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, glatulathione, etc., are commercially available. 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 costs. 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 administration. 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) (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 intensify the difficulties of oral delivery as pH 2.0–4.0 in stomach, pH 5.5 in duodenum, pH 6.0 in jejunum, pH 7.2–8.0 in ileum, pH 6.5 in colon.

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 release. However, the enteric polymer coated may not dissolve completely in the small
which generated nanosized CO2 bubbles while water passes through
strategies applied above, to optimize the ef
and particle size is necessary64. Surface charge plays a critical role in
often the tuning of physicochemical properties like electrical property
was shown by thiolated polymers at pH 3.0. Other tested polymers
bioavailability. Chuang et al.61 developed a bubble carrier system
contacting with the mucus and
carrier system produced steady plasma glucose levels
in the partly dissolved shell, which may lower their oral
the bubble carrier system which generated nanosized CO2 bubbles while water passes through the gelatin shell to saturate the compounds and continue to expand till
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. Barbieri et al.66 prepared ultrasmall (<15 nm), monodispersed and water-dispersible NPs applying a simple and

### 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    |
| Myrcludex 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   |
| Elasilipesi            | A synthetic marine-derived cyclic peptide with a molecular weight of 83 kDa          | 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 decapetide 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)  | An 8-amino-acid peptide                                                               | A specific antigenic peptide                                                    | 31   |
| Exendrin-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.
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 administrations. To pass the epithelial barriers two strategies applied have achieved considerable successes: paracellular pathway by opening TJs and transcellular endocytosis ways by clathrin-mediated endocytosis, caveolae-mediated endocytosis, macropinocytosis or phagocytosis. 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 GIT. For instance, Gaowa et al. 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 delivery. 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 pathways. 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 intestine. 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. 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. orally co-delivered...
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, meritotive 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. Summary of lipid-based particles applied in oral delivery of therapeutic peptides/proteins was shown in Table 5.

| Experimental Techniques         | Advantages/Improvements                                                                 | Ref. |
|--------------------------------|----------------------------------------------------------------------------------------|------|
| CARS microscopy                | To image protein and lipid distributions without prior labeling or destructive sample  | 26   |
|                               | preparation                                                                              |      |
| Microfluidics technique        | Emulsions are formed with an exquisite control and a quite high encapsulation efficiency | 39,40|
|                               | compared to the conventional production methods                                          |      |
| 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      | 66   |
|                               | water-dispersible NPs                                                                  |      |
| 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 poly(methacrylate Eudragit<sup>®</sup> NE as a flexible film, superdisintegrant sodium starch glycolate Explotab<sup>®</sup> as a pore former and applied to a HPMC coating of reduced thickness delays the drug release | 88   |

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

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

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 fusion<sup>91</sup>, well-designed liposomes are extremely essential for effective delivery.

Niu et al.<sup>52,80,81</sup> 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 ± 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.<sup>92</sup> 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. Thiomer-coated liposomes present favorable characteristics of mucoadhesion, TJs-opening effect, efflux pumps inhibition, and enzyme inhibition<sup>93–95</sup>. Gradauer et al.<sup>14</sup> coupled CS and thioglycolic acid (TGA) to coat sodium calcitonin (sCT)-loaded liposomes, these thiomer-coated liposomes causing no immunogenic reactions in mice showed enhanced permeation of
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-glycerolcaldityl 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
3.4% whereas free hGH administered orally was only 0.01%\textsuperscript{8}. What's more, liposomes with 25% TEL could improve the oral bioavailability of octreotide more than 4-fold compared with free octreotide\textsuperscript{9}. Uhl et al.\textsuperscript{5} found that an almost two-fold elevation in vancomycin uptake of TEL-liposomes over conventional liposomes was detected in Wistar rats. He and his group\textsuperscript{77} further showed that myrtulix 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.\textsuperscript{10} 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 (DLS), 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 nanocarriers\textsuperscript{79}. Roger et al.\textsuperscript{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 biodegradable\textsuperscript{101}. Christophersen et al.\textsuperscript{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.\textsuperscript{102} dissolved Ins into the inner aqueous phase and then emulsified in an organic phase to prepare a biodhesive 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.\textsuperscript{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 SLP 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 hydrophilicity of SLPs. Yuan et al.\textsuperscript{104} evaluated that the permeation ability of PEGylated (MW = 2 kDa) SLPs were decreased through Caco-2 cell monolayer while increased through a mucus-secreted co-cultured Caco-2/HT29 cells. The relative oral bioavailability of PEGylated SLPs elevated 1.99-folder 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 water\textsuperscript{105}. Karamanidou et al.\textsuperscript{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.\textsuperscript{106} prepared Ins–phospholipid complex-loaded SNEDDS and then coated with Eudragit\textsuperscript{R} 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.\textsuperscript{107} designed a successful SNEDDS for oral delivery of polypeptide-k (PPK) which exhibited stable characters focusing on the protective effects against protease degradation.

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**Figure 6** Schematic illustration of transepithelial transport of Ins from DNP to overcome multiple barriers of the intestinal epithelium by exploiting the bile acid pathway\textsuperscript{168}. Adapted from Fan et al.\textsuperscript{78} with permission © 2017 Elsevier Ltd.
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 Dose | PD Ref. | Ref. |
|----------------------------------------------|------------|-------------------------------|------------------------------------------------------------------------------------------------|---------|---------|------|
| 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     | 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     | 7    |
| Liposomes containing bio-enhancers and tetraether lipids | hGH        | N/A                           | Size: 229.7 ± 12.8 nm; ZP: 40.0 ± 1.2 mV; EE: 31.2 ± 0.5%                                             | 8 mg    | 3.4     | 8    |
| Liposomes with 25% TELs                      | Octreotide | N/A                           | Size: 130–207 nm; EE: 13.0%                                                                       | N/A     | N/A     | 9    |
| Octreotide-DOCA SEDDS                        | Octreotide | N/A                           | Size: 152 nm; ZP: −3.7 mV                                                                            | 50 mg   | 5.21    | 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    | 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 | 38   |
| Liposomes containing SGC, STC, STC respectively | Ins        | Transcellular way              | Size: 157 ± 19 nm; EE: 29.8 ± 1.7%                                                                   | 20 IU/kg| 8.5 ± 2.1 (the optimal formulation) | 52, 81 |
| Biotinylated liposomes (BLPs)                | Ins        | Biotin receptor mediated transport | Size: ~150 nm; EE: 35%–42%                                                                            | 20 IU/kg| 12.09   | 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     | 98   |
| VA incorporated SLN nanoparticles            | Ins        | N/A                           | Size: 172–281 nm; ZP: −40 mV; EE: 54.5%                                                              | 50 IU/kg| 5.1     | 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 | 106  |

ZP, zeta potential; EE, encapsulation efficiency; F, relative bioavailability; PGL, plasma glucose levels; TGA, thiglycolic acid; MNA, 6-mercaptonicotinamide-conjugate; Pt, protamine; TELs, tetraether lipids; GCTE, glycerylcaldityltetraether lipids; VA, viscosity-enhancing agent; SNEDDS, self-nanoemulsifying drug delivery systems; DOC, sodium docusate; DOCA, deoxycholate
Hetényi et al. verified that SNEDDS provided a perfect protection towards protease degradation and deactivation by GSH. Zupancic et al. 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. 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 ± 3.07% and caused a significant (P<0.05) decrease in PGL. Bonengel et al. 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 hydrophobic drugs including therapeutic peptides/proteins is considered extremely challenging due to their hydrophobic nature.

2.4. Multimulsion

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 carriers.

Dogru et al. 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. prepared Ins-loaded NPs in multimulsion with particle size of 300–400 nm, which, in different pH conditions, protected cargos from destruction. Griffin et al. 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.

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/proteins. 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 (pKₐ=6.5) composed of N-acetyl glucosamine (GlcNAc) 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 TJ.s. CS-based NPs are attracting increased attentions for their abilities to orally deliver therapeutic peptides/proteins. Poly-γ-glutamic acid (γPGA), a natural peptide carrying negative charge, has been used to deliver protein vaccines. Sonaje et al. prepared Ins-loaded CS NPs mixing with anionic γPGA with a mean particle size of 218.0 ± 3.4 nm and EE of 71.8 ± 1.1%. During the preparation of NPs, MgSO₄ and TIP were introduced to raise their stabilities in an extensive range of pH. The NPs demonstrated a relative bioavailability of 15.1 ± 0.9% and a reductive trend in PGL in 10 h in diabetic rats. It is well known that valent 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. covalently conjugated DTPA on γPGA mixing with CS for oral delivery of Ins. The CS/γPGA 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 ± 1.3%. Besides, ethylene glycol tetracetic acid (EGTA) is a Ca²⁺-specific chelating agent. Chuan et al. synthesized CS/PGA–EGTA NPs for oral delivery of Ins, which ultimately produced a prolonged hypoglycemic effect in vivo with a relative bioavailability of 21.3 ± 1.5%. They further demonstrated that combination therapy by co-loading Ins with exendin-4 delivered by CS/PGA NPs can be more effective than its monotherapy counterparts in achieving preferable glycemic control and undergoing oral glucose tolerance test (OGTT). 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 stability. Sukyung et al. conjugated cysteinylated exendin-4 to low molecular weight chitosan (LMWC) via a disulfide bond which is cleavable under physiological conditions. 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. The negatively charged NGs exhibited a higher mucoadhesion and better intestinal permeability than positively charged ones in vitro intestinal studies, which can be accounted for the attenuated surface charge of CMCS/CS-NGs (+) and weaker contact with mucosal epithelial cell in alkalyscence environment (pH>6.5) of jejunum. It is known that transition metal ions such as Fe³⁺ 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. prepared CS/PGA NPs, in the presence of Fe³⁺, the EE of NPs significantly enhanced 2.5-fold compared to NPs without Fe³⁺. 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±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. 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. |
|--------------------------------------------------------------|-----------------------------------|---------------------------------------|-----------------------------------------------------------------------------------------------|-----------------|-----------------------------|------|
| 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                          | 32                           |
| Conjