitis                                                   | The Boots Company PLC (UK)           |
| Pancrelipase | Creon®/Creon®/Creos® | Delayed release; acts locally | Exocrine pancreatic insufficiency                             | AbbVie Inc. (USA)                   |
| Tilactase    | Lacteeze®  | Chewable tablets; acts locally                   | Lactose intolerance                                           | Lacteese (USA)                      |
| Sacrosidase  | Sucraid®   | Oral solutions; acts locally                     | Congenital sucrase-isomaltase deficiency                     | QOL Medical, LLC (USA)               |
| Diamine oxidase | DAOSiN®   | Acts locally                                     | Histamine intolerance                                         | SCIOTE (Austria)                    |

### Table 6  Oral PP products under clinical trials (clinical trials.gov).

| PPs          | Phase | Technology                                      | Company                                                   |
|--------------|-------|-------------------------------------------------|-----------------------------------------------------------|
| Insulin      | I     | Enteric coating; bioadhesive calcium phosphate nanoparticles (Nodlin™) | NOD Pharmaceuticals, Inc. (China)                         |
| Insulin      | I     | Gastrointestinal permeation enhancement technology (GIPET™) | Merrion Pharmaceuticals Ltd. (Ireland) with Novo Nordisk (Denmark) |
| Insulin      | I     | Permeation enhancer (Eligen™)                    | Emisphere (USA) with Novo Nordisk (Denmark)               |
| GLP-1 analog | I     | Permeation enhancer (sodium caprate)             | Merrion Pharmaceuticals Ltd. (Ireland) with Novo Nordisk (Denmark) |
| Insulin      | II    | Silica-based nanoparticles                      | Oshadi (Israel)                                           |
| Insulin      | II    | Enteric coating; enzyme inhibitor; permeation enhancer | Merrion Pharmaceuticals Ltd. (Ireland) with Novo Nordisk (Denmark) |
| Insulin      | II    | Permeation enhancers (Axess™, Capsulin™)         | Promigina Concepts Ltd/Diabetology (UK)                   |
| PTH          | II    | Permeation enhancers (Axess™, CaPTHymone™)       | Promigina Concepts Ltd/Diabetology (UK)                   |
| rhPTH(1−31)  | II    | Permeation enhancers; enzyme inhibitor (Pepelligence™) | Enteris Biopharma, Inc. (USA)                            |
| CsA          | II    | Oil in water emulsion                           | Sigmoid Pharma (Ireland)                                  |
| Dolcanatide  | II    | Chemical modification                           | Synergy Pharmaceuticals Inc. (USA)                        |
| Acylone      | II    | Gastrointestinal permeation enhancement technology (GIPET™) | Merrion Pharmaceuticals Ltd. (Ireland)                    |
| Leuprolide   | II    | Permeation enhancer; pH modulator; enzyme inhibitor | Enteris Biopharm (USA)                                   |
| Insulin      | III   | Liver-targeted liposomes                        | Diasome Pharma (USA)                                      |
| Insulin      | III   | Chemical modification (PEGylated insulin)        | Biocon (India)                                            |
| sCT           | III   | Permeation enhancers (Axess™, Capsition™)        | Promigina Concepts Ltd/Diabetology (UK)                   |
| sCT           | III   | pH modulator (Pepellation™)                      | Tarsa therapeutics, Inc. (USA)                            |
| sCT           | III   | Permeation enhancer (5-CNC)                      | Emisphere (USA)                                           |
| Plenacatide  | III   | Chemical modification; local delivery            | Chiasma (Israel)                                          |
Insulin loaded the microneedles exhibited encouraging hypoglycemic effect with oral bioavailability of 50% in preclinical trials. Likewise, intestinal patches have also been attempted to orally delivery sCT, exenatide, insulin and interferon-α. The patches can be prepared as the size of millimeters or micrometers coated with pH-responsive polymer and contain drug reservoir. This device could also integrate absorption enhancers or enzyme inhibitors to further improve oral absorption of PPs. Permeation enhancers are the main components in products of oral PPs in market or clinical trials. Until now, a number of companies are still developing various permeation enhancers. For instance, Emisphere developed a series of derivatives of caprylic acid as permeation enhancers, such as SNAC and 5-CNAC. Aegis Therapeutics (CA, USA) developed a category of permeation enhanced excipients named Intravail which are a group of alkylsaccharides composed of disaccharides and alkyl chain substituents with lengths between 10 and 16 carbons. They have been used in oral delivery of various peptides including octreotide and D-Leu-OB3. The results showed high systemic bioavailability after combination of peptides and Intravail in rodents compared to sc administration.

### 6. Conclusions and future perspectives

Oral delivery of PPs has become more attractive in drug research and development since the increasing market share in the last decade. Therefore, various new technologies are emerging for improving oral bioavailability of PPs by overcoming obstacles of PPs in terms of stability and permeability. Some technologies have been successfully used in oral marketed products of PPs, such as enteric coating, enzyme inhibitors, permeation enhancers, as well as chemical modification. Currently, nanotechnology-based approaches have shown potential for developing oral PPs formulation. Moreover, intestinal microdevices were also developed for delivering PPs by oral route, such as intestinal microneedles. However, the safety, efficacy and reproducibility in preparation technique of these new technologies are needed to be improved and evaluated thoroughly. Furthermore, the oral bioavailability of PPs for systemic delivery is still very low, even lower than 1% for some products despite using new technology to improve the stability and permeability.

Nanoparticles are able to facilitate the intestinal transport of PPs, which has been reported by numerous papers. However, it is more important to shed light on well-understanding the detailed mechanism of interaction between these nanocarriers and PPs or the intestinal milieu. Moreover, elucidation of PPs transport in the intestinal epithelium and the influence of physiological factors on absorption of PPs are also indispensable. Therefore, elucidating the in vivo fate of nanoparticles and PPs after oral administration is the necessary prerequisite to development of highly efficient oral systems of PPs.

### Acknowledgments

This work is financially supported by National Natural Science Foundation of China (No.s 81872815, 81872826 and 82073801), Science and Technology Commission of Shanghai Municipality (No. 18ZR1404100, China), Shanghai Pujiang Program (No. 18PJJD001, China), and Key Subject of Shanghai Skin Disease Hospital (No. 2019ZDXK03, China).

### Author contributions

Quangang Zhu, Zhongjian Chen and Pijush Kumar Paul collected references and drafted the manuscript. Yi Lu collected references. Jianping Qi and Wei Wu proposed the concept and revised the references and drafted the manuscript. All of the authors have read and approved the final manuscript.

### Conflicts of interest

The authors have no conflicts of interest to declare.

### References

1. Patel A, Patel M, Yang X, Mitra AK. Recent advances in protein and peptide drug delivery: a special emphasis on polymeric nanoparticles. *Protein Pept Lett* 2014;21:1102–20.
2. Rengasamy KRR, Khan H, Ahmad I, Lobine D, Mahomoodally F, Suroowan S, et al. Bioactive peptides and proteins as alternative antiplatelet drugs. *Med Res Rev* 2019;39:2153–71.
3. Choi YA, Yoon YH, Choi K, Kwon M, Goo SH, Cha JS, et al. Enhanced oral bioavailability of morin administered in mixed micelle formulation with pluronic F127 and tween 80 in rats. *Biol Pharm Bull* 2015;38:208–17.
4. Anselmo AC, Gokarn Y, Mitragotri S. Non-invasive delivery strategies for biologics. Nat Rev Drug Discov 2019; 18:19–40.
5. Van Der Walle C. Peptide and protein delivery. 1st ed. London: Academic Press; 2011.
6. Ismail R, Csoka I. Novel strategies in the oral delivery of anti-diabetic peptide drugs—insulin, GLP1 and its analogs. Eur J Pharm Biopharm 2017; 115:257–67.
7. Rader AFB, Weinmuller M, Reichart F, Schumacher-Klinger A, Merzbach S, Gilon C, et al. Orally active peptides: is there a magic bullet? Angew Chem Int Ed 2018; 57:14414–38.
8. Drucker DJ. Advances in oral peptide therapeutics. Nat Rev Drug Discov 2020; 19:277–89.
9. Di L. Strategic approaches to optimizing peptide adme properties. AAPS J 2015; 17:134–43.
10. Bohley M, Haunberger A, Goepferich AM. Intracellular availability of poorly soluble drugs from lipid nanocapsules. Eur J Pharm Biopharm 2019; 139:23–32.
11. Remukunja J, Vadlapudi AD, Patel A, Boddu SH, Mitra AK. Approaches for enhancing oral bioavailability of peptides and proteins. Int J Pharm 2013; 447:75–93.
12. Goldberg M, Gomez-Orellana I. Challenges for the oral delivery of macromolecules. Nat Rev Drug Discov 2003; 2:289–95.
13. Morishita M, Peppas NA. Is the oral route possible for peptide and protein drug delivery? Drug Discov Today 2006; 11:905–10.
14. Jain D, Mahammod SS, Singh PP, Kodipyaka R. A review on parenteral delivery of peptides and proteins. Drug Dev Ind Pharm 2019; 45:1403–20.
15. Chung SW, Hii-lal TA, Byun Y. Strategies for non-invasive delivery of biologics. J Drug Target 2012; 20:481–501.
16. Hwang SR, Byun Y. Advances in oral macromolecular drug delivery. Expert Opin Drug Deliv 2014; 11:1955–67.
17. Brayden DJ, Alonso MJ. Oral delivery of peptides: opportunities and issues for translation. Adv Drug Deliv Rev 2016; 106:193–5.
18. Moro E, Matoori S, Leroux JC. Oral delivery of macromolecular drugs: where we are after almost 100 years of attempts. Adv Drug Deliv Rev 2016; 101:108–21.
19. Shaikh S, Jaiswal P. Oral proteins and peptides market overview. 2018. Available from: https://www.alliedmarketresearch.com/ oral-proteins-peptides-market.
20. Mahmoud A, Bernkop-Schnurch A. SEDDS: a game changing approach for the oral administration of hydrophilic macromolecular drugs. Adv Drug Deliv Rev 2019; 142:91–101.
21. Mahes S, Misra RJ, Brayden DJ. Intestinal permeation enhancers for oral peptide delivery. Adv Drug Deliv Rev 2016; 106:277–319.
22. Liu C, Kou Y, Zhang X, Chen H, Chen X, Mao S. Strategies and industrial perspectives to improve oral absorption of biological macromolecules. Expert Opin Drug Deliv 2018; 15:223–33.
23. Duran-Lobato M, Niu Z, Alonso MJ. Oral delivery of biologics for precision medicine. Adv Mater 2020; 32:e1901935.
24. Smart AL, Gaisford S, Basit AW. Oral peptide and protein delivery: intestinal obstacles and commercial prospects. Expert Opin Drug Deliv 2014; 11:3323–35.
25. Cao SJ, Xu S, Wang HM, Ling Y, Dong J, Xia RD, et al. Nanoparticles: oral delivery for protein and peptide drugs. AAPS PharmaSciTech 2019; 20:190.
26. Tan X, Liu X, Zhang Y, Zhang H, Lin X, Pu C, et al. Silica nanoparticles on the oral delivery of insulin. Expert Opin Drug Deliv 2018; 15:805–20.
27. Heinemann L, Jacques Y. Oral insulin and buccal insulin: a critical reappraisal. J Diabetes Sci Technol 2009; 3:568–84.
28. Harrison GA. Insulin in alcoholic solution by the mouth. Br Med J 1923; 2:1204–5.
29. Ferguson JEA. Oral blood sugar lowering compositions. 1965. US3172814 A.
30. Wong CY, Martinez J, Dass CR. Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities. J Pharm Pharmacol 2016; 68:1093–108.
31. Muheem A, Shakeel F, Jahangir MA, Anwar M, Mallick N, Jain GK, et al. A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives. Saudi Pharm J 2016; 24:413–28.
32. Stillhart C, Vucićević K, Augustijns P, Basit AW, Batchelor H, Planagan TR, et al. Impact of gastrointestinal physiology on drug absorption in special populations—an ungap review. Eur J Pharmaceut Sci 2020; 147:105280.
33. Koziolek M, Grimm M, Becker D, Iordanov V, Zou H, Shimizu J, et al. Investigation of pH and temperature profiles in the GI tract of fasted human subjects using the InteliCap® system. J Pharm Sci 2015; 104:2855–63.
34. Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 1988; 29:1035–41.
35. Khan MS, Roberts MS. Challenges and innovations of drug delivery in older age. Adv Drug Deliv Rev 2018; 135:3–38.
36. Mooij MG, de Koning BAE, Huisman ML, de Wildt SN. Ontogeny of oral drug absorption processes in children. Exp Opin Drug Metab Toxicol 2012; 8:1293–303.
37. Deng J, Zhu X, Chen Z, Fan CH, Kwan HS, Wong CH, et al. A review of food—drug interactions on oral drug absorption. Drugs 2017; 77:1833–55.
38. Sasaki Y, Hada R, Nakajima H, Fukuda S, Munakata A. Improved localizing method of radiolipid in measurement of entire gastrointestinal ph profiles: colonic luminal pH in normal subjects and patients with Crohn’s disease. Am J Gastroenterol 1997; 92:114–8.
39. Fallborg J, Christensen LA, Jacobsen BA, Rasmussen SN. Very low intraluminal colonic pH in patients with active ulcerative colitis. Dig Dis Sci 1993; 38:1969–93.
40. Lu PJ. Gastric juice acidity in upper gastrointestinal diseases. World J Gastroenterol 2010; 16:5496–501.
41. Wei W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. Int J Pharm 1999; 185:129–88.
42. Gracia R, Yus C, Abian O, Mendoza G, Iusts A, Sebastian V, et al. Enzyme structure and function protection from gastrointestinal degradation using enteric coatings. Int J Biol Macromol 2018; 119:413–22.
43. Zhang W, Li Y, Zou P, Wu M, Zhang Z, Zhang T. The effects of pharmaceutical excipients on gastrointestinal tract metabolic enzymes and transporters—an update. AAPS J 2016; 18:830–43.
44. Bernkop-Schnürch A. The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. J Control Release 1998; 52:1–16.
45. Whitcomb DC, Lowe ME. Human pancreatic digestive enzymes. Dig Dis Sci 2007; 52:1–17.
46. Sánchez J, Fernández-Tomé S, Miralles B, Hernández-Ledesma B, Tomé D, Gaudichon C, et al. Protein degradation and peptide release from milk proteins in human jejenum: comparison with in vitro gastrointestinal simulation. Food Chem 2018; 239:486–94.
47. Woodley JF. Enzymatic barriers for GI peptide and protein delivery. Crit Rev Ther Drug Carrier Syst 1994; 11:61–95.
48. Minner-Williams WM, Stevens BR, Moughan PJ. Are intact peptides absorbed from the healthy gut in the adult human?. Nutr Res Rev 2015; 27:308–29.
49. Cui M, Wu W, Hovgaard L, Lu Y, Chen D, Qi J. Liposomes containing cholesterol analogues of botanical origin as drug delivery systems to enhance the oral absorption of insulin. Int J Pharm 2015; 489:277–84.
50. He H, Lu Y, Qi J, Zhao W, Dong X, Wu W. Biomimetic thiamine- and niacin-decorated liposomes for enhanced oral delivery of insulin. Acta Pharm Sinica B 2018; 8:966.
51. Wang J, Yadav V, Smart AL, Tajiri S, Basit AW. Toward oral delivery of biopharmaceuticals: an assessment of the gastrointestinal stability of 17 peptide drugs. Mol Pharm 2015; 12:966–73.
52. Wu L, Shan W, Zhang Z, Huang Y. Engineering nanomaterials to overcome the mucosal barrier by modulating surface properties. Adv Drug Deliv Rev 2018; 124:150–63.
Oral delivery of proteins and peptides

53. Varum FJ, Veiga F, Sousa JS, Basit AW. Mucus thickness in the gastrointestinal tract of laboratory animals. *J Pharm Pharmacol* 2012;64:218–27.

54. Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. *Adv Drug Deliv Rev* 2012;64:557–70.

55. Bansil R, Turner BS. The biology of mucus: composition, synthesis and organization. *Adv Drug Deliv Rev* 2018;124:3–15.

56. Corfield AP, Carroll D, Myrsicough N, Probert C. Mucins in the gastrointestinal tract in health and disease. *Front Biosci* 2001;6:D1321–57.

57. Murty V, Sarosiek J, Slomiany A, Slomiany B. Effect of lipids and proteins on the viscosity of gastric mucous glycoprotein. *Biochem Biophys Res Commun* 1984;121:521–9.

58. Demoueaux B, Gouyer V, Grottrand F, Narita T, Desseyn JL. Gel-forming mucin interactome drives mucus viscoelasticity. *Adv Colloid Interface Sci* 2018;252:69–82.

59. Lichtenberger L. The hydrophobic barrier properties of gastrointestinal mucus. *Annu Rev Physiol* 1995;57:565–83.

60. Homayun B, Lin X, Choi HJ. Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics* 2019;11:129.

61. Boegh M, Nielsen HM. Mucus as a barrier to drug delivery—understanding and mimicking the barrier properties. *Basic Clin Pharmacol Toxicol* 2015;116:179–86.

62. Zhang X, Cheng H, Dong W, Zhang M, Liu Q, Wang X, et al. Design and intestinal mucus penetration mechanism of core-shell nanocomplex. *J Control Release* 2018;272:29–38.

63. QI J, Zhuang J, Ly Y, Ly Y, Wu W. Exploiting or overcoming the dome trap for enhanced oral immunization and drug delivery. *J Control Release* 2018;275:92–106.

64. Cheng H, Leblond C. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine: unitarian theory of the origin of the four epithelial cell types, *Am J Anat* 1974;141:537–61.

65. Capaldo CT, Powell DN, Kalman D. Layered defense: how mucus and tight junctions seal the intestinal barrier. *J Mol Med* 2017;95:927–34.

66. Vancambleneke M, Vermeire S. The intestinal barrier: a fundamental and translational challenge observed in a stabilized form of RSV prefusion F protein. *Eur J Pharm Biopharm* 2018;124:179–86.

67. Singh B, Maharjan S, Jiang T, Kang SK, Choi YJ, Cho CS. Combinatorial approach of antigen delivery using M cell-homing peptide and mucoadhesive vehicle to enhance the efficacy of oral vaccine. *Mol Pharm* 2015;12:8371–9.

68. Ménard S, Lebréton C, Schumann M, Matsuiya-Budnik T, Dugave C, Bouhnik Y, et al. Paracellular versus transcellular intestinal permeability to gliadin peptides in active celiac disease. *Am J Pathol* 2012;180:608–15.

69. Tyagi P, Pechenov S, Subramony JA. Oral peptide delivery: translational challenges due to physiological effects. *J Control Release* 2018;287:167–76.

70. Boronikolos GC, Menge BA, Schenker N, Breuer TG, Otte JM, Heckermann S, et al. Upper gastrointestinal motility and symptoms in individuals with diabetes, prediabetes and normal glucose tolerance. *Diabetologia* 2015;58:1175–82.

71. Sugihara M, Takeuchi S, Sugita M, Higaki K, Kataoka M, Yamashita S. Analysis of intra- and inter-subject variability in oral drug absorption in human bioequivalence studies of 113 generic products. *Mol Pharm* 2015;12:4405–13.

72. Jamal Azam Y, Machavaram KK, Rostami-Hodjegan A. The modulating effects of endogenous substances on drug metabolism enzymes and implications for inter-individual variability and quantitative prediction. *Curr Drug Metabol* 2014;15:599–619.

73. Bois FY, Jamei M, Clewell HJ. PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. *Toxicology* 2010;278:256–67.

74. Gervasi V, Agnol RD, Cullen S, McCoy T, Vucen S, Crean A. Parenteral protein formulations: an overview of approved products within the European Union. *Eur J Pharm Biopharm* 2018;131:87–84.

75. Krause ME, Sahin E. Chemical and physical instabilities in manufacturing and storage of therapeutic proteins. *Curr Opin Biotechnol* 2019;60:159–67.

76. Duerr C, Friess W. Antibody—drug conjugates—stability and formulation. *Eur J Pharm Biopharm* 2019;139:168–76.

77. Wang W, Ohtake S. Science and art of protein formulation development. *Int J Pharm* 2019;568:118505.

78. Pace CN, Fu H, Fryar KL, Landua J, Trevino SR, Shirley BA, et al. Contribution of hydrophobic interactions to protein stability. *J Mol Biol* 2011;408:514–28.

79. Nick Pace C, Scholtz JM, Grimsley GR. Forces stabilizing proteins. *FEBS Lett* 2014;588:2177–84.

80. Ouytay AO, Méndez-Lucio O, Bender A, Kiefer H. Diversity selection, screening and quantitative structure-activity relationships of osmolyte-like additive effects on the thermal stability of a monoclonal antibody. *Eur J Pharm Sci* 2017;97:151–7.

81. Singh SN, Kumar S, Bondar V, Wang N, Forcino R, Colandene J, et al. Unexplored benefits of controlled ice nucleation: lyophilization of a highly concentrated monoclonal antibody solution. *Int J Pharm* 2018;552:171–9.

82. Qian J, Yearley E, Tian S, Jing L, Balsaraf A, Lo Surdo P, et al. Predicting protein stability in highly concentrated monoclonal antibody solutions using dilute osmolyte-like additive effects on the thermal stability of a monoclonal antibody. *Anal Chem* 2018;90:10897–902.

83. Tardini MA, Ghosh R, Saluja F, Roberts CJ. Predicting protein-protein interactions of concentrated antibody solutions using dilute solution data and coarse-grained molecular models. *J Pharm Sci* 2018;107:1269–81.

84. Shi S, Hashemi V, Wang SC, Yang J, Yang M, Semple A, et al. Novel screening and optimization pathway observed in a stabilized form of RSV prefusion F protein. *Anal Chem* 2018;90:10897–902.

85. Calero-Rubio C, Ghosh R, Saluja F, Roberts CJ. Predicting protein-protein interactions of concentrated antibody solutions using dilute solution data and coarse-grained molecular models. *J Pharm Sci* 2018;107:1269–81.

86. Duerr C, Friess W. Antibody—drug conjugates—stability and formulation. *Eur J Pharm Biopharm* 2019;139:168–76.

87. Wang W, Ohtake S. Science and art of protein formulation development. *Int J Pharm* 2019;568:118505.

88. Pace CN, Fu H, Fryar KL, Landua J, Trevino SR, Shirley BA, et al. Contribution of hydrophobic interactions to protein stability. *J Mol Biol* 2011;408:514–28.

89. Nick Pace C, Scholtz JM, Grimsley GR. Forces stabilizing proteins. *FEBS Lett* 2014;588:2177–84.

90. Ouytay AO, Méndez-Lucio O, Bender A, Kiefer H. Diversity selection, screening and quantitative structure-activity relationships of osmolyte-like additive effects on the thermal stability of a monoclonal antibody. *Eur J Pharm Sci* 2017;97:151–7.

91. Singh SN, Kumar S, Bondar V, Wang N, Forcino R, Colandene J, et al. Unexplored benefits of controlled ice nucleation: lyophilization of a highly concentrated monoclonal antibody solution. *Int J Pharm* 2018;552:171–9.

92. Qian J, Yearley E, Tian S, Jing L, Balsaraf A, Lo Surdo P, et al. Predicting protein stability in highly concentrated monoclonal antibody solutions using dilute osmolyte-like additive effects on the thermal stability of a monoclonal antibody. *Anal Chem* 2018;90:10897–902.
current dosage form profile and formulation strategies. *J Drug Target* 2020;28:339–55.

98. Bane J, Mozziconacci O, Yi L, Wang YI, Seedhara A, Schöneich C. Photo-oxidation of IGG1 and model peptides: detection and analysis of triply oxidized his and trp side chain cleavage products. *Pharm Res* 2017;34:229–42.

99. Kamerzell TJ, Esfandiary R, Joshi SB, Middaugh CR, Volkin DB. Protein–exipient interactions: mechanisms and biophysical characterization applied to protein formulation development. *Adv Drug Deliv Rev* 2011;63:1118–59.

100. Forney-Stevens KM, Bogner RH, Pikal MJ. Addition of amino acids to further stabilize lipophilized sucrose-based protein formulations: I. screening of 15 amino acids in two model proteins. *J Pharm Sci* 2016;105:697–704.

101. Bhattacharjya BS, Bogner RH, Pikal MJ. Protein stability during freezing: separation of stresses and mechanisms of protein stabilization. *Pharmaceut Dev Technol* 2007;12:505–23.

102. Arsicchio A, Pisano R. Surfactants as stabilizers for bio-pharmaceuticals: an insight into the molecular mechanisms for inhibition of protein aggregation. *Eur J Pharm Biopharm* 2018;128:98–106.

103. Semenyuk P, Morozov V. Protein interaction with charged macromolecules: from model polymers to unfolded proteins and post-translational modifications. *Int J Mol Sci* 2019;20:1252.

104. Yahyaei M, Mehmehmad F, Naderi-Manesh H, Rezayan AH. Protein adsorption onto polysaccharides: comparison of chitosan and chitin polymers. *Carbohydr Polym* 2018;191:191–7.

105. Mat DJL, Cattaneo T, Tsuchon I, Michon C, Le Feunteun S. Monitoring protein hydrolysis by pepsim using pH-stat: in vitro gastric digestions in static and dynamic pH conditions. *Food Chem* 2018;239:268–75.

106. Xu B, Zhang W, Chen Y, Wang B, Zong L. Eudragit L100-coated mannansoylated chitosan nanoparticles for oral protein vaccine delivery. *Int J Biol Macromol* 2018;113:534–42.

107. Schilling RJ, Mitra AK. Degradation of insulin by trypsin and alpha-chymotrypsin. *Pharm Res* 1991;8:721–7.

108. Welling SH, Hubidel F, Jacobsen J, Brayden DJ, Rahbek UL, Buckley ST. The role of citric acid in oral peptide and protein formulations: relationship between calcium chelation and proteolysis inhibition. *Eur J Pharm Biopharm* 2014;86:54–11.

109. Lee YH, Perry BA, Labruno S, Lee HS, Stern W, Falzone LM, et al. Impact of regional intestinal pH modulation on absorption of peptide drugs: oral absorption studies of salmon calcitonin in beagle dogs. *Pharm Res* 1999;16:233–9.

110. Binkley N, Bolognese M, Sidorowicz-Bialynicka A, Vally T, Schilling RJ, Mitra AK. Degradation of insulin by trypsin and alpha-chymotrypsin. *Pharm Res* 2017;34:229–42.

111. Choonoara BF, Choonoara YE, Kumar P, Biukumar D, du Toit LC, Pillay V. A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. *Biotechnol Adv* 2014;32:1269–82.

112. Niu M, Lu Y, Hovgaard L, Wu W. Liposomes containing glyco-cholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation. *Int J Nanomed* 2011;6:1155.

113. Agrwal V, Reddy IK, Khan MA. Oral delivery of proteins: effect of chicken and duck ovomucoid on the stability of insulin in the presence of α-chymotrypsin and trypsin. *Pharm Pharmocol Commun* 2000;6:223–9.

114. Arbit E, Kidron M. Oral insulin delivery in a physiologic context. *J Diabetes Sci Technol* 2017;11:825–32.

115. Del Curto MD, Maroni A, Palugan L, Zema L, Gazzaniga A, Sangalli ME. Oral delivery system for two-pulse colonic release of protein drugs and protease inhibitor/absorption enhancer compounds. *J Pharm Sci* 2011;100:3251–9.
Oral delivery of proteins and peptides

136. Gentileucci L, De Marco R, Cerisoli L. Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. Curr Pharmaceut Des 2010;16:3185–203.

137. Conibear AC, Chaoussis S, Durek T, Johan Rosengren K, Craik DJ, Schroeder CI. Approaches to the stabilization of bioactive epitopes by grafting and peptide cyclization. Pept Sci 2016;106:89–100.

138. Zhang RY, Thapa P, Espiritu MI, Menon V, Bingham JP. From nature to creation: going around in circles, the art of peptide cyclization. Bioorg Med Chem 2018;26:11355–50.

139. Matsui K, Kimura T, Ota K, Itake K, Shoji M, Inoue M, et al. Resistance of 1-deamino[8-o-arginine]-vasopressin to in vitro degradation as compared with arginine vasopressin. Endocrinol Jpn 1985;32:547–57.

140. White CJ, Yudin AK. Contemporary strategies for peptide macrocyclization. Nat Chem 2011;3:509.

141. Bogdanowich-Knipp SJ, Chakrabarti S, Siahaan TJ, Williams TD, Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric self-assembled nanoparticles for oral delivery of insulin. J Control Release 2017;244:35–46.

142. Maisel K, Ensign L, Reddy M, Cone R, Hanes J. Effect of surface particle shapes on the oral delivery of drug nanocrystals: mucus permeation, transepithelial transport and bioavailability. J Control Release 2019;307:64–75.

143. Yu M, Wang J, Yang Y, Zhu C, Su Q, Guo S, et al. Rotation-facilitated rapid transport of nanorods in mucosal tissues. Nano Lett 2016;16:7176–82.

144. Dünnschnitt B, Kammons O, Waldner C, Kiparissides C, Bernkop-Schnürch A. Nano-carrier systems: strategies to overcome the mucus gel barrier. Euro J Pharm Biopharm 2015;96:447–53.

145. Zupančič O, Griješnjak JA, Rohrer J, de Sousa JP, Dannerling A, Lathenhaus A, et al. Development, in vitro and in vivo evaluation of a self-emulsifying drug delivery system (seds) for oral enoxaparin administration. Eur J Pharm Biopharm 2016;109:113–21.

146. Suk JS, Lai SK, Boylan NJ, Dawson MR, Boyle MP, Hanes J. Rapid diffusion barrier of mucus and absorption barrier of epithelium by mucus-penetrating nanoparticles to sequentially overcome mucus penetration, transepithelial transport and bioavailability. J Control Release 2019;307:64–75.

147. Florey HW. Secretion and function of intestinal mucus. Gastroenterology 1962;43:326–9.

148. Chickering lii D, Jacob J, Desai T, Harrison M, Harris W, Morrell C, et al. Bioadhesive microspheres. III. an in vivo transit and bioavailability study of drug-loaded alginate and poly(fumaric-co-sebacic anhydride) microspheres. J Control Release 1997;48:35–46.

149. Eldridge JH, Hammond CJ, MeBrlock IA, Staas JK, Gilley RM, Tice TR. Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the payer’s patches. J Control Release 1990;11:205–14.

150. Chen F, Zhang ZR, Yuan F, Qin X, Wang M, Huang Y. In vitro and in vivo study of n-trimethyl chitosan nanoparticles for oral protein delivery. Int J Pharm 2008;349:226–33.

151. Bernkop-Schnürch A. Thiomers: a new generation of mucoadhesive polymers. Adv Drug Deliv Rev 2005;57:1569–82.

152. Bernkop-Schnürch A, Horinof M, Zoidl T. Thiolated polymer−thiomers: synthesis and in vitro evaluation of chitosan−2-iminothiolane conjugates. Int J Pharm 2003;260:29−37.

153. Yen L, Ding J, He C, Cui L, Tang C, Yin C. Drug permeability and mucoadhesion properties of thiolated trimethyl chitosan nanoparticles in oral insulin delivery. Biomaterials 2009;30:5691–700.

154. Lehr CM. Lectin-mediated drug delivery: the second generation of bioadhesives. J Control Release 2000;65:19−29.

155. Leong KH, Chung LY, Noordin MI, Onuki Y, Morishita M, Takayama K. Lectin-functionalized carboxymethylated kappacarrageenan microparticles for oral insulin delivery. Carbohydr Polym 2018;202:555–65.

156. Zhang N, Ping Q, Huang G, Xu W, Cheng Y, Han X. Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. Int J Pharm 2006;327:153–9.

157. Teutonico D, Ponchel G. Patches for improving gastrointestinal absorption: an overview. Drug Discov Today 2011;16:991−7.

158. Banerjee A, Mitragotri S. Intestinal patch systems for oral drug delivery. Curr Opin Pharmacol 2017;36:58–65.

159. Shen Z, Mitragotri S. Intestinal patches for oral drug delivery. Pharm Res 2002;19:391−5.

160. Gupta V, Hwang BH, Lee J, Anselmo AC, Doshi N, Mitragotri S. Intestinal patches for oral delivery of salmon calcitonin. J Control Release 2013;172:753–62.

161. Whitehead K, Shen Z, Mitragotri S. Oral delivery of macromolecules using intestinal patches: applications for insulin delivery. J Control Release 2004;98:37−45.

162. Banerjee A, Wong J, Gogoi R, Brown T, Mitragotri S. Intestinal micropatches for oral insulin delivery. J Drug Target 2017;25:608−15.

163. Toorisioka E, Watanabe K, Ono H, Hirata M, Kamiya N, Goto M. Intestinal patches with an immobilized solid-in-oil formulation for oral protein delivery. Acta Biomater 2012;8:653−8.

164. Gupta V, Hwang BH, Doshi N, Banerjee A, Anselmo AC, Mitragotri S. Delivery of exenatide and insulin using mucoadhesive intestinal devices. Ann Biomed Eng 2016;44:1993−2007.

165. Peppas NA, Wood KM, Blanchette JO. Hydrogels for oral delivery of therapeutic proteins. Expet Opin Biol Ther 2004;4:881−7.

166. Kamei N, Morishita M, Chiba H, Kavimandan NJ, Peppas NA, Takayama K. Complexation hydrogels for intestinal delivery of interferon β and calcitonin. J Control Release 2009;134:98−102.
179. Nakamura K, Murray RJ, Joseph JL, Peppas NA, Morishita M, Lowman AM. Oral insulin delivery using p(MAA-x-EG) hydrogels: effects of network morphology on insulin delivery characteristics. J Control Release 2004;95:589–99.

180. Dorkoosh F, Verhoef JC, Borchard G, Rafiee-Tehrani M, Verheijden J, Junginger H. Intestinal absorption of human insulin in pigs using delivery systems based on superporous hydrogel polymers. Int J Pharm 2002;247:47–55.

181. Yin L, Fei L, Cui F, Tang C, Yin C. Superporous hydrogels containing poly(acrylic acid-co-acrylamide)/O-carboxymethyl chitosan interpenetrating polymer networks. Biomaterials 2007;28:1258–66.

182. Pauletti GM, Gangwar S, Siahaan TJ, Jeffrey A, Borchardt RT. Polymer integrity related absorption mechanism of superporous hydrogel containing interpenetrating polymer networks for oral delivery of insulin. Biomaterials 2010;31:3347–56.

183. Borchardt RT. Optimizing oral absorption of peptides using produgs strategies. J Control Release 1999;62:231–8.

184. Hashizume M, Douen T, Murakami M, Yamamoto A, Takada K, Muranishi S. Improvement of large intestinal absorption of insulin by chemical modification with palmitic acid in rats. J Pharm Pharmacol 1992;44:555–9.

185. Shen WC. Oral peptide and protein delivery: unfilled promises?. Drug Discov Today 2003;8:607–8.

186. Ekrami HM, Kennedy AR, Shen WC. Water-soluble fatty acid derivatives as acylating agents for reversible lipidization of polypeptides. FEBS Lett 1995;371:283–6.

187. Wang J, Chow D, Heiti H, Shen WC. Reversible lipidization for the oral delivery of salmon calcitonin. J Control Release 2003;88:369–80.

188. Niu M, Lu Y, Uzzau S, Wang W, Margerrett K, Pazzani C, et al. Zonula occludens toxin modulates tight junctions through protein kinase c-dependent actin reorganization, in vitro. J Clin Invest 1995;96:710–20.

189. Fasano A, Fiorentini C, Donelli G, Uzzau S, Kaper JB, Margerrett K, et al. Zonula occludens toxin modulates tight junctions through protein kinase c-dependent actin reorganization, in vivo. J Clin Invest 2003;112:191–20.

190. Fasano A, Nataro JP. Intestinal epithelial tight junctions as targets for enteric bacteria-derived toxins. Adv Drug Deliv Rev 2004;56:795–807.

191. Niu M, Tan Y, Uzzau S, Wang W, Margerrett K, Pazzani C, et al. Zonula occludens toxin structure-function analysis. Identification of the fragment biologically active on tight junctions and of the zonulin receptor binding domain. J Biol Chem 2001;276:19160–5.

192. Gopalakrishnan S, Pandey T, Taniz AP, Vere J, Carrasco R, Somervile R, et al. Mechanism of action of ZOT-derived peptide AT-1002, a tight junction regulator and absorption enhancer. Int J Pharm 2009;365:121–30.

193. Kelly CP, Green PH, Murray JA, Colatrella A, Leffler DA, et al. Larazotide acetate in patients with coeliac disease undergoing a gluten challenge: a randomised placebo-controlled study. Aliment Pharmacol Ther 2013;37:252–62.

194. Tavelin S, Hashimoto K, Malkinson J, Lazorova L, Toth I, Margarrett K, et al. Zonula occludens toxin increases transport across the intestinal barrier. Adv Drug Deliv Rev 2009;61:1350–90.

195. Niu M, Lu Y, Uzzau S, Sasaki H, Yonenuma S, Katakura J, Horiguchi Y, et al. Clostridium perfringens enterotoxin fragment removes specific claudins from tight junction strands: evidence for direct involvement of claudins in tight junction barrier. J Cell Biol 1999;147:195–204.

196. Böhmová E, Machová D, Pechár M, Pola R, Venclíková K, Janousková O, et al. Cell-penetrating peptides: a useful tool for the delivery of various cargos into cells. Physiol Res 2018;67:5267–79.

197. Derossi D, Joliot AH, Chassaing G, Prochiantz A. The third helix of the antennapedia homeodomain translocates through biological membranes. J Biol Chem 1994;269:10444–50.

198. Sánchez-Navarro M, García J, Giralt E, Teixidó M. Using peptides to increase transport across the intestinal barrier. Adv Drug Deliv Rev 2016;106:355–66.

199. Danielsen EM, Hansen GH. Impact of cell-penetrating peptides (CPPs) melitin and HIV-1 TAT on the enterocyte brush border using a mucosal explant system. Biochim Biophys Acta-Biomembranes 2018;1860:1589–99.

200. Zhang L, Shi Y, Song Y, Sun X, Zhang X, Sun K, et al. Use of low molecular weight peramine to enhance oral absorption of exenatide. Int J Pharm 2018;547:265–73.

201. Kamei N, Shigei C, Hasegawa R, Takeda-Morishita M. Exploration of oral delivery of insulin. Diabetes Technol Therapeut 2010;12:78–8.
B12-coated dextran nanoparticles. *J Control Release* 2007;122: 141–50.

219. Verma A, Sharma S, Gupta PK, Singh A, Teja BV, Dwivedi P et al. Vitamin B12 functionalized layer by layer calcium phosphate nanoparticles: a mucosadhesive and pH responsive carrier for improved oral delivery of insulin. *Acta Biomater* 2016;31:288–300.

220. Zhang J, Tang C, Yin C. Galactosylated trimethyl chitosan–cytosteine nanoparticles loaded with MAP4K4 siRNA for targeting activated macrophages. *Biomaterials* 2013;34:3667–77.

221. Byeon JC, Lee SE, Kim H, Ahn JB, Kim DH, Choi JS, et al. Design of novel proliposome formulation for antioxidant peptide, glutathione with enhanced oral bioavailability and stability. *Drug Deliv* 2019;26:216–25.

222. East L, Isacke CM. The mannose receptor family. *Biochim Biophys Acta Gen Subj* 2002;1572:364–86.

223. Liu D, Jiang G, Yu W, Li L, Tong Z, Kong X, et al. Oral delivery of insulin using CaCO3-based composite nanocarriers with hyaluronic acid coatings. *Mater Lett* 2017;188:263–6.

224. Amet N, Wang W, Shen WC. Human growth hormone–transferrin fusion protein for oral delivery in hypophysectomized rats. *J Control Release* 2010;141:177–82.

225. Pridgen EM, Alexis F, Kuo TT, Levy-Nissenbaum E, Karnik R, Blumberg BS, et al. Transepithelial transport of Fc-targeted nanoparticles by the neonatal Fc receptor for oral delivery. *Sci Transl Med* 2013;5:213ra167.

226. Zhang N, Ping QN, Huang GH, Xu WF. Investigation of lectin-modified insulin liposomes as carriers for oral administration. *Int J Pharm* 2005;294:247–59.

227. Liu C, Shan W, Liu M, Zhu X, Xu J, Xu Y, et al. A novel ligand conjugated nanoparticles for oral insulin delivery. *Drug Deliv* 2016;23:2015–25.

228. Jin Y, Song Y, Zhu X, Zhou D, Chen C, Zhang Z, et al. Golbet cell-targeting nanoparticles for oral insulin delivery and the influence of mucus on insulin transport. *Biomaterials* 2012;33:1573–82.

229. Russell-Jones G. Intestinal receptor targeting for peptide delivery: an expert’s personal perspective on reasons for failure and new opportunities. *Ther Deliv* 2011;2:1575–93.

230. Hamman JH, Demana PH, Olivier EI. Targeting receptors, transporters and site of absorption to improve oral drug delivery. *Drug Target Insights* 2007;2:71–81.

231. Xie S, Gong YC, Xiong XY, Li ZL, Luo YY, Li YP. Targeted folate-conjugated phoronic P85/poly(allylamine–hydrazide) polymersome for the oral delivery of insulin. *Nanomedicine* 2018;13:2527–44.

232. Kokrashvili Z, Messinger B, Margolkske RF. Taste signaling elements expressed in gut enterocendocyte cells regulate nutrient-responsive secretion of gut hormones. *Am J Clin Nutr* 2009;90:822s–5s.

233. Li Y, Yang B, Zhang X. Oral delivery of imatinib through galactosylated polycrymic nanoparticles to explore the contribution of a saccharide ligand to absorption. *Int J Pharm* 2019;568:118508.

234. Fievez V, Plapied L, des Rieux A, Pourcelle V, Freichels H, Wascotte V, et al. Targeting nanoparticles to m cells with non-peptidic ligands for the oral delivery of insulin. *Acta Biomater* 2017;151:13–22.

235. Wu L, Liu M, Shan W, Zhu X, Li L, Zhang Z, et al. Bioinspired butyrate-functionalized nanovehicles for targeted oral delivery of biomacromolecular drugs. *J Control Release* 2017;262:73–83.

236. Kunisawa J, Kurashima Y, Kiyono H. Gut-associated lymphoid tissues for the development of oral vaccines. *Adv Drug Deliv Rev* 2012;64:523–30.

237. Brayden DJ, Jepson MA, Baird AW. Intestinal peyer’s patch m cells and oral vaccine targeting. *Drug Discov Today* 2005;10:1145–57.

238. Clark MA, Blair H, Liang L, Brey RN, Brayden D, Hirst BH. Targeting polymerised liposome vaccine carriers to intestinal M cells. *Vaccine* 2001;20:206–17.

239. Kim BY, Jeong JH, Park J, Kim JD. Bioadsive interaction and hypoglycemic effect of insulin-loaded lectin-microparticle conjugates in oral insulin delivery system. *J Control Release* 2005;102:525–38.

240. Yoo MK, Kang SK, Choi JH, Park IK, Na HS, Lee HC, et al. Targeted delivery of chitosan nanoparticles to peyer’s patch using M cell-homing peptide selected by phage display technique. *Biomaterals* 2010;31:7738–47.

241. Zhang P, Xu Y, Zhu X, Huang Y, Golbet cell-targeting nanoparticles containing drug-loaded micelle cores for oral delivery of insulin. *Int J Pharm* 2013;496:993–1005.

242. Mohamadzadeh M, Duong T, Sandwick SJ, Hoover T, Klaenhammer TR. Dendritic cell targeting of bacillus anthraxis protective antigen expressed by lactobacillus acidophilus protects mice from lethal challenge. *Proc Natl Acad Sci U S A* 2009;106:4331–6.

243. Kathania M, Zadeh M, Lightfoot YL, Roman RM, Sahay B, Abbott JR, et al. Colonic immune stimulation by targeted oral vaccine targeting. *Adv Drug Deliv Rev* 2005;57:106–10.

244. Ryan GM, Kaminskas LM, Porter CJ. Nano-chemotherapeutics: maximising lymphatic drug exposure to improve the treatment of lymph-metastatic cancers. *J Control Release* 2014;193:241–56.

245. Supersaxo A, Hein WR, Steffen H. Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcaneous administration. *Pharm Res* 1990;7:167–9.

246. Oussoren C, Zuidema J, Crommelin D, Storm G. Lymphatic uptake and biodistribution of liposomes after subcutaneous injection: II. Influence of liposomal size, lipid composition and lipid dose. *Biochim Biophys Acta Biomembr* 1997;1328:261–72.

247. Reddy ST, Van Der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, Blumberg RS, et al. Transepithelial transport of Fc-targeted nanoparticles: a mucoadhesive and pH responsive carrier for targeting polymerised liposome vaccine carriers to intestinal M cells. *Adv Drug Deliv Rev* 2005;57:106–10.

248. Verma A, Gupta PK, Singh A, Teja BV, Dwivedi P et al. Vitamin B12 functionalized layer by layer calcium phosphate nanoparticles: a mucosadhesive and pH responsive carrier for improved oral delivery of insulin. *Acta Biomater* 2016;31:288–300.

249. Marino I, Liu D, Fontana F, Ferreira M, Correia A, Valentino S, et al. Microfluidic nanoscaffold assembly of bioengineered chitosan-modified FcRn-targeted porous silicon nanoparticles @ hypromellose acetate succinate for oral delivery of anti-diabetic peptides. *ACS Appl Mater Interfaces* 2018;10:44354–67.

250. Sun AQ, Zhu L, Luo Y, Xu S, Suchy FJ. Human soluble transporter (host): protein interaction and membrane sorting process. *Int J Biochem Mol Biol* 2012;3:290–301.

251. Varghese Gupta S, Gupta D, Sun J, Dahan A, Tsume Y, Hilfinger J, et al. Enhancing the intestinal membrane permeability of ranamivir: a carrier mediated produg approach. *Mol Pharm* 2011;8:2358–67.

252. Fan W, Xia D, Zhu Q, Li X, He S, Zhu C, et al. Functional nanoparticles exploit the bile acid pathway to overcome multiple barriers of the intestinal epithelium for oral insulin delivery. *Biomaterials* 2017;151:13–22.

253. Wu L, Liu M, Shan W, Zhu X, Li L, Zhang Z, et al. Bioinspired butyrate-functionalized nanovehicles for targeted oral delivery of biomacromolecular drugs. *J Control Release* 2017;262:73–83.

254. Kunisawa J, Kurashima Y, Kiyono H. Gut-associated lymphoid tissues for the development of oral vaccines. *Adv Drug Deliv Rev* 2012;64:523–30.
Oral delivery of proteins and peptides

301. Gupta PN, Vyas SP. Investigation of lectinized liposomes as M-cell targeted carrier-adjuvant for mucosal immunization. *Colloids Surf, B* 2011; 82:118–25.

302. Hegi J, Amighi K, Goole J. Development and evaluation of insulin-loaded cationic solid lipid nanoparticles for oral delivery. *J Drug Deliv Sci Technol* 2016; 36:192–200.

303. Fan T, Chen C, Guo H, Xu J, Zhang J, Zhu X, et al. Design and evaluation of solid lipid nanoparticles modified with peptide ligand for oral delivery of protein drugs. *Eur J Pharm Biopharm* 2014; 88: 518–28.

304. Li X, Qi J, Xie Y, Zhang X, Hu S, Xu Y, et al. Nanoemulsions coated with alginate/chitosan as oral insulin delivery systems: preparation, characterization, and hypoglycemic effect in rats. *Int J Nanomed* 2013; 8:23–32.

305. Lin FY, Chen KH, Miao YB, Chen HL, Lin KJ, Chen CT, et al. Phase-changeable nanoemulsions for oral delivery of a therapeutic peptide: toward targeting the pancreas for antiobesity treatments using lymphatic transport. *Adv Funct Mater* 2019; 29:1809015.

306. Abeer MM, Meka AK, Pujara N, Kumeria T, Strounina E, Nunes R, et al. Rationally designed dendritic silica nanoparticles for oral delivery of exenatide. *Pharmaceutics* 2019; 11:418.

307. Tan X, Zhang Y, Wang Q, Ren T, Gou J, Guo W, et al. Cell-penetrating peptide together with PEG-modified messtructured silica nanoparticles promotes mucous permeation and oral delivery of therapeutic proteins and peptides. *Biomater Sci* 2019; 7:2934–50.

308. Lamson NG, Berger A, Fein KC, Whitehead KA. Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability. *Nat Biomed Eng* 2020; 4:84–96.

309. Scaramuzzi K, Tanaka GD, Neto FM, Garcia PRAF, Gabrili JJM, Kumeria T, et al. Characterization of water-in-oil microemulsion for oral delivery of calcitonin. *Eur J Pharm Biopharm* 2014; 92:369–75.

310. Cao P, Han FY, Grundahl L, Xu ZP, Li L. Enhanced oral vaccine efficacy of polysaccharide-coated calcium phosphate nanoparticles. *ACS Omega* 2020; 5:18185–97.

311. Cho HJ, Oh J, Choo MK, Ha JI, Park Y, Maeng HJ. Chondroitin sulfate-capped gold nanoparticles for the oral delivery of insulin. *Int J Biol Macromol* 2014; 63:15–20.

312. Lundquist P, Artursson P. Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations, and studies in human tissues. *Adv Drug Deliv Rev* 2016; 106:256–76.

313. Sonaje K, Chuang EY, Lin KJ, Yen TC, Su FY, Tseng MT, et al. Opening of epithelial tight junctions and enhancement of paracellular permeation by chitosan: microscopic, ultrastructural, and computed-tomographic observations. *Mol Pharm* 2012; 9:1271–9.

314. Yu Z, Ma L, Ye S, Li G, Zhang X. Construction of an environmentally friendly octenylsuccinic anhydride modified pH-sensitive chitosan nanoparticle drug delivery system to alleviate inflammation and oxidative stress. *Carbohydr Polym* 2020; 236:115972.

315. Thanou M, Nihot M, Jansen M, Verhoef JC, Junginger H. Mono-n-carboxymethyl chitosan (MCC), a polycationic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in *in vitro* and *in vivo*. *J Pharm Sci* 2001; 90:38–46.

316. Gradauer K, Barthelmes J, Vonach C, Almer G, Mangge H, Teubl B, et al. Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats. *J Control Release* 2013; 172:872–8.

317. Sonaje K, Lin KJ, Wang JH, Mi FL, Chen CT, Juang JH, et al. Self-assembled pH-sensitive nanoparticles: a platform for oral delivery of protein drugs. *Adv Funct Mater* 2010; 20:3695–700.

318. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. *Pharm Res* 2007; 24:2198–206.

319. Thanou M, Florea BI, Langemeyer MWE, Verhoef JC, Junginger HE. *N*-Trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug buserelin in *in vitro* (Caco-2 cells) and in *vivo* (rats). *Pharm Res* 2000; 17:27–31.

320. Moghasemi S, Parnian E, Hakanimala A, Darzianiazizi M, Vardanjani MM, Kashanian S, et al. Uptake and transport of insulin across intestinal membrane model using trimethyl chitosan coated insulin niosomes. *Mater Sci Eng C* 2015; 46:333–40.

321. Tsai LC, Chen CH, Lin CW, Ho YC, Mi FL. Development of multifunctional nanoparticles self-assembled from trimethyl chitosan and fucoidan for enhanced oral delivery of insulin. *Int J Biol Macromol* 2019; 126:141–50.

322. Zhang H, Huang X, Sun Y, Xing J, Yamamoto A, Gao Y. Absorption-improving effects of chitosan oligomers based on their mucocadhesive properties: a comparative study on the oral and pulmonary delivery of calcitonin. *Drug Deliv* 2016; 23:2419–27.

323. Zhang X, Sun M, Zheng C, Cao D, Bi Y, Sun J. Preparation and characterization of insulin-loaded bioadhesive PGA nanoparticles for oral administration. *Eur J Pharmaceut Sci* 2012; 45:632–8.

324. Paul PK, Nopparat J, Nuanplub M, Treetong A, Suedee R. Improvement in insulin absorption into gastrointestinal epithelial cells by using molecularly imprinted polymer nanoparticles: microscopic evaluation and ultrastructure. *Int J Pharm* 2017; 530:279–90.

325. Lamson NG, Berger A, Fein KC, Whitehead KA. Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability. *Nat Biomed Eng* 2020; 4:84–96.

326. Abeer MM, Meka AK, Pujara N, Kumeria T, Strounina E, Nunes R, et al. Rationally designed dendritic silica nanoparticles for oral delivery of exenatide. *Pharmaceutics* 2019; 11:418.

327. Tan X, Zhang Y, Wang Q, Ren T, Gou J, Guo W, et al. Cell-penetrating peptide together with PEG-modified messtructured silica nanoparticles promotes mucous permeation and oral delivery of therapeutic proteins and peptides. *Biomater Sci* 2019; 7:2934–50.

328. Lamson NG, Berger A, Fein KC, Whitehead KA. Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability. *Nat Biomed Eng* 2020; 4:84–96.

329. Scaramuzzi K, Tanaka GD, Neto FM, Garcia PRAF, Gabrili JJM, Oliveira DCA, et al. Nanostructured SBA-15 silica: an effective protective vehicle to oral hepatitis B vaccine immunization. *Nano medicine* 2016; 12:2241–50.

330. Cao P, Han FY, Grundahl L, Xu ZP, Li L. Enhanced oral vaccine efficacy of polysaccharide-coated calcium phosphate nanoparticles. *ACS Omega* 2020; 5:18185–97.

