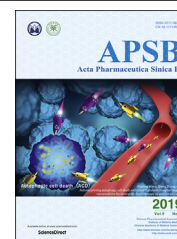




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REVIEW

Multifunctional oral delivery systems for enhanced bioavailability of therapeutic peptides/proteins



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Abstract In last few years, therapeutic peptides/proteins are rapidly growing in drug market considering their higher efficiency and lower toxicity than chemical drugs. However, the administration of therapeutic peptides/proteins is mainly limited in parenteral approach. Oral therapy which was hampered by harsh gastrointestinal environment and poorly penetrating epithelial barriers often results in low bioavailability (less than 1%–2%). Therefore, delivery systems that are rationally designed to overcome these challenges in gastrointestinal tract and ameliorate the oral bioavailability of therapeutic peptides/proteins are seriously promising. In this review, we summarized various multifunctional delivery systems, including lipid-based particles, polysaccharide-based particles, inorganic particles, and synthetic multifunctional particles that achieved effective oral delivery of therapeutic peptides/proteins.

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1. Introduction

Therapeutic peptides/proteins presenting superior potencies and fewer side effects compared to chemical drugs are springing up like mushrooms in drug market recently. With the common physicochemical characteristics of high molecular weight (MW), hydrophilicity and enzyme/pH sensitivity, protein drugs presenting poor oral bioavailability are almost administrated parenterally, while only few of them, for instance, desmopressin, glutathione, etc., are commercially available¹. The development of oral administration for peptides/proteins as a non-invasive therapeutic method has emerged as an attractive alternate to the parenteral route in recent years, considering long-term dosing, safety, convenience, less pain and fewer burdens of medical costs². It is the most advantageous approach of drug delivery, in particular for the treatment of chronic diseases such as diabetes and hepatitis B, which demand long-term drug administration. Especially, the pharmacokinetics (PK) of insulin (Ins) delivered orally mimics the pulsatile secretion pattern and physiological fate of endogenous Ins in the body, which is delivered to the liver exploiting the first-pass metabolism rather than peripheral tissues leading to a lot side effects compared with parenteral administration³. Moreover, in paediatric use, human growth hormone (hGH) has to be given per day during a long time, which makes the parenteral administration unacceptable, particularly when applying to younger children. Several efforts have been made in recent years for oral delivery of therapeutic peptides/proteins. Part of promising oral delivery systems of peptides/proteins have progressed to clinical trials, as indicated in Table 1. However, peptides/proteins usually have poor oral bioavailability (<2%) due to the unfavorable physiological environment (pH, enzymes) and complex biological barriers (mucus layer, epithelial cells, tight junctions) in the gastrointestinal tract (GIT)⁴ (Fig. 1). To overcome the harsh environments in GIT for oral delivery of therapeutic peptides/proteins and improve their oral bioavailability, increasing number of multifunction systems are designed and researched. Summary of representative therapeutic peptides/proteins under research for oral delivery are listed in Table 2.

1.1. Gastrointestinal barriers

Multiple digestive enzymes such as pepsin, chymotrypsin, trypsin and bile salts in the GIT may lead to early leakage and degradation of the cargos. Besides, complex pH values varying in different regions of the GIT intensified the difficulties of oral delivery as pH 2.0–4.0 in stomach, pH ~5.5 in duodenum, pH ~6.0 in jejunum,

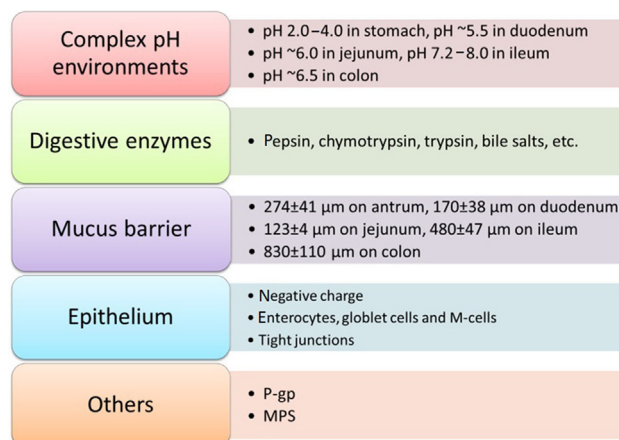


Figure 1 The physiological barriers during oral absorption of peptides/proteins.

pH 7.2–8.0 in ileum and pH ~6.5 in colon⁵⁶. Even if these challenges above are safely undergone, the mucosal layer covering the entire GIT, which lubricates and protects the epithelial layer, would keep out most foreign particles. The mean thicknesses of mucus in different regions of the GIT alter greatly by $274 \pm 41 \mu\text{m}$ on antrum, $170 \pm 38 \mu\text{m}$ on duodenum, $123 \pm 4 \mu\text{m}$ on jejunum, $480 \pm 47 \mu\text{m}$ on ileum and $830 \pm 110 \mu\text{m}$ on colon⁵⁷. In addition, the glycosylation of threonine, proline and serine domains in mucins endow the epithelium with negative charge. The intestinal epithelium mainly composed of three kinds of cells: enterocytes, goblet cells and Microfold cells (M-cells), in which enterocytes are the majority, goblet cells that secrete mucin occupied 10%–20% of epithelial cells, M-cells mainly located in Peyer's patches in the ileum representing less than 1% of the total epithelial surface are capable of transporting drugs from the epithelium to the underlying lymphoid tissues⁵⁸. Tight junctions (TJs) localized between the intestinal epithelial cells are another restrictions towards the absorption of hydrophilic peptides/proteins *via* paracellular way, of which the gap is about 7–9 Å for the jejunum, 3–4 Å for the ileum, and 8–9 Å for the colon⁵⁹.

1.2. Strategies to overcome the barriers in GIT

To safely pass the gastric acid in stomach, enteric materials have been applied to encapsulate drug carriers to make them more resistant to the environment as well as control their release⁶⁰. However, the enteric polymer coated may not dissolve completely in the small

Table 1 Examples of clinically delivery systems for oral peptides/proteins (clinicaltrials.gov).

Technology	Drug	Phase	Company
Eligen [®]	Insulin	I	Emisphere Technologies, Inc. (USA)
Mycapssa [™]	Octreotide	III	Chiasma, Inc. (USA)
Peptelligence [™]	Leuprolide	II	Enteris BioPharma, Inc. (USA)
Eligen [®]	GLP-1	II	University Hospital, Basel (Switzerland)
Peptelligence [™]	PTH	II	Entera Bio Ltd. (Israel)
Eligen [®]	Semaglutide	III	Novo Nordisk A/S (Denmark)
Eligen [®]	Salmon calcitonin	III	Nordic Bioscience A/S (Denmark) and Novartis (Switzerland)
POD [™]	Insulin	II	Oramed, Ltd. (Israel)
Oshadi Icp	Insulin	I	Oshadi Drug Administration (Israel)
Peptelligence [™]	Salmon calcitonin	II	Tarsa Therapeutics, Inc. (USA)

Table 2 Representative therapeutic peptides/proteins under research for oral delivery.

Name	Structure/composition	Primary therapy	Ref.
EGFR targeted hybrid peptide	In a total of 32 amino acid residues and a molecular weight of 3774 Da	Highly selective activity toward EGFR-positive cancer cells	5
Vancomycin	A branched tricyclic glycopeptide	Infections by Gram-positive bacteria	6
Myrccludex B	A linear myristoylated peptide composed of 47 amino acids	Hepatitis B	7
hGH	A 191 amino acids protein	Adult growth hormone deficiency and children's growth disorders	8
Octreotide	An 8 amino acids synthetic analogue of somatostatin	Acromegaly, psoriasis and gastro-intestinal disorders	9,10
Urokinase	A protein consisting of 411-amino acid residues	A thrombolytic agent	11
Rituxan	A whole antibody with a molecular weight of about 144 kDa	Non-Hodgkin's lymphoma, chronic lymphocytic leukemia	11
sCT	With a molecular weight of 3431 Da and composed of 32 amino acids	Paget's disease	11–14
Polypeptide-k	Contained 9 out of 11 essential amino acids, among a total of 17 types, 168 amino acids	Antidiabetic	15
Dalargin	A model opioid peptide composed of 6 amino acids with a molecular weight of 725 Da	Immunoregulation	16
Elisidepsin	A synthetic marine-derived cyclic peptide	Antitumor	17
Cholera toxin	An oligomeric complex made up of six protein subunits with a molecular weight of 83 kDa	As a neuronal tracer	18
Ovalbumin	Consisted of 385 amino acids and has a relative molecular mass of 42.7 kDa	Used in proteomics and immunology	18–20
BSA	With a molecular weight of 66.5 kDa and composed of 607 amino acids	Often used as a blocker in immunohistochemistry	21–25
Lysozyme	With a molecular weight of 14 kDa and consisting of 130 amino acids	Diarrhea	21,26,27
SOD	A homodimer of molecular weight-33 kDa	Inflammatory bowel diseases	28
Antide	A decapeptide with an antagonist of GnRH	Endometriosis and uterine fibrosis	29
IAPP	A 37-residue peptide hormone	Obesity	30
Irisin	A peptide hormone composed of 111 amino acids	Obesity	30
SIINFEKL (OVA257-264)	A 8-amino-acid peptide	A specific antigenic peptide	31
Exendin-4	A 39-amino-acid peptide	Type 2 diabetes	32–38
GLP-1	A 30 amino acid long peptide hormone	Type 2 diabetes	39,40
Insulin	A dimer of an A-chain and a B-chain composed of 51 amino acids and has a molecular weight of 5808 Da	Diabetes	41–55

hGH, human growth hormone; IAPP, islet amyloid polypeptide; GLP-1, glucagon-like peptide-1; GnRH, gonadotropin hormone-releasing hormone; SOD, superoxide dismutase; BSA, albumin from bovine serum; sCT, salmon calcitonin; EGFR, epidermal growth factor receptor.

intestine leading to a certain number of cargos sticking or aggregating in the partly dissolved shell, which may lower their oral bioavailability. Chuang et al.⁶¹ developed a bubble carrier system which generated nanosized CO₂ bubbles while water passes through the gelatin shell to saturate the compounds and continue to expand till contacting with the mucus and finally burst and liberate the contents. The bubble carrier system produced steady plasma glucose levels (PGL) for over 10 h and a relative bioavailability of $21.7 \pm 1.7\%$. To penetrate through the widely distributed mucus, mucosal adhesive agents as well as mucosal penetrate agents are extensively used. Mucoadhesive materials represented by chitosan (CS), alginate (ALG), methacrylate can be incorporated to prolong the residence time of peptides/proteins in intestine⁶². The highest mucoadhesion was shown by thiolated polymers at pH 3.0. Other tested polymers like cellulose derivatives, polyvinylpyrrolidone and polyethyleneglycole showed low to almost no mucoadhesion⁶³. Beyond those strategies applied above, to optimize the efficacy in oral delivery, often the tuning of physicochemical properties like electrical property and particle size is necessary⁶⁴. Surface charge plays a critical role in

the uptake of nanoparticles (NPs) because of the anionic intestinal barrier. Czuba et al.⁶⁵ proved that formulation of negatively charged NPs represents a promising approach to improve NPs' uptake and oral bioavailability of Ins. Furthermore, particle size is thought to be a critical factor affecting the bioavailability of NPs following oral exposure since larger size may be intercepted by the steric barriers of mucosal network. Barbari et al.⁶⁶ prepared ultrasmall (<15 nm), monodispersed and water-dispersible NPs applying a simple and reproducible water-in-oil (w/o) nanoemulsion technique. Results exhibited 15%–19% enhanced transshipment of Ins across the cell monolayer. He et al.⁶⁷ reported a new method, termed flash nanocomplexation (FNC), to fabricate smaller size (45 nm) and higher encapsulation efficiency (EE, >90%) of Ins loaded NPs by infusing aqueous solutions of CS, tripolyphosphate (TPP), and Ins under rapid mixing condition ($r > 1600$) in a 4-inlet vortex mixer. Results showed that smaller NPs could regulate the PGL more effectively than the larger. When evaluating particle characteristics that influence their uptake, agglomerate state in the GIT must be assessed. Hinkley et al.⁶⁸ demonstrated that different from uncoated

gold NPs that are tend to be agglomerated, polyethylene glycol (PEG)-coated gold NPs can be observed as primary, un-agglomerated particles throughout the GIT using transmission electron microscopy (TEM). Strategies of co-administration with protease inhibitors (PIs) such as aprotinin and calcium chelators could help NPs safely undergo the enzymes in GIT. However, relatively high doses of the PIs may cause safety dangers following repeated administrations⁶⁹. To pass the epithelial barriers two strategies applied have achieved considerable successes: paracellular pathway by opening TJs and transcellular transcytosis ways by clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis or phagocytosis⁷⁰. The primary approaches that the peptides/proteins loaded NPs traverse the intestinal epithelium are manifested in Fig. 2. Strategies of co-administration with penetrate enhancers (PEs) such as bile salts, fatty acids, surfactants, CS and derivatives, chelating agents and other enhancers could help traverse poorly penetrating intestinal barriers in GIT⁶⁹. For instance, Gaowa et al.⁵ prepared epidermal growth factor receptor (EGFR)-targeted hybrid peptide/bile acid complexes *via* electrostatic interactions. The *in vitro* permeability of the complexes across Caco-2 cell monolayers was 5.0-fold higher than free peptide.

Furthermore, after treated with the complexes *in vivo*, the mean tumor volume reduced 1.6-fold than that of the free peptide. Cell-penetrating peptides (CPPs) composed of 5–30 amino acid residues have been investigated for several years as PEs through electrostatic or covalent conjugation with hydrophilic macromolecules for oral delivery⁷¹. The most extensively applied synthetic CPPs include MAP, octa-arginine (8R), CADY, and polylysine. CPP-mediated delivery has been reported to take place *via* multiple endocytosis ways including macropinocytosis, caveolae mediated and clathrin-mediated pathways⁷². In addition, more efficient and specific delivery can be realized by incorporating actively targeted ligands into CPP based systems. There are a variety of special receptors such as vitamins, transferrins, amino acids and sugar receptors expressing on the epithelium of intestine⁷³. Being more effective with low poisonous, strategies containing targeted ligands *via* receptor-mediated transport have become the major impetus towards the oral delivery that can overcome the intestinal barriers^{74,75}. Representative ligand-mediated transports in oral delivery are shown in Table 3. In addition, drug co-loading technique can be applied to enhance the therapeutic effect and bioavailability of peptides. Araújo et al.^{39,40} orally co-delivered

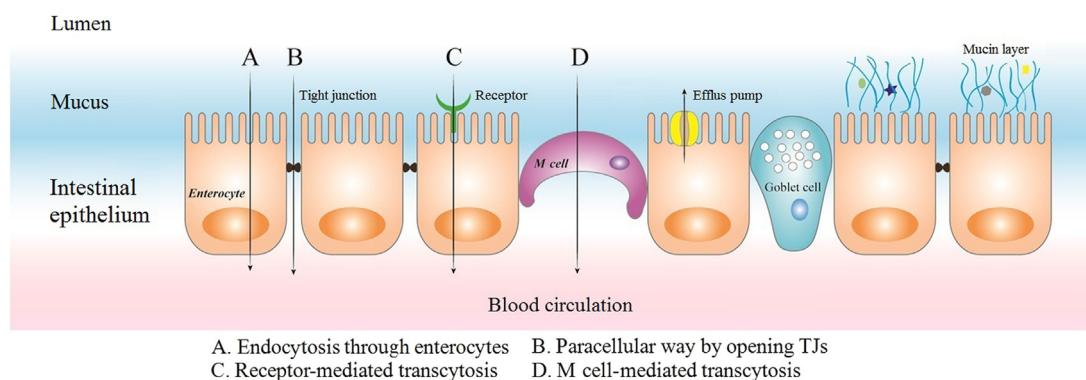


Figure 2 Primary approaches for the peptides/proteins loaded nanoparticles traversing the epithelium.

Table 3 Representative ligand-mediated transport in oral delivery of therapeutic peptides/proteins.

Name	Distribution/Function	Characteristics	Ref.
Bile acid transporters	In the epithelium of ileum	The ASBT in the small intestine transports bile acids into epithelial cells for bile acid recycling	5,48,52,76–81
UEA-1	In M-cell	M-cell selective molecular signature	20
Lectin-like protein receptors	In the intestine	Proteins or glycoproteins specifically recognize the carbohydrate moieties on the intestine	42
Biotin (vitamin B7) receptor	In the intestine	The biotin receptor distributes throughout the small intestine	55
Proton-coupled oligopeptide transporters PepT1 and PepT2	In the brush border membrane of the small intestine	Driven by the presence of an inward H^+ gradient and a negative membrane potential. Transports various natural di/tri-peptides and comprehensive peptide-mimetics. High capacity, low affinity	82,83
CSK peptide transporters	In goblet cells	CSK peptide specifically recognize goblet cells	84
AT-1002 peptide	Open TJs	A hexamer peptide derived from ZOT open the TJs transiently and reversibly	85
Monocarboxylate transporter	In the intestine	Cellular uptake of SCFAs efficiently, among which butyrate is in majority, a key mediator of physiological function in the intestine	86
CD44 receptor	In the intestine	A highly heterogeneous single-stranded transmembrane glycoprotein widely expressed on the membrane	87

ASBT, apical sodium-dependent bile acid transporter; CSK, CSKSSDYQC; ZOT, zonula occludens toxin; TJs, tight junctions; UEA-1, ulex europaeus agglutinin-1; SCFAs, short chain fatty acids.

glucagon-like peptide-1 (GLP-1) and dipeptidyl peptidase 4 inhibitor (DPP4I) by a multifunctional tailorable composite system. In the presence of DPP4I, the permeability of GLP-1 across the cell monolayers was even higher, with 1.5-fold increase for the porous silicon (PSi) systems and 5-fold increase for the poly(lactide-co-glycolide) (PLGA) systems.

Above all, we can see that versatile formulation strategies have been evolved to enhance the oral bioavailability of peptides/proteins. The objective of our review is to put forward a systematic overview on recently published promising delivery systems represented by lipid-based particles, polysaccharide-based particles, inorganic particles, and synthetic functional particles for oral delivery of therapeutic peptides/proteins. Typical structures of peptides/proteins carriers are demonstrated in Fig. 3. These works are in particular concerned with formulation strategies for controlled drug release, meliorative pharmacokinetics/pharmacodynamics and other modulated physicochemical characteristics. Several novel techniques developed to optimize the preparation of oral delivery carriers are shown in Table 4. We also discuss the strategies to enhance the oral bioavailability of peptides/proteins and make suggestions for further design in oral delivery systems (Figs. 4–6).

2. Lipid-based particles

Lipid-based carriers have attracted much attention for their excellent biocompatibility to cross the intestinal barrier^{89,90}. Summary of lipid-based particles applied in oral delivery of therapeutic peptides/proteins was shown in Table 5.

2.1. Liposomes

Liposomes with micro-vesicular structures are composed of aqueous cores and amphiphilic bilayers. Considering that conventional phospholipid/cholesterol liposomes are compromised to the hostile environment in GIT by phospholipid hydrolysis or oxidation and moreover restricted by aggregation, sedimentation, and fusion⁹¹, well-designed liposomes are extremely essential for effective delivery.

Niu et al.^{52,80,81} compared the effect of three kinds of liposomes loaded with SGC, sodium taurocholate (STC) or sodium deoxycholate (SDC). The hypoglycemic effect was size-dependent with the highest at 150 or 400 nm and oral bioavailability were in the order of SGC ($8.5 \pm 2.1\%$) > STC > conventional liposomes > SDC. Liposomes containing bile salts showed prolonged residence time and enhanced permeation across the membranes. They further investigated the transiting fate of bile acid-liposomes and verified the absorption of intact liposomes rather than free Ins. Additionally, ergosterol was found to be a substitute for cholesterol and bile salt derivatives in liposomes. Cui et al.⁹² screened liposomes contained ergosterol (Er-Lip) of botanical origin rather than cholesterol as the stabilizer. Results indicated that Er-Lip was more stable and enhanced oral bioavailability of Ins more significantly. Thiomers-coated liposomes present favorable characteristics of mucoadhesion, TJs-opening effect, efflux pumps inhibition, and enzyme inhibition^{93–95}. Gradauer et al.¹⁴ coupled CS and thioglycolic acid (TGA) to coat sodium calcitonin (sCT)-loaded liposomes, these thiomers-coated liposomes causing no immunogenic reactions in mice showed enhanced permeation of

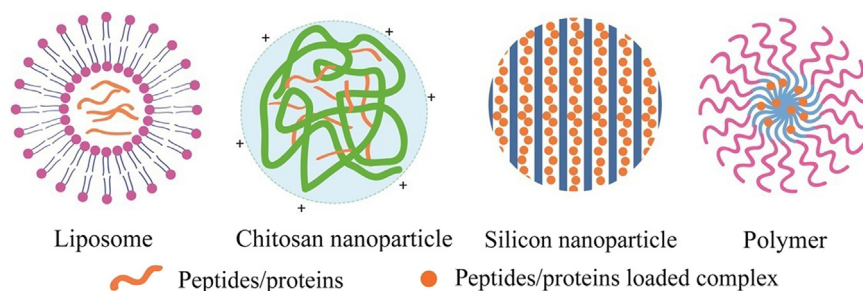


Figure 3 Typical structures of peptides/proteins loaded nanocarriers.

Table 4 Novel techniques for oral delivery of therapeutic peptides/proteins.

Experimental Techniques	Advantages/Improvements	Ref.
CARS microscopy	To image protein and lipid distributions without prior labeling or destructive sample preparation	26
Microfluidics technique	Emulsions are formed with an exquisite control and a quite high encapsulation efficiency compared to the conventional production methods	39,40
Oral dual-delivery of GLP-1 and DPP4 inhibitor	The permeability of GLP-1 across the cell monolayers was higher while coloaded DPP4 inhibitor	39,40
A novel w/o nanoemulsion technique	A simple and reproducible technique making ultrasmall (<15 nm), monodispersed and water-dispersible NPs	66
FNC	The optimized FNC process produces NPs with a smaller size (45 nm) and higher encapsulation efficiency (90%) compared with the bulk-mixing method	67
Three-layer release technology	The platform consisting of neutral polymethacrylate Eudragit® NE as a flexible film, superdisintegrant sodium starch glycolate Explotab® as a pore former and applied to a HPMC coating of reduced thickness delays the drug release.	88

GLP-1, peptide glucagon-like peptide-1; DPP4, dipeptidyl peptidase 4; NPs, nanoparticles; CS, chitosan; CARS, coherent anti-stokes Raman scatteri; HPMC, hydroxypropyl methylcellulose.

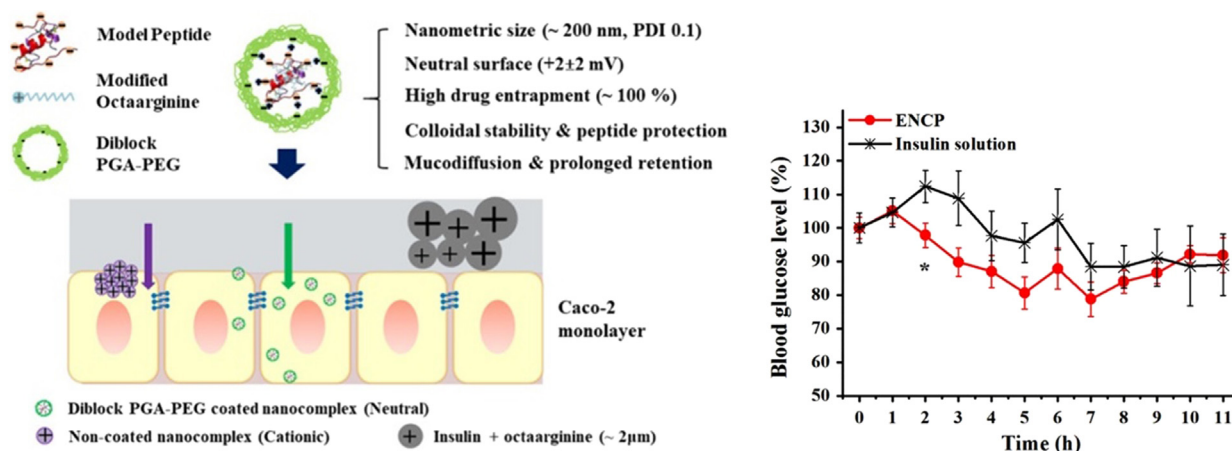


Figure 4 Schematic illustration of rational design of octaarginine-based nanoparticles and their hypoglycemic effect in rats⁷⁸. Adapted from Niu et al.¹⁶⁸ with permission © 2018 Elsevier Ltd.

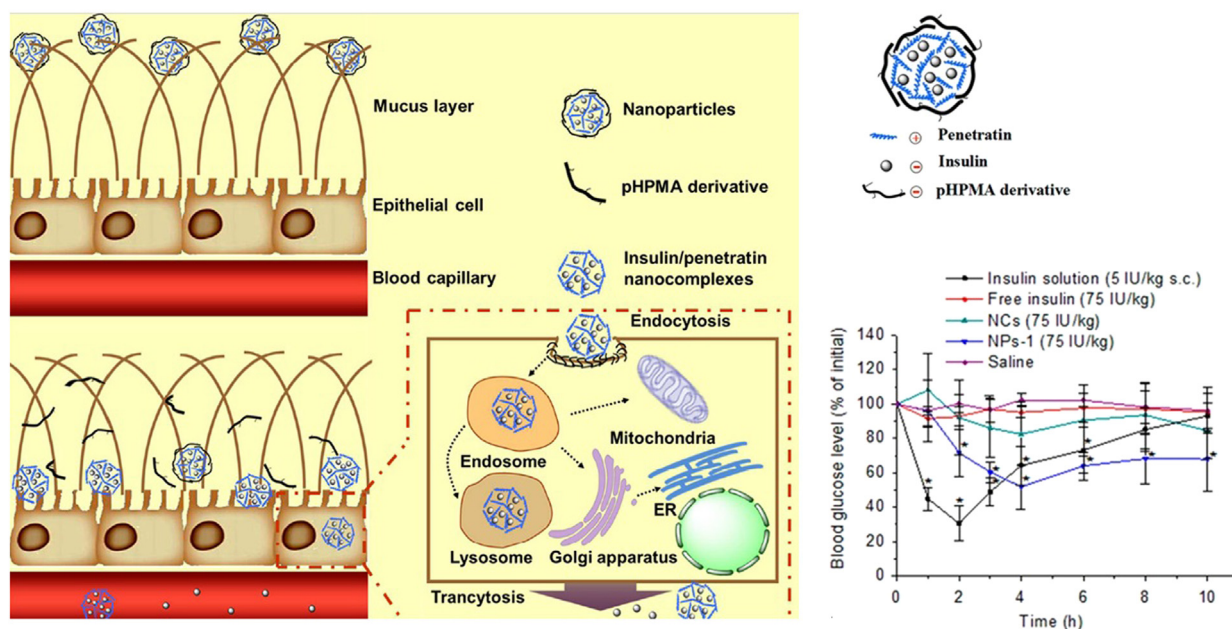


Figure 5 Schematic illustration of the self-assembled NPs overcoming the mucus barrier and epithelium barrier¹⁶³. Adapted from Shan et al.¹⁶³ with permission © 2015 American Chemical Society.

membranes and inhibitory properties of efflux pump. These coated liposomes reduced the blood calcium level to a minimum of 65% of the initial value after 6 h. Comparing the areas under curves (AUC) of the blood calcium levels, these coated liposomes led to an 8.2-fold increase compared to the free sCT solution. Biotin (vitamin B7) is a promising ligand for oral delivery because its receptor distributes throughout the small intestine. Through the incorporation of biotin-conjugated 1,2-distearoyl-*sn*-glycero-3-phosphatidyl ethanolamine (DSPE) into the liposome membranes, Zhang et al.⁵⁵ produced biotinylated liposomes (BLPs) for oral delivery of Ins and this BLPs achieved a relative bioavailability of 12.09%, approximately twice than that of conventional liposomes.

To enhance the oral bioavailability of encapsulated peptides/proteins, liposomes should maintain their vesicular form and avoid early leakage of loaded cargos by withstanding the destruction of

bile salts, pancreatic enzymes and the acidic conditions in GIT⁹⁶. Separated from prokaryotic microorganisms, archaea with unique membrane lipids can survive extreme environments in GIT. These tetraether lipids containing membrane spanning hydrocarbon chains linked through ether bonds are much more stable than conventional phospholipids in harsh circumstances. Two major groups of archaeal membrane lipids are known as diphytanylglycerol diether lipids (DELs) and its derivatives, dibiphytanylglycerol tetraether lipids (TELs) and its derivatives. Parmentier et al.⁹⁷ tested *in vitro* that particular TEL-glycerylcaldityl tetraether (GCTE)-stabilised liposomes could maintain the integrity of membrane and protect encapsulated proteins from degradation. They further confirmed that the relative oral bioavailability of human growth hormone (hGH) loaded tetraether lipids containing cetylpyridinium chloride (CpCl) as a bio-enhancer was around

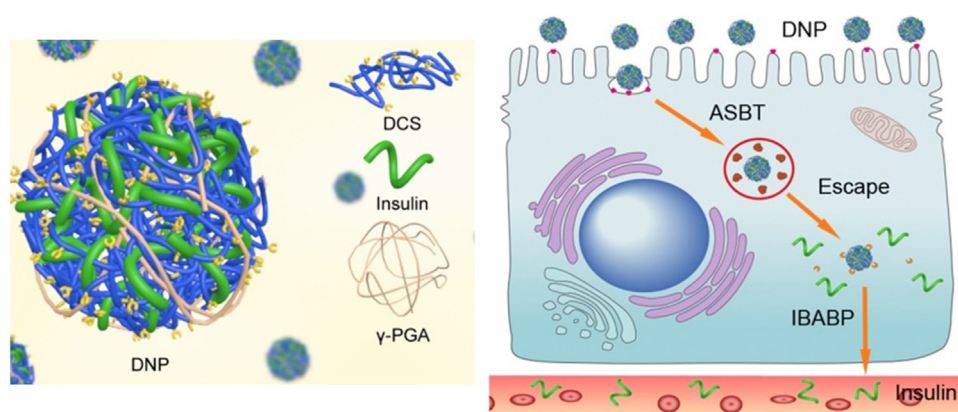


Figure 6 Schematic illustration of transepithelial transport of Ins from DNP to overcome multiple barriers of the intestinal epithelium by exploiting the bile acid pathway¹⁶⁸. Adapted from Fan et al.⁷⁸ with permission © 2017 Elsevier Ltd.

3.4% whereas free hGH administered orally was only 0.01%⁸. What's more, liposomes with 25% TEL could improve the oral bioavailability of octreotide more than 4-fold compared with free octreotide⁹. Uhl et al.⁶ found that an almost two-fold elevation in vancomycin uptake of TEL-liposomes over conventional liposomes was detected in Wistar rats. He and his group²⁸ further showed that myrcludex B-loaded GCTE-liposomes contributed 1.6-fold higher of relative oral bioavailability than the standard liposomes. Proliposomes, prepared by adsorption of drug and phospholipids on to the carriers having microporous matrix like mannitol and sorbitol, are free-flowing powdered particles superior to conventional liposomes. Sharma et al.⁹⁸ employed protamine sulphate (Pt) as a PE and prepared Pt-rhIns proliposomes encased in Eudragit S100, which ameliorated the cellular uptake of rhIns almost 4.0-fold compared to free rhIns.

The fate of these nanocarriers (NCs) after oral delivery is still unknown, which can be explained by the difficulty to find relevant approaches to confirm the real integrity of the carriers, that is whether the cargos are still inside the carriers or not. Indeed, several techniques such as dynamic light scattering (DCS), confocal laser scanning microscopy (CLSM) and Fourier transform infrared spectroscopy (FTIR) could characterize the whole structure of carrier or its individual parts but not the integrity. A novel technique, Förster resonance energy transfer (FRET), which is highly sensitive to donor–acceptor distances and indicates preserved nanoscale environment can quantify the integrity of nanocarriers⁹⁹. Roger et al.¹⁰⁰ studied the fate of lipid nanocapsules after their transportation across Caco-2 cell model employing FRET and Nanoparticle Tracking Analysis. Results showed that the presence of NPs in the basolateral side have a measurable FRET signal after 2 h, which verifies the intact crossing of the NCs.

2.2. Solid lipid particles

Solid lipid particles (SLPs) are made of natural, semi-synthetic or synthetic lipids containing triglycerides, fatty acids, partial glycerides, phospholipids and steroids, which are widely considered as safe and biodegradable¹⁰¹.

Christophersen et al.^{26,27} investigated that the SLPs with different types of lipid excipients exhibited a lipase-mediated degradation mechanism on release of proteins. He and his colleagues further proved that lysozyme incorporated SLPs in an aqueous solution released lysozyme much faster than in a solid.

However, the hydrophobic nature of SLPs limits its encapsulation of hydrophilic peptides accounting for low EE. Hecq et al.¹⁰² dissolved Ins into the inner aqueous phase and then emulsified in an organic phase to prepare a bioadhesive cationic SLPs, which increased 2.5-fold the transshipment of capped Ins through co-cultured Caco-2/HT29 cells compared to free Ins. Boushra et al.¹⁰³ adopted three different hydrophilic viscosity-enhancing agents (VAs): propylene glycol (PG), PEG 400 and PEG 600 within SLP cores to develop Ins-loaded NCs with enhanced viscosity. The highest EE was achieved by 70% (w/w) PG contained NCs (54.5%) compared to only 20.4% in unmodified SLN and achieved good hypoglycemic effect with a relative bioavailability of 5.1% following oral administration. It should be noted that the mucosal layer has a significant impact on determining the efficiency of oral nanoformulations. It is also shown that low MW and high surface coverage of PEG could minimize mucoadhesion and enhance the hydrophilic of SLPs. Yuan et al.¹⁰⁴ evaluated that the permeation ability of PEGylated (MW=2 kDa) SLPs were decreased through Caco-2 cell monolayer while increased through a mucus-secreting co-cultured Caco-2/HT29 cells. The relative oral bioavailability of PEGylated SLPs elevated 1.99-fold compared to unmodified ones.

2.3. Self-nanoemulsifying drug delivery system (SNEDDS)

SNEDDS is an (o/w) nanoemulsion spontaneously formed by isotropic mixtures of oil, surfactant and cosurfactant through mixing with water¹⁰⁵.

Karamanidou et al.⁴⁹ developed a new mucus permeating SNEDDS formulation, incorporating a hydrophobic ion pair of Ins/dimyristoyl phosphatidylglycerol (DMPG), which exhibited enhanced mucus penetration and an EE of 70.89%. Li et al.¹⁰⁶ prepared Ins–phospholipid complex-loaded SNEDDS and then coated with Eudragit[®] L100, the EE of which was increased from 18.6% of single Ins to 73.1%. Oral administration of complex-loaded SNEDDS enhanced 2.7-fold the relative bioavailability and 3.4-fold the reduction of PGL separately compared to Ins-loaded carriers. Garg et al.¹⁵ designed a successful SNEDDS for oral delivery of polypeptide-k (PPK) which exhibited stable characters and promising antidiabetic potentials compared to its native form. It is reported that smaller SNEDDS have higher mucus permeating abilities and anionic SNEDDS demonstrated a better permeation rate than positively charged ones¹⁰⁷. Recently, several studies are focusing on the protective effects against protease degradation.

Table 5 Summary of lipid-based particles in oral delivery of therapeutic peptides/proteins.

Formulation composition	Model drug	Main transport mechanisms	Characterization (size, ZP, EE)	PK		PD	Ref.
				Dose	<i>F</i> (%)		
GCTE-liposomes	Vancomycin	N/A	Size: 134.0 ± 9.7 nm; ZP: -4.43 ± 0.81 mV; EE: 58.53 ± 1.76%	N/A	N/A	N/A	6
GCTE-liposomes	Myrcludex B	N/A	Size: 140.7 ± 4.3 nm; ZP: -4.20 ± 0.48 mV; EE: 65.67 ± 2.91%	N/A	N/A	3.5-Fold increase compared to the free peptide	7
Liposomes containing bio-enhancers and tetraether lipids	hGH	N/A	Size: 229.7 ± 12.8 nm; ZP: 41.0 ± 1.2 mV; EE: 31.2 ± 0.5%	8 mg	3.4	N/A	8
Liposomes with 25% TELs	Octreotide	N/A	Size: 130–207 nm; EE: 13.0%	N/A	N/A	4-Fold the hypoglycemic effect compared with free octreotide	9
Octreotide-DOCA SEDDS	Octreotide	N/A	Size: 152 nm; ZP: -3.7 mV	50 mg (pig)	5.21	N/A	10
CS-TGA-MNA-coated liposomes	sCT	TJs opening	Size: 604.8 ± 29.6 nm; ZP: 27.9 ± 1.1 mV	40 µg	4.04	A minimum of 65% of PGL value after 6 h	14
Exenatide/DOC SNEDDS	Exendin-4	N/A	Size: 45.87 ± 2.9 nm; ZP: 0.7 ± 0.1 mV	150 µg	14.62 ± 3.07	20.6% decrease of PGL in 5 h	38
Liposomes containing SGC, STC, STC respectively	Ins	Transcellular way	Size: 157 ± 19 nm; EE: 29.8 ± 1.7% (the optimal formulation)	20 IU/kg	8.5 ± 2.1 (the optimal formulation)	60% decrease of PGL in 20 h with peak time around 8–12 h	52,81
Biotinylated liposomes (BLPs)	Ins	Biotin receptor mediated transport	Size: ~150 nm; EE: 35%–42%	20 IU/kg	12.09	64% reduction of the PGL in 24 h with peak time around 5–12 h	55
Proliposomes encased in Eudragit S100	Ins	Paracellular way	Size: 583.2 ± 10.2 nm; ZP: 28.3 ± 3.7 mV; EE: 17.6 ± 2.4%	N/A	N/A	N/A	98
VA incorporated SLN nanoparticles	Ins	N/A	Size: 172 ~ 281 nm; ZP: -40 mV; EE: 54.5%	50 IU/kg	5.1	~50% decrease of PGL in 4 h	103
Ins-phospholipid complex loaded SNEDDS	Ins	TJs opening	Size: multi-dispersed peaks; ZP: -4.1 ± 0.3 mV ; EE: 73.1%	50 IU/kg	0.43 ± 0.13	38% decrease of PGL in 10 h	106

ZP, zeta potential; EE, encapsulation efficiency; *F*, relative bioavailability; PGL, plasma glucose levels; TGA, thioglycolic acid; MNA, 6-mercaptopurine-conjugate; Pt, protamine; TELs, tetraether lipids; GCTE, glycerylcholate-tetraether lipids; VA, viscosity-enhancing agent; SNEDDS, self-nanoemulsifying drug delivery systems; DOC, sodium docusate; DOCA, deoxycholate

Hetényi et al.¹⁰⁸ verified that SNEDDS provided a perfect protection towards protease degradation and deactivation by GSH. Zupancic et al.¹⁶ evaluated SNEDDS for oral delivery of a opioid peptide, dalargin. Results showed that the established SNEDDS exhibited mucus penetrating properties and protective effects against enzymatic degradation by trypsin, α -chymotrypsin, and elastase, etc. Menzel et al.³⁸ developed an oral SNEDDS loading exenatide *via* hydrophobic ion pairing with sodium docusate (DOC). After oral administration, exenatide/DOC SNEDDS showed a relative bioavailability of $14.62 \pm 3.07\%$ and caused a significant ($P < 0.05$) decrease in PGL. Bonengel et al.¹⁰ investigated the impact of different hydrophobic ion pairs (SDC, decanoate and docusate) for SNEDDS on the oral bioavailability of octreotide in pigs. *In vivo* studies showed that octreotide-SDC and octreotide-docusate SNEDDS resulted in 17.9-fold and 4.2-fold higher bioavailability separately than free octreotide. According to these results, hydrophobic ion pairing might be an important factor for SNEDDS to elevate the oral bioavailability.

Despite all this, SNEDDS used to deliver hydrophilic drugs including therapeutic peptides/proteins is considered extremely challenging due to their hydrophobic nature.

2.4. Multiemulsion

Multiemulsions, liquid or semisolid disperse systems of a simple emulsion in an external phase, can protect drug from enzymatic hydrolysis and increase absorption through the intestinal barriers as drug carriers¹⁰⁹.

Dogru et al.¹¹⁰ designed a w/o/w multiple emulsion formulation for oral delivery of sCT, which produced analogous effects of serum calcium compared to the commercial preparations in rats. Venkata et al.¹¹¹ prepared Ins-loaded NPs in multiemulsion with particle size of 300–400 nm, which, in different pH conditions, protected cargos from destruction. Griffin et al.¹¹² prepared Ins-loaded tripalmitin nanoparticles by water/oil/water (w/o/w) multiple emulsion technique. The nanoparticles coated by PEG–stearate were proved to be more stable from pancreatin. Li et al.¹¹³ prepared w/o/w nanoemulsions coated with alginate/chitosan, which demonstrated great protection for Ins in SIF and the bioavailability of Ins was 8.19%. Agrawal et al.¹¹⁴ formulated Ins entrapped Eudragit S100 microspheres by w/o/w multiemulsion solvent evaporation technique, which showed a relatively high EE (76.84%) and pH-dependant controlled release. Although multiemulsion is a common approach for oral delivery of peptides/proteins, the main drawbacks of instability and increased particle sizes restricts its development.

3. Polysaccharide-based delivery systems

Polysaccharides are considered as highly safe, biocompatible, and biodegradable natural biomaterials with high MW. Most polysaccharides have hydrophilic groups such as hydroxyl, carboxyl, and amino groups, which could form non-covalent bonds with intestinal mucus to facilitate the absorption of therapeutic peptides/proteins¹¹⁵. Summary of polysaccharide-based delivery systems applied in oral delivery of therapeutic peptides/proteins are shown in Table 6.

3.1. Chitosan and its derivatives

Obtained from alkaline deacetylation of chitin, CS is a polycation copolymer ($pK_a = 6.5$) composed of *N*-acetyl glucosamine (Glc-

Nac) and glucosamine (GlcN) presenting pH responsive property with low poisonousness, which embraces mucoadhesion by interacting with anionic sialic acid residues on mucosal surfaces and permeation enhancing effect by reversibly opening TJs. CS-based NPs are attracting increased attentions for their abilities to orally deliver therapeutic peptides/proteins. Poly- γ -glutamic acid (γ PGA), a natural peptide carrying negative charge, has been used to deliver protein vaccines. Sonaje et al.⁵⁴ prepared Ins-loaded CS NPs mixing with anionic γ PGA with a mean particle size of 218.0 ± 3.4 nm and EE of $71.8 \pm 1.1\%$. During the preparation of NPs, $MgSO_4$ and TPP were introduced to raise their stabilities in an extensive range of pH. The NPs demonstrated a relative bioavailability of $15.1 \pm 0.9\%$ and a reductive trend in PGL in 10 h in diabetic rats. It is well known that divalent metal ions play a key role in developing the apical junctions and preserving protease activity. Diethylene triamine pentaacetic acid (DTPA), a complexant, is able to disrupt TJs and restrain protease activity by chelating divalent metal ions. Su et al.¹¹⁶ covalently conjugated DTPA on γ PGA mixing with CS for oral delivery of Ins. The CS/ γ PGA NPs protected the loaded cargos from enzymatic attacks and kept intact when $pH < 7.0$ in the intestine, which *in vivo* achieved a relative bioavailability of $19.7 \pm 1.3\%$. Besides, ethylene glycol tetraacetic acid (EGTA) is a Ca^{2+} -specific chelating agent. Chuang et al.¹¹⁷ synthesized CS/ γ PGA–EGTA NPs for oral delivery of Ins, which ultimately produced a prolonged hypoglycemic effect *in vivo* with a relative bioavailability of $21.3 \pm 1.5\%$. They further demonstrated that combination therapy by co-loading Ins with exendin-4 delivered by CS/ γ PGA NPs can be more effective than its monotherapy counterparts in achieving preferable glycemic control and undergoing oral glucose tolerance test (OGTT)¹¹⁸. It is well known that CS NPs with smaller size demonstrated better absorption and transportation in GIT. Apart from size, the MW and deacetylation degree of CS in NPs also associated with its performance and stability¹¹⁹. Sukyung et al.³³ conjugated cysteinylated exendin-4 to low molecular weight chitosan (LMWC) *via* a disulfide bond which is cleavable under *in vivo* circumstances. The LMWC–exendin-4 conjugate have a mean particle size of 101 ± 41 nm and a relative bioavailability of 6.4%. Besides, surface charge properties may determine the absorption sites of NPs in small intestine. Two Ins-loaded CMCS/CS nanogels (NGs) with similar shape, size, but opposite surface charge were prepared by Wang et al.¹²⁰. The negatively charged NGs exhibited a higher mucoadhesion and better intestinal permeability than positively charged ones in *ex vivo* intestinal studies, which can be accounted for the attenuated surface charge of CMCS/CS-NGs (+) and weaker contact with mucosal epithelium in alkalescence environment ($pH > 6.5$) of jejunum. It is known that transition metal ions such as Fe^{3+} may form coordinate-covalent bonds with glycosidic, carboxylic, and hydroxyl oxygen atoms, which could be used to increase the EE of NPs. Nguyen et al.³⁴ prepared CS/ γ PGA NPs, in the presence of Fe^{3+} , the EE of NPs significantly enhanced 2.5-fold compared to NPs without Fe^{3+} . Oral administration of the NPs increased the blood level of Ins in a slower but prolonged manner in 12 h and the bioavailability, *versus* the s.c. counterpart, was found to be $14.0 \pm 1.8\%$. However, the oral bioavailability of peptide-loaded NPs is still far from satisfactory. One main reason is the fast leakage of cargos from the NPs in GIT. Therefore, to improve the oral bioavailability, peptides should not only be protected from enzymatic digestion, but also possess the ability to traverse the epithelial barriers. Ins–LMWP conjugates were prepared by Sheng et al.¹²¹ and then loaded into trimethyl chitosan (TMC)-coated

Table 6 Summary of polysaccharide-based particles in oral delivery of therapeutic peptides/proteins.

Formulation composition	Model drug	Main transport mechanisms	Characterization (size, ZP, EE)	PK		PD	Ref.
				Dose	<i>F</i> (%)		
Matrix tablets prepared by 6-MNA protected TGA-CS	Antide	Paracellular way	N/A	2 mg	10.88 ± 4.22	N/A	29
BSA/dextran NPs cross-linked with STMP	Exendin-4	Lymphatic uptake (not confirmed)	Size: 192.7 ± 3.5 nm; ZP: 39.5 mV	165 µg/kg	77	N/A	32
Conjugated with LMWC through disulfide bonds	Exendin-4	N/A	Size: 101 ± 41 nm; ZP: 44.36 mV	400 µg/kg	6.39	22.90 ± 2.0% decrease of PGL in 3 h for 4 µg/kg, while 41.07 ± 4.7% for 40 µg/kg	33
CS/Fe ³⁺ -γ-PGA NPs	Exendin-4	Paracellular way	Size: 260.6 ± 26.4 nm; EE: 60.92%	300 µg/kg	14.0 ± 1.8	25% decrease of PGL in a slower but prolonged manner in 12 h	34
CS/TPP	Exendin-4	Paracellular way	Size: 303.1 ± 10.36 nm; ZP: 18.37 ± 1.15 mV; EE: 38.02.6%	N/A	N/A	N/A	37
PLGA/CS-CPP (PSi/CS-CPP)	GLP-1coloaded with DPP4 inhibitor	N/A	Size: 277.2 ± 3.8 nm (320.0 ± 9.8 nm); ZP: 21.6 ± 3.8 mV (19.1 ± 1.0 mV); EE: 59.7 ± 0.7% (75.0 ± 0.5%)	N/A	N/A	44% decrease of PGL in 8 h	39,40
CS/γ-PGA	Ins	Paracellular way	Size: 218.0 ± 3.4 nm; ZP: 25.3 ± 0.9 mV; EE: 71.8 ± 1.1%	30 IU/kg	15.1 ± 0.9	Low impact on PGL	54
DOCA-modified CS nanoparticles	Ins	Bile acid receptor-mediated transport	Size: ~226.1 nm; ZP: 9.4 mV	30 IU/kg	15.9	A slower but prolonged 50% reduction of PGL in 12 h	78
TMC-CM-GG and TMC-CM-AA NPs	Ins	Opening TJs and actively transported by oligopeptide transporters	Size: 157.3–197.7 nm; ZP: 24.35–34.37 mV; EE: 70.60–86.52%	20 IU/kg	17.19	45.1% decrease of PGL in 8 h of TMC-CM-AA NPs	82
CMCS-PBA-LV	Ins	Paracellular way and transcellular way	EE: 67%	75 IU/kg	7.55 ± 1.32	60% decrease of PGL in 12 h	83
PGA-g-DA micelles with CSK peptide conjugated TMC	Ins	Clathrin-dependent and caveolae-dependent endocytosis	Size: 184.5 ± 13.5 nm; ZP: 24.70 ± 2.45 mV; EE: 83.51 ± 4.24%	50 IU/kg	7.05	50% decrease of PGL in 12 h, totally 1.19-fold higher than T-NPs	84
AT-1002 peptide-CS dual pluronic-based nanocarrier	Ins	TJs opening	Size: ~150 nm; ZP: 20.0 ± 3.4 mV; EE: > 95%	75 IU/kg	~10	~50% decrease of PGL in 20 h	85
CS/γ-PGA-DTPA NPs	Ins	Paracellular way	Size: 246.6 ± 4.8 nm; ZP: 37 ± 0.3 mV; EE: 75.7 ± 0.7 %	30 IU/kg	19.7 ± 1.3	50% decrease of PGL in 10 h	116

Table 6 (continued)

Formulation composition	Model drug	Main transport mechanisms	Characterization (size, ZP, EE)	PK		PD	Ref.
				Dose	<i>F</i> (%)		
CS/ γ PGA-EGTA NPs	Ins	Paracellular way	Size: 328.6 ± 2.3 nm; ZP: 38.7 ± 0.2 mV; EE: $78.7 \pm 0.4\%$	30 IU/kg	21.3 ± 1.5	60% decrease of PGL in 12 h	117
PLGA/FA-CS	Ins	N/A	Size: 252.4 ± 4.6 nm; ZP: 5.99 ± 2.85 mv; EE: 41%	70 IU/kg	7.77 ± 1.3	50% decrease of PGL in 12 h	128
TMC/ pHPMA	Ins	TJs opening	Size: 163.1 ± 3.99 nm; ZP: -3.35 ± 1.0 mv; EE: $54.1 \pm 1.9\%$	50 IU/kg	8.56	36% decrease of PGL in 4 h	131
PSA coated protamine NCs	Ins	Paracellular way and caveolae (predominantly) and Clathrin mediated endocytosis	Size: 301 ± 84 nm; ZP: -4 ± 1 mV; EE: $51 \pm 9\%$	N/A	N/A	20% decrease of PGL in 9 h	135
CS/ALG	Ins	TJs opening	Size: 104 nm; ZP: $+3.89$ mV; EE: 78.3%	50 IU/kg	~ 8.11	70% decrease of PGL in 9 h	136
Dual chitosan/albumin-coated alginate/dextran sulfate nanoparticles	Ins	Clathrin-mediated endocytosis.	Size: 300.8 ± 3.8 nm; ZP: 28.9 ± 0.9 mV; EE: $30.7 \pm 3.4\%$	N/A	N/A	N/A	138
CS/HPMCP	Ins	Paracellular pathway and adsorptive endocytosis and in part by clathrin-mediated vesicles	Size: 255 nm; ZP: 30.1 ± 0.8 mV; EE: $60.88 \pm 1.09\%$	12.5 U	8.47 ± 1.59	60% decrease of PGL in 12 h	140
HP55-coated capsule containing PLGA/RS NPs	Ins	N/A	Size: 285.6 ± 4.5 nm; ZP: $+42.9 \pm 1.4$ mV; EE: 73.9%	50 IU/kg	9.2 ± 2.4	40% decrease of PGL in 15 h	142
PAA/S-CS hydrogel	Ins	N/A	EE: $\sim 76\%$	50 IU/kg	~ 4.43	$\sim 55\%$ decrease of PGL in 6 h	144

ZP, zeta potential; EE, encapsulation efficiency; *F*, relative bioavailability; PGL, plasma glucose levels; NPs, nanoparticles; TMC, trimethyl chitosan; TMC-CM-GG, glycyl-glycine conjugated nanoparticles; TMC-CM-AA, alanyl-alanine conjugated nanoparticles; TJs, tight junctions; INS, insulin; TPP, sodium tripolyphosphate; CS, chitosan; NCs, nanocomplexes; γ -PGA, poly- γ -glutamic acid; DTPA, diethylene triamine pentaacetic acid; GLP-1, glucagon-like peptide-1; DPP4, dipeptidyl peptidase 4; PEs, permeability enhancers; BSA, bovine serum albumin; STMP, sodium trimetaphosphate; LMWC, low molecular weight chitosan; PSi, mesoporous silicon; CPP, cell penetrating peptides; CMCS, carboxymethyl chitosan; PBA, phenylboronic acid; LV, L-valine; LMWH, low molecular weight heparin; DOCA, sodium deoxycholate; ASBT, sodium-dependent bile acid transporter; PSA, polysialic acid; BGL, blood glucose level; C12, lauric acid; Chol, cholesterol; r8, octaarginine; SGC, sodium glycocholate; TMC, *N*-trimethylated chitosan; CS-6-MNA, chitosan-6-mercaptopnicotinic acid; TGA, thioglycolic acid; PAA, polyacrylamide; S-chitosan, succinyl chitosan; SOD, superoxide dismutase; ALG, alginate; PGL, plasma glucose level; FA, folic acid

PLGA NPs. The oral bioavailability of delivered conjugate-loaded NPs, relative to s.c. injected solution of Ins was $17.98 \pm 5.61\%$, 2-fold higher over native Ins-loaded NPs. CS-based NCs conjugated with SAR6EW, a novel CPP, are prepared and evaluated by Li et al.¹²². The SAR6EW/CS/Ins-NPs displayed sufficient hypoglycemic effect with no significant toxicity in diabetic rats and induced a significantly higher internalization of Ins *via* clathrin- and caveolae-mediated endocytosis.

NPs decorated with specific ligand are expected to generate better binding with the epithelium and enhance the oral bioavailability. L-Valine is a target ligand distributed all over the small intestine. Li et al.⁸³ evaluated the CS-based NCs modified by L-valine and phenylboronic acid (a glucose-responsive unit). Results showed a corresponding bioavailability of $7.55 \pm 1.32\%$ after oral administration. CSKSSDYQC (CSK) peptide has been identified to specifically recognize goblet cells, the second large cell population on epithelium. Zhang et al.⁸⁴ developed Ins-loaded dodecylamine-graft-g-polyglutamic acid (PGA-g-DA) micelles coated in CSK peptide-conjugated TMC. The NPs exhibited excellent hypoglycemic effect following oral administration with a relative bioavailability of 7.05%, 1.2-fold higher than that of unmodified NPs and the total decrease of PGL was 1.19-fold higher than unmodified NPs. AT-1002 is a hexamer peptide derived from zonula occludens toxin (ZOT), which has been shown to open the TJs reversibly and enhance the absorption of peptides across the epithelium. Lee et al.⁸⁵ developed AT-1002 peptide-CS dual ligand functionalized pluronic-based NCs for oral delivery of Ins. Results revealed that the penetration of FITC-labeled Ins-loaded dual ligand NCs across the Caco-2 cell monolayer was nearly 7%, 1.75-fold higher than single ligand conjugated NCs. *In vivo* experiment showed that the relative bioavailability of Ins-loaded dual ligand functionalized NCs significantly increased almost 10%, 6-fold higher than that of single ligand functionalized NCs. What's more, intracellular lysosomal degradation is detrimental to transepithelial transport of peptides/proteins, which cannot be overstated in the study of oral delivery. Apical sodium-dependent bile acid transporter (ASBT)^{123,124} is different from the common receptor-mediated transport such as transferrin, vitamin B12 and lectin receptor-mediated pathways, which not only deal with the apical membrane barrier but also responsible for the intracellular trafficking and basolateral release¹²⁵. SDC-conjugated CS NPs (DNPs) were synthesized by Fan et al.⁷⁸ and loaded with Ins. They focused on exploring the mechanism of functional NPs exploiting the bile acid pathway to overcome multiple barriers of the intestinal epithelium through CLSM, intravital two-photon microscopy and other techniques. The apical membrane was overcome through ASBT-mediated endocytosis. Moreover, DNPs escaped from the endolysosome to avoid lysosomal degradation of Ins by bonding with ileal bile acid-binding protein (IBABP) in cytoplasmic trafficking. Eventually, Ins were excreted from the basolateral membrane. Results showed that the relative bioavailability of enteric-coated DNPs was 15.9%, 2.2-folds of NPs without SDC. A major limitation impeding the oral delivery of NPs apart from permeating rate is the elimination of NPs by the mononuclear phagocyte system (MPS)¹²⁶. Sarmiento et al.¹²⁷ investigated the ability of CS-coated SLNs to survive phagocytosis by the MPS after intestinal uptake using RAW 264.7 macrophage cell line. Results showed that this system demonstrated potential ability to prolong the half-life of Ins in blood and provided stealth properties by MPS after intestinal uptake. As folate receptor is significantly expressed on epithelial cells, macromolecules conjugated with folic acid (FA) can enhance their

uptake and targeting abilities. PLGA- and FA-modified CS were fabricated *via* electrostatic self-assembly method by Xu et al.¹²⁸. The relative bioavailability of orally delivered Ins-loaded PLGA/FA-CS is 7.22%, which is 2.76-fold higher than that of Ins solution.

However, CS is inadequate for opening TJs in neutral pH environments, which limits its potential use as a PE only in the duodenum section. In addition, CS is only dissolved in acid solutions and has limited mucoadhesive abilities. A series of CS derivatives such as TMC, *O*- and *N*-carboxymethyl CS, *N*-methylene phosphonic CS, carbohydrate branched CS and alkylated CS are synthesized to solve these problems. Many researches have been done on TMC, which is soluble in a wide range of pH in aqueous solutions so that it could carry peptides in different organs regardless of the pH changes. CS and its derivatives such as TMC enhance the absorption of NPs *via* paracellular way by opening TJs. Omid et al.⁸² indicated that Glycyl-glycine (GG)- and alanylalanine (AA)-conjugated TMC NPs showed enhanced 2.5–3.3-fold permeability of Ins in Caco-2 cell line than unmodified TMC NPs and demonstrated increased relative bioavailability of 17.19% and 15.46% separately compared with TMC NPs (14.15%). This enhanced absorption is explained by the presence of proton-coupled oligopeptide transporters PepT1 and PepT2 that could actively transport di/tri-peptides like GG and AA in the brush border of membrane of the small intestine. CS–6-mercaptopicolonic acid (MNA) is a thiolated CS with strong mucoadhesive properties and a pH-independent reactivity. Millott et al.¹²⁹ evaluated *in vivo* the potential of CS–6-MNA for oral delivery of Ins. Results showed that CS–6-MNA tablets were at least 84-fold stronger mucoadhesive than unmodified ones. The relative bioavailability of thiolated formulations ($M=20$ kDa) was 15.3%, 4.79-fold higher compared with non-thiolated ones, while thiolated CS of 400 kDa mass was 12.8%, 21.3-fold higher compared with non-thiolated ones. However, unless sealed under inert conditions, thiomers are prone to thiol oxidation at physiological pH¹³⁰. The protection of thiol groups on the thiomers prevents an early oxidation before contacting with mucosa. Dünhaupt et al.²⁹ prepared sulfhydryl-protected thiolated CS for oral delivery of antide. They utilized thioglycolic acid (TGA) as sulfhydryl ligand protected by the thiolated aromatic residue 6-MNA. The permeation enhancing effect of sulfhydryl-protected CS was enhanced approximately 1.2-fold than its corresponding thiomers and 2.8-fold compared to unmodified ones. Oral administration of antide incorporated TGA–MNA matrix tablets reached a relative bioavailability of 10.9%. Suksamran et al.¹⁹ successfully synthesized *N*-(4-*N,N*-dimethylaminocinnamyl) CS (TM₆₅CM₅₀CS) as a surface coating for ovalbumin (OVA)-loaded calcium-alginate and calcium-alginate-yam microparticles, which exhibited the greatest immune responses compared with other modified CS. Although TMC-based NPs have been demonstrated to facilitate the paracellular transport of peptides across the epithelial barriers, the trapping and clearance of these NPs by the mucus layer was often overlooked. Different from other commonly used hydrophilic “mucus-inert” materials like PEG, the physicochemical properties of *N*-(2-hydroxypropyl) methacrylamide copolymer (pHPMA) can be easily tuned by manipulating the monomers. Liu et al.¹³¹ designed NPs composed of Ins-loaded TMC-based polyelectrolyte complex core, and a dissociable coating of pHPMA to overcome the epithelial barriers. Results revealed that the outer pHPMA gradually dissociated from the TMC-based NP core as the NPs permeated through mucus using CLSM. At the dose of 50 IU/kg, these NPs exhibited a relative bioavailability of 8.56%, 2.8-fold higher than that of uncoated NPs.

3.2. Other polysaccharide-based systems

Dextran (DEX), a complex branched glucan, is consisted of chains with extensive lengths (3–2000 kDa). Soudry-Kochavi et al.³² markedly improved the oral relative bioavailability of exendin-4 to 77% compared to a s.c. injection of ByettaTM. He and coworkers designed a nano-in-micro encapsulation delivery system, in which the inside nano-systems are a mixture of bovine serum albumin (BSA) and DEX NPs cross-linked with sodium trimetaphosphate (STMP) and the micro-systems are composed of an appropriate ratio of Eudragit[®] L100-55 (Eudragit L) and hydroxypropylmethylcellulose (HPMC) for additional protection. They hypothesized that the significantly improved oral absorption is due to DEX that increased lymphatic uptake and circumvented the first-pass effect. Heparin is a negatively charged biomolecular material mainly used as a natural anticoagulant. Park et al.⁷⁷ designed Pt nanocomplex-loaded heparin-SDC NPs with size of 150.8 ± 16.5 nm and zeta potential of -29.1 ± 3.9 mV. They showed that the orally administered NPs were absorbed by ASBT in the epithelium of ileum, which could be applied for delivering peptides avoiding accumulative problems. Recently, PEG is questioned by inducing an immune response in human body, particularly when given repeatedly. Polysialic acid (PSA), a highly hydrophilic polysaccharide primarily composed of α -2,8-linked 5-*N*-glycolyneuraminic acid, could be used as a suitable alternate of PEG as an endogenous substance. The PSA-based carrier systems are non-immunogenic and can be used to extend the half-lives similar to PEG-conjugates^{132,133}. Wu et al.¹³⁴ modified the uricase to obtain the PSA-PEG-uricase conjugates, which exhibited lower immunogenicity and stronger water absorbency. Thwala et al.¹³⁵ indicated that coating the nanosystem with PSA improved its stability and mucosal penetration ability. They designed double layer PSA-Pt NCs. The formulations, administered intra-jejunal to healthy rats resulted in a moderate reduction of PGL (20% reduction) and lasted for 4 h.

Alginate (ALG) extracted from brown seaweed are natural water-soluble linear polysaccharides composed of α -L-guluronic acids and β -D-mannuronic acids. They are anionic compounds featured by the capability to form hydrogels in the presence of divalent cations such as Ca^{2+} and have significant advantages in oral delivery for their pH sensitivity and low cost⁴⁴. Lee et al.²⁸ encapsulated superoxide dismutase (SOD) in zein-ALG NPs (ZAN) via a phase separation method. Carboxyl groups in ALG are protonated at low pH and the ZAN swell slightly in the stomach. ZAN (w/w 200:40) releases $90.8 \pm 1.2\%$ of encapsulated SOD at pH 7.4 in 2 h, while only $11.4 \pm 0.4\%$ of SOD was released at pH 1.3. Mukhopadhyay et al.¹³⁶ developed Ins-loaded CS/ALG core-shell NPs with an average particle size of 100–200 nm. After oral administration, a significant reduction of PGL with a sustained effect at least 9 h and a relative bioavailability of nearly 8.11% were detected. The fact that some virus having hydrophilic and neutral surface composed of both positive and negative charges less hindered in mucus offers a new idea for designing mucus-penetrating NPs. Zhang et al.¹³⁷ designed an intestinal mucus-penetrating core-shell nanocomplex through self-assembly between positive Ins-loaded CS/TPP with negative ALG. These nanocomplexes with negative ALG coating were confirmed to have 1.6–2.5 times higher mucus penetration abilities than CS NPs. However, compared to PEGylated NPs which have 20-fold mucus penetrating ability than uncoated NPs, ALG-coated nanocomplexes needs further improving. Lopes et al.¹³⁸ formulated Ins-loaded ALG/DEX NPs and dual-coated with CS and ALB, in which

ALB provided electrostatic stabilization and enhanced dissolution rate of Ins from NPs. It was demonstrated to transport actively by clathrin-mediated endocytosis. Eldin et al.²³ synthesized pH-sensitive L-arginine grafted alginate (Arg-g-Alg) hydrogel beads and utilized it as a new carrier for BSA. Arg-g-Alg showed sufficient release profile with about 300% in acidic media compared to pure alginate hydrogel beads. However, attention should be poured into the process of introducing negatively charged shell into drug loaded core because competitive interactions among different materials may lead to a decrease in drug loading.

Cellulose is one of the most widely used natural substances and commercial biopolymers. Microcrystalline cellulose and cellulose derivatives, such as HPMC, hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), and carboxymethyl cellulose (CMC) are recognized as the natural materials with good tolerance in body and often used as pH-sensitive coating materials to protect encapsulated peptides¹³⁹. Song et al.¹³⁹ prepared the oppositely charged CMC and quaternized cellulose NPs by changing the ratio of anionic-to-cationic polymers and selected two proteins, lysozyme (*pI* 11.4) and BSA (*pI* 4.8) with opposite charges. Results revealed that positively charged NPs were more efficiently internalized. However, the negatively charged NPs may be more appropriately applied in delivering drugs such as Ins, which needs the longer half-life. An oral multiple-unit formulation for colonic release of Ins was proposed by Maroni et al.⁵¹. The system comprises an immediate-release minitabiet core containing the protein and SGC coated by HPMC as a swellable internal layer. Oral administration of the novel formulation to diabetic rats elicited a peak in PGL after 6 h associated with a sharp decrease in the PGL. The relative bioavailability of such formulation was 2.2-fold higher than that of uncoated ones. Makhlof et al.¹⁴⁰ formulated CS NPs with hydroxypropyl methylcellulose phthalate (HPMCP, pK_a 5.2) as a pH-sensitive polymer. Fluorescently-labeled CS/HPMCP NPs showed 2–4 times improvement in the intestinal mucoadhesion and penetration compared to CS/TPP NPs. Following oral administration, CS/HPMCP NPs with a relative bioavailability of $8.47 \pm 1.59\%$ increased the hypoglycemic effect by more than 2.8-fold compared to Ins-loaded CS/TPP NPs. In addition, Singh et al.¹⁴¹ developed an ileum-targeted protein delivery system using HPMCP. Initially, they attuned pH-sensitive property of HPMCP for controlled dissolution at ileum pH (≥ 7.4) by thiolation, which prevented the early release of protein in acidic pH in stomach and duodenum but at ileal pH in a controlled manner. Wu et al.¹⁴² developed a two-stage delivery system composed of PLGA/ Eudragit[®] RS NPs coated with pH-sensitive hydroxypropyl methylcellulose phthalate (HP55, pK_a 5.5) for the oral delivery of Ins, in which HP55 was designed to overcome the first barrier. PLGA/RS NPs, as the second stage, adhered to the intestine mucosa and improved the absorption of Ins. The hypoglycemic effect and relative bioavailability of the enteric-coated capsule were 32.9% and 9.2%, respectively. For further insight, Wang et al.¹⁴³ investigated the structure–function relationship of PLGA/HP55 NPs in different conditions by dissipative particle dynamics simulations (DPD). It can be seen that all polymeric molecules formed spherical core-shell NPs with PVA molecules as a stabilizer adsorbed on the PLGA/HP55 matrixes.

4. Inorganic particles

Some of the inorganic NCs have been successfully applied in oral delivery of therapeutic peptides/proteins, gold NPs (Au NPs)^{145,146}, selenium NPs (Se NPs)⁴¹, silica NPs (Si NPs)^{43,147,148}, alumina¹⁴⁹,

Table 7 Summary of inorganic particles in oral delivery of therapeutic peptides/proteins.

Formulation composition	Model drug	Main transport mechanisms	Characterization (size, ZP, EE)	PK		PD	Ref.
				Dose	<i>F</i> (%)		
NiMOS-loaded HNTs	Albuimn	N/A	Size: 215.3 ± 2.5 nm; EE: $63.16 \pm 4.66\%$	N/A	N/A	N/A	25
SiNPs-PEG	Ins	N/A	Size: 493.7 ± 89.10 nm; ZP: -15.2 ± 0.0 mV; EE: 85.4%	N/A	N/A	N/A	43
A bubble carrier system loading DTPA, SBC, SDS and Ins	Ins	Transcellular way and paracellular way in free-form insulin	Size: 150 nm	30 IU/kg	21.7 ± 1.7	A steady decrease of PGL for over 10 h with maximum decrease of 50% 4–5 h	61
Chondroitin sulfate capped AuNPs	Ins	CD44 receptor-mediated endocytosis	Size: 122.90 ± 7.12 nm; ZP: -33.69 ± 3.39 mV; EE: $90.19 \pm 3.42\%$	N/A	N/A	50% decrease of PGL in 4 h	87
Montmorillonite coated with TiO ₂	Ins	N/A	Size: 50 nm; ZP: -54.5 mV	N/A	N/A	N/A	150
SeNPs	Ins	Clathrin-dependent endocytosis	Size: 100–200 nm; ZP: 25 mV; EE: 95.97%	50 IU/kg	9.15	50% decrease of initial PGL maintaining for 10 h	152
Silica coating HP55	Ins	N/A	Size: 50 nm; EE: 27.4%	N/A	N/A	PGL maintained from 40% to 70% for a period of 2–7 h.	156
Ins/ZrP coated with TiO ₂	Ins	N/A	Size: 364.2 ± 53.9 nm; ZP: 27.3 ± 2.4 mV	N/A	N/A	N/A	159

ZP, zeta potential; EE, encapsulation efficiency; *F*, relative bioavailability; PGL, plasma glucose levels; NiMOS, nanotubes-in-microgel oral system; HNTs, halloysite nanotubes

TiO₂¹⁵⁰, zirconium phosphate (ZrP)^{145,151}, for instance. In comparison with organic matrices, these materials are born with noticeable stabilities in acidic and enzymatic environment. Summary of inorganic particles applied in oral delivery of therapeutic peptides/proteins are showed in Table 7.

Chondroitin sulfate capped Au NPs/Ins with around 120 nm were prepared by Cho et al.⁸⁷. Chondroitin sulfate was used as a stabilizing agent for synthesis of Au NPs. The mean concentration of Ins in plasma at 2 h after oral treatment was 6.61-fold enhanced than that of Ins solution. It was shown that Se has similar hypoglycemic effect with Ins by improving pancreatic islet function and glucose utilization in latest research⁴¹. Deng et al.¹⁵² fabricated Ins-loaded Se NPs around 120 nm by ionic cross-linking/*in situ* reduction technique. Ins-SeNPs (50 IU/kg) resulted in a relative bioavailability of 9.15% and a decreased PGL of 50% of starting levels sustaining 10 h. Hydroxyapatite (HAP), a biocompatible and porous material with no obvious poisonous effects, might be an ideal drug carrier. Zhang et al.¹⁵³ prepared HAP NPs wrapped by PEG which conjugated with Ins and gallic acid (GA) and showed a downward trend of PGL after directly administered to the ileum.

Si NPs with large specific surface area and high porosity are promising candidates for the excellent biocompatibility and biodegradability¹⁵⁴. What's more, Si NPs possess residual silanol groups (Si–OH) at surface that can be functionalized by different organic groups¹⁵⁵. Zhao et al.¹⁵⁶ prepared Ins-loaded Si NPs coated with HP55. *In vivo* evaluation showed a significant hypoglycemic effect that was maintained from 40% to 70% in

2–7 h. However, Andreani et al.^{43,157} produced PEG-coated Si NPs for oral administration of Ins. Si NPs-PEG_{20,000} showed a faster diffusion followed by Si NPs-PEG₆₀₀₀ and Si NPs. They further reported the development of Ins-Si NPs coated with mucoadhesive polymers such as CS, ALG or PEG of low and high MW. Si NPs coated with ALG or CS showed higher contact with mucin compared to non-coated Si NPs and Si NPs-PEG. Si could prevent coalescence of emulsion droplets by forming stable networks and a rigid protective barrier. A hybrid nanocapsule using liposomes as template for the deposition of Si NPs or CS was developed by Mohanraj et al.¹⁵⁸. The results exhibited increased EE up to 70% and controlled release.

In addition, hybrid carriers coated by inorganic materials are applied to achieve controlled release. Safari et al.¹⁵⁹ synthesized a series of Ins/ZrP composites coated with TiO₂ by sol-gel means. The TiO₂-coated composites prolonged drug release and enhanced EE considerably compared to the Ins/ZrP composites. Similarly, Kamari et al.¹⁵⁰ successfully prepared hybrid nanocomposites composed of montmorillonite (Mt)/Ins/TiO₂. Mt has a large surface area and a high capacity of cation exchange. Results revealed that nanocomposites without and with TiO₂ coating released cargos after 60 min and 22 h in pH 7.4, respectively. In addition, microencapsulation approaches, such as prilling protein into microspheres, may protect it from enzymatic degradation in GIT. Kruif et al.²⁵ demonstrated a nanotubes-in-microgel oral system prepared by prilling, a mild process embedding BSA-loaded halloysite nanotubes, which demonstrated a higher enzymatic protection than pure nanotube.

5. Synthetic macromolecular polymer delivery systems

A new range of biodegradable polymeric NPs that are easily functionalized have been synthesized and recently applied in oral delivery of therapeutic proteins/peptides in order to enhance their stability and realize controlled release¹⁶⁰. Summary of synthetic macromolecular polymers applied in oral delivery of therapeutic peptides/proteins are shown in Table 8.

Mucosal layer acts as a protective barrier can trap foreign particulates and clear them subsequently, which consequently diminished the possibilities for NPs to traverse the absorptive membrane of the intestine. PEG would render more hydrophilic the NPs to pass through the mucus and prevents from aggregation due to its steric hinderance effect. Inchaurreaga and coworkers¹⁶¹ evaluated *in vivo* the mucus-penetrating abilities of PEG-coated poly(anhydride) NPs, which was clearly influenced by both the MW and surface density of coated PEG. The mucus-penetrating abilities were higher for PEG₂₀₀₀ or PEG₆₀₀₀ coated NPs than PEG_{10,000} and lower for excessive densities of coated PEG. However, a dilemma occurs when selecting proper materials between high mucus permeation and high epithelial transshipment. LMWP (VSRRRRRRGGRRRR), composed of 10 arginine residues, could function as a CPP to achieve effective intracellular transport¹⁶². Shan et al.¹⁶³ demonstrated that the NPs co-loaded with CPP as a core and then coating dissociable pHMPA could successfully solve the dilemma. The NPs exhibited 20-fold higher absorption than free Ins on epithelial cells that secrete mucus. They further indicated that organelles including endoplasmic reticulum (ER), Golgi apparatus and lysosome were all participants in the intracellular trafficking of NPs. He et al.⁴⁵ developed monomeric Ins/LMWP conjugates (w/w, 1:1) by using succinimidyl-[(N-maleimido-propionamido)-polyethyleneglycol] ester as an intermediate cross-linker. It is demonstrated that transport of the conjugates across the mucosal monolayer was almost 5-fold higher than free Ins. The *in vivo* bioavailability of the *in situ* loop administered conjugates was 7.08%, 3.9-fold higher than physical mixture. It has been reported that Zn²⁺ improves the thermal stability of cargos by forming Zn²⁺-imidazole coordination complexes *via* modulating Zn²⁺ at the active site of proteins. Zhang et al.³⁶ used PEG-PLGA as the forming polymer to orally deliver LMWP-exenatide-Zn²⁺ complexes. The relative bioavailability of LMWP-exenatide-Zn²⁺ NPs was augmented by 1.74-fold compared to LMWP free NPs. Additionally, the AUC of exematide-Zn²⁺ NPs was 3.27-fold higher than NPs contained no Zn²⁺, which confirmed the function of Zn²⁺ in improving the biological activity of exematide.

PLGA is a promising drug delivery vehicle as it has been approved by the US Food and Drug Administration for applying in biomedicine. The instability of Ins-loaded PLGA microparticles was reported due to deamidation. To incorporate antacids could help overcome such problems by preventing aggregation and increasing pH of microclimate. Therefore, Sharma et al.⁵³ developed antacid-Ins co-encapsulated PLGA NPs for oral delivery, the oral bioavailability of which augmented 6-fold compared with native Ins in healthy rats. The hydrophobic nature of PLGA hampers its effective load of hydrophilic drugs. Hence, amphiphilic block copolymers are designed to increase the EE of hydrophilic proteins. Hosseininasab et al.⁴⁷ synthesized Ins-loaded PLGA-PEG copolymer NPs using the double-emulsion method (w/o/w), in which PEG₂₀₀₀ showed higher EE (62.3 ± 5.6%) than PEG₄₀₀₀. Although PEG has been widely used as a hydrophilic segment of diblock copolymers, it might lead a protein-repelling effect. García-Díaz et al.¹⁶⁴ incorporated premixing amphiphilic lipids-Ins into PLGA NPs, resulting in a

significantly enhanced EE of 90% than 24% in the absence of lipids. Besides, DEX is peptides-friendly to use in block copolymers as a hydrophilic segment. Alibolandi et al.⁴² synthesized Ins-encapsulated DEX-PLGA amphiphilic copolymers, which showed an average EE more than 90% with sustained release of Ins at pH 7.4. The polymeric lipid NPs combined properties of both polymeric and liposomes realized a relative bioavailability of 9.77%. Ma et al.²⁰ reported ulex europaeus agglutinin-1 (UEA-1) conjugated PLGA-lipid NPs to orally deliver OVA, which contained an M-cell selective molecular signature toll-like receptor (TLR)-agonist monophosphoryl lipid (MPL) to effectively transport through M-cells.

Recent studies have revealed that NPs coated with hydrophilic polymers exhibit less adhesion to mucus layer. To further improve the mucus penetration of NPs, Li et al.⁵⁰ designed core shell corona nanolipoparticles (CSC) containing CS NPs as core, pluronic F127-lipid vesicles as shell and polyethylene oxide (PEO) as a corona. The cellular level of Ins after CSC treatment was 10-fold higher compared to CS NPs, exhibiting significantly higher efficiency of mucosal penetration. Studies in diabetic rats showed 2.5 times the hypoglycemic effects of CSC and 2.1-fold times the relative bioavailability than CS NPs. Salvioni et al.⁸⁸ applied a three-layer colonic system to prepare polyethylene imine (PEI) coated Ins-DEX NPs. The three-layer release technology platform was consisting of a flexible film composed of a neutral polymethacrylate Eudragit[®] NE and sodium starch glycolate Explotab[®], applied to a HPMC coating of reduced thickness in order to delay the drug liberation. Compared with s.c. Ins that led to 25% of the starting level of glucose concentration at 1 h post-treatment, the PGL in rats administered with the particles gradually decreased and remained at 45%.

Mucus is incessantly secreted, shed and digested as a dynamic gel. In many cases, the mucus clears the drug carriers before loaded cargos reaching the underlying cells and entering the blood circulation. A promising technique occurred to reach the epithelial layer by cleavage of mucoglycoprotein substructures on mucus through proteases like papain (PAP) and bromelain (BRO). Poly (acrylic) acid with weak mucoadhesion bears carboxylic groups that enzymes can be conjugated with. Müller et al.¹⁶⁵ prepared PAP-grafted PAA NPs *via* ionic gelation. Permeation studies revealed that PAP conjugated particles diffused 3.0-fold higher across mucosal layer than unmodified ones. PAP and BRO were separately conjugated to PAA by Pereira de Sousa et al.¹⁶⁶ BRO modified NPs exhibited higher permeating abilities by altering the structure of mucosal layer compared to PAP conjugated ones. Koetting et al.^{11,13} synthesized pH-responsive hydrogels composed of itaconic acid (IA) copolymerized with *N*-vinylpyrrolidone (NVP) to orally deliver therapeutic proteins with high pI. NVP was chosen to be a hydrogen-bond acceptor as it offers enhanced protection for proteins. Results showed that these hydrogels rapidly and completely release sCT within 1 h in simulated intestinal fluid (SIF) while undetectable release in simulated gastric fluid (SGF). Arginine-rich polymers have received increasing attentions in oral delivery for their higher efficiency in crossing epithelial cells than the other polycationic polymers. He et al.⁴⁶ synthesized a new arginine-based poly(ester amide) (Arg-PEA) combined with PEA-COOH for the protection of Ins. PEA is a biodegradable polymer with good mechanical and thermal properties. *In vivo* test revealed that the PGL can be effectively controlled in 10 h, and the oral bioavailability was 5.89 ± 1.84% in healthy rats.

Ligand-functionalization can increase the affinities of NPs with targeted cells. Butyrate functionalization PEG (Bu-PEG) NPs,

Table 8 Summary of synthetic macromolecular polymers in oral delivery of therapeutic peptides/proteins.

Formulation composition	Model drug	Main transport mechanisms	Characterization (size, ZP, EE)	PK		PD	Ref.
				Dose	<i>F</i> (%)		
mPEG- <i>g</i> -AA	sCT	Transcellular way	Size: 72.1 ± 0.5 nm; EE: 72.8%	N/A	N/A	77.7% decrease of serum calcium level was observed within 1 h and last 7 h	12
LMWP-PEG-PLGA	Exendin-4	Transcellular way	Size: 114.4 ± 10.5 nm; ZP: 2.5 ± 0.2 mV; EE: 71.3 ± 4.3 %	100 µg/kg	7.44	55% decrease of PGL in 24 h	36
Dextran ₅₀₀₀ - <i>b</i> -PLGA ₁₃₀₀₀ polymersome	Ins	Lectin-like protein receptors (not confirmed)	Size: 139.2 ± 12.23 nm; EE: 90.42 ± 1.39 %	100 IU/kg	9.77	75% decrease of PGL in 12 h	42
INS-PEG-LMWP conjugate	Ins	N/A	N/A	50 IU/kg	7.08	The BGL dropped considerably by 70% in 10 h	45
PEA-COOH/Arg-PEA microspheres	Ins	N/A	Size: 13.4 ± 5.8 µm; EE: 80.2 ± 1.3 %	50 IU/kg	5.89 ± 1.84	50% decrease of PGL in 10 h	46
CS/pluronic F127-lipid vesicles/PEO core shell corona nanolipoparticles	Ins	N/A	Size: 195.3 ± 32.9 nm; ZP: 4.3 ± 5.4 mV; EE: 76.6 ± 5.8 %	50 IU/kg	7.8	50% decrease of PGL in 12 h	50
Antacid (magnesium hydroxide or zinc carbonate)-Ins co-encapsulated PLGA NPs	Ins	Via the M cells of the Peyer's patches (not confirmed)	Size: ~ 136 – 143 nm; EE: 81%–85%	120 IU/kg	1.2	74% decrease of PGL in 30 h	53
Bu-PEG NPs	Ins	MCT1-mediated endocytosis	Size: 90.8 ± 1.73 nm; ZP: -9.89 ± 0.11 mV; EE: 57.47 ± 0.03 %	50 IU/kg	9.28	58.8% decrease of PGL after 4 h	86
Ins-LMWP conjugates loaded TMC-coated PLGA nanoparticles	Ins	Paracellular way and clathrin-dependent endocytosis and adsorptive endocytosis.	Size: 253.8 ± 6.4 nm; ZP: 47.5 ± 3.8 mV; EE: 49.3 ± 2.1 %	20 IU/kg	17.98 ± 5.61	50% decrease of PGL in 9 h	121
Insulin/CPP NCs coated pHPMA	Ins	Paracellular way	Size: 177.3 ± 15.2 nm; ZP: -10 mV; EE: 94.9 ± 1.1 %	75 IU/kg	3.02 ± 0.66	50% decrease of PGL in 10 h	163
ConA-PEG-PLGA	Ins	Lectin-receptor mediated transport	Size: 196.3 ± 4.5 nm; ZP: -25.6 ± 1.68 mV; EE: 44.6 ± 3.5 %	N/A	N/A	60% decrease of PGL in 20 h	167
C12(Chol)-r8-Ins loaded diblock PEG-PGA NPs	Ins	N/A	Size: 236 ± 27 nm (225 ± 10 nm); ZP: 2 ± 2 mV (2 ± 3 mV); EE: 99 ± 0 % ; (92 ± 9 %)	N/A	N/A	There are not statistically significant differences between the Ins and the ENCP formulation	168

ZP, zeta potential; EE, encapsulation efficiency; *F*, relative bioavailability; PGL, plasma glucose levels; LMWP, low molecular weight protamine; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PGA, poly (glutamic acid); pHPMA, *N*-(2-hydroxypropyl) methacrylamide copolymer; PGA-*g*-DA, dodecylamine-graft-*g*-polyglutamic acid; PEO, polyethylene oxide; PGL, plasma glucose level; Bu-PEG, butyrate-conjugated PEG; MCT1, monocarboxylate transporter 1; mPEG-*g*-AA, mPEG grafted alginate acid; Con A, concanavalin A; PEA, poly(ester amide); ARG, arginine; DOCA, deoxycholic acid; TMC, *N*-trimethyl chitosan.

established by Wu et al.⁸⁶ generated a stronger hypoglycemic effect in diabetic rats and a relative bioavailability of 9.28%, which increased 2.87-fold than bare PEG NPs. Enhanced cellular uptake was achieved *via* specific interaction between butyrate and the monocarboxylate transporter (MCT) on cell membranes. Li et al.¹² demonstrated that the mPEG grafted alginate micelles with size of 72 nm could significantly improve ($P < 0.001$) the oral absorption of sCT by transcellular way. Lectins (concanavalin A) are proteins or glycoproteins that specifically recognize carbohydrate moieties and trigger active vesicular transport by endocytosis. Concanavalin A anchored PEG–PLGA diblock copolymers were synthesized by Sharma et al.¹⁶⁷. The established system showed a delayed response (2–4 h) in the reduction of PGL within an acceptable range. Samstein et al.⁷⁹ hypothesized that bile salts could be used to enhance the bioavailability of PLGA NPs by protection and elevated absorption. Oral administration of Ins loaded PLGA NPs to mice, using a SDC emulsion, produced sustained levels of the PGL over 24–48 h with a relative bioavailability of 1.81%.

Although numerous oral protein/peptide delivery systems using synthetic polymers as carriers have been established, these polymers still face considerable challenges in toxicological evaluation, biocompatibility, and biodegradability.

6. Conclusions and future perspectives

Obviously, the oral bioavailabilities of therapeutic proteins/peptides are seriously hampered by the atrocious conditions in GIT. It is increasingly recognized that in order to overcome these physiological barriers for successful delivery, drug delivery systems embracing protection ability for drug carriers, modified release behavior for cargos, enhanced stability by PIs, elevated uptake by PEs, and escape ability from MPS are somehow required. The multifunctional materials utilized to modify the surface of NPs for oral delivery can produce “smart” NCs such as pH-triggered and targeted release systems. Although a considerable number of formulated multifunctional delivery systems have shown the potential for oral delivery of therapeutic proteins/peptides, further issues concerning the safety and side effect, EE and LC of drug loaded carriers, typicality of *in vitro* models, reproducibility of fabrication technique, feasibility of storage condition, and reproducible therapeutic effect in human need to be addressed. What's more, the influence of anesthetics and sampling methods for evaluating oral drug delivery systems of peptides/proteins cannot be overstated¹⁶⁹. At present, lack of information being available about the detailed absorption mechanism of established delivery systems *in vivo* is a major barrier to progress in further study. More mechanism studies are needed to shed new insights on the details of intestinal absorption of NPs to realize the rational design of NPs and enhanced oral bioavailability¹⁷⁰. After being endocytosed, NCs interact with different organelles, including endosomes, lysosomes, ER, and the Golgi apparatus, and transport *via* diverse routes, such as the endolysosomal, ER/Golgi, and cytoplasmic routes, resulting in totally different destinies. Whether these interactions of NCs with organelles are beneficial to the oral delivery of peptides/proteins remains to be investigated¹⁷¹. In particular, intracellular lysosomal degradation is detrimental to transepithelial transport of peptides/proteins, which should be paid real attention to in the field of oral peptides/protein delivery in future study. All in all, these new delivery systems paved a new way in oral delivery of therapeutic peptides /proteins.

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