331. Choj HJ, Oh J, Choo MK, Ha JI, Park Y, Maeng HJ. Chondroitin sulfate-capped gold nanoparticles for the oral delivery of insulin. *Int J Biol Macromol* 2014; 63:15–20.

332. Lundquist P, Artursson P. Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations, and studies in human tissues. *Adv Drug Deliv Rev* 2016; 106:256–76.

333. Sonaje K, Chuang EY, Lin KJ, Yen TC, Su FY, Tseng MT, et al. Opening of epithelial tight junctions and enhancement of paracellular permeation by chitosan: microscopic, ultrastructural, and computed-tomographic observations. *Mol Pharm* 2012; 9:1271–9.

334. Yu Z, Ma L, Ye S, Li G, Zhang X. Construction of an environmentally friendly octenylsuccinic anhydride modified pH-sensitive chitosan nanoparticle drug delivery system to alleviate inflammation and oxidative stress. *Carbohydr Polym* 2020; 236:115972.

335. Thanou M, Nihot M, Jansen M, Verhoef JC, Junginger H. Mono-n-carboxymethyl chitosan (MCC), a polycationic chitosan derivative, enhances the intestinal absorption of low molecular weight heparin across intestinal epithelia in *in vitro* and *in vivo*. *J Pharm Sci* 2001; 90:38–46.

336. Gradauer K, Barthelmes J, Vonach C, Almer G, Mangge H, Teubl B, et al. Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats. *J Control Release* 2013; 172:872–8.

337. Sonaje K, Lin KJ, Wang JH, Mi FL, Chen CT, Juang JH, et al. Self-assembled pH-sensitive nanoparticles: a platform for oral delivery of protein drugs. *Adv Funct Mater* 2010; 20:3695–700.

338. Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D. Alginate/chitosan nanoparticles are effective for oral insulin delivery. *Pharm Res* 2007; 24:2198–206.

339. Thanou M, Florea BI, Langemeyer MWE, Verhoef JC, Junginger HE. *N*-Trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug buserelin in *in vitro* (Caco-2 cells) and in *vivo* (rats). *Pharm Res* 2000; 17:27–31.
pharmacokinetiks to parenteral octreotide and effective growth hormone suppression. J Clin Endocrinol Metab 2012;97:2362–9.

365. https://www.clinicaltrials.gov/ct2/show/results/NCT01923181?term=oral+semaglutide&phase=1&draw=1&rank=1.

366. Busby RW, Bryant AP, Bartolini WP, Cordero EA, Hannig N, Kessler MM, et al. Linacotide, through activation of guanylate cyclase C, acts locally in the gastrointestinal tract to elicit enhanced intestinal secretion and transit. Eur J Pharmacol 2010; 649:328–35.

367. Rao S, Kupfer Y, Pagala M, Chapnick E, Tessler S. Systemic absorption of oral vancomycin in patients with clostridium difficile infection. Scand J Infect Dis 2011;43:386–8.

368. Fuhrmann G, Leroux J-C. Improving the stability and activity of oral therapeutic enzymes—recent advances and perspectives. Pharm Res 2014;31:1099–105.

369. Company Announcement: financial report for the period 1 January 2015 to 30 June 2015. Novo Nordisk. Available from: https://www.novonordisk.com/content/dam/Denmark/HQ/investors/irmaterial/quarterly_financial_reports/2015/20150806_finanical%20report_ Q2%202015%20UK.pdf [Accessed August 12, 2019].

370. Li J, Wang Y, Han L, Sun X, Yu H, Yu Y. Time—action profile of an oral enteric insulin formulation in healthy Chinese volunteers. Clin Therapeut 2012;34:2333–8.

371. Eldor R, Arbi E, Corcos A, Kidron M. Glucose-reducing effect of the ORMD-0801 oral insulin preparation in patients with uncontrolled type 1 diabetes: a pilot study. PLoS One 2013;8:e59524.

372. Zijlstra E, Heinenmann L, Plum-Mörschel L. Oral insulin released: a structured approach. J Diabetes Sci Technol 2014;8:456–65.

373. Khedkar A, Lebovitz H, Fleming A, Cherrington A, Jose V, Athalye SN, et al. Impact of insulin treagopil and its permeation enhancer on pharmacokinetics of metformin in healthy volunteers: randomized, open-label, placebo-controlled, crossover study. Clin Transl Sci 2012;5:276–82.

374. Khedkar A, Lebovitz H, Fleming A, Cherrington A, Jose V, Athalye SN, et al. Pharmacokinetics and pharmacodynamics of insulin treagopil in relation to premeal dosing time, between meal interval, and meal composition in patients with type 2 diabetes mellitus. Clin Pharmacol Drug Develop 2020;9:74–86.

375. Geho WB, Geho HC, Lau JR, Gana TJ. Hepatic-directed vesicle insulin: a review of formulation development and preclinical evaluation. J Diabetes Sci Technol 2009;3:1451–9.

376. Geho WB, Rosenberg LN, Schwartz SL, Lau JR, Gana TJ. A single-blind, placebo-controlled, dose-ranging trial of oral hepatitis C, acts locally in the gastrointestinal tract to elicit enhanced intestinal secretion and transit. Eur J Pharmacol 2010; 649:328–35.

377. Karsdal MA, Byrjalsen I, Henriksen K, Riis BJ, Lau EM, Arnold M, et al. The effect of oral salmon calcitonin delivered with 5-CNAC on bone and cartilage degradation in osteoarthritic patients: a 14-day randomized study. Osteoarthr Cartilage 2010;18:150–9.

378. https://www.protagonist-inc.com/pipeline/product-candidates/.

379. http://avaxiabiologics.com/programs/avx-470.php.

380. http://protalix.com/pipeline/.

381. Sung HW, Liao ZX, Peng SF, Tu H, inventors; GP Medical, Inc., assignee. Nanoparticles for protein delivery. J Biomed Mater Res B 2015;133:120–39.

382. Transgene reports excellent results from TrabiOral™ oral insulin study. Adv Drug Deliv Rev 2019;127:233–8.

383. Thwala LN, Préat V, Csaba NS. Emerging delivery platforms for oral administration of biopharmaceuticals: a critical update on recent advances and perspectives. J Diabetes Sci Technol 2012;6:1451–9.

384. Zhao R, Lebovitz H, Fleming A, Cherrington A, Jose V, Athalye SN, et al. Pharmacokinetics and pharmacodynamics of insulin treagopil in relation to premeal dosing time, between meal interval, and meal composition in patients with type 2 diabetes mellitus. J Diabetes Sci Technol 2014;8:551–9.

385. Karsdal MA, Byrjalsen I, Henriksen K, Riis BJ, Lau EM, Arnold M, et al. The effect of oral salmon calcitonin delivered with 5-CNAC on bone and cartilage degradation in osteoarthritic patients: a 14-day randomized study. Osteoarthr Cartilage 2010;18:150–9.

386. https://www.protagonist-inc.com/pipeline/product-candidates/.

387. http://avaxiabiologics.com/programs/avx-470.php.

388. http://protalix.com/pipeline/.

389. Sung HW, Liao ZX, Peng SF, Tu H, inventors; GP Medical, Inc., National Tsing Hua University, assignee. Nanoparticles for protein drug delivery. United States patent US No. 8283317B1. 2012 Oct 9.

390. Transgene reports excellent results from TrabiOral™ oral insulin studies, Available from: http://www.transgenebiotek.com/images/news/news/press_release_120521_chinese_insulin_efficacy_studies.pdf [Accessed 7 December, 2015].

391. Thwala LN, Prett V, Csaba NS. Emerging delivery platforms for mucosal administration of biopharmaceuticals: a critical update on nasal, pulmonary and oral routes. Expert Opin Drug Deliv 2017;14:23–36.

392. https://emisphere.com/technology/.

393. Maggio ET. Novel formulations for non-invasive delivery & stabilization of peptides. Biopolymers 2013;13:68–75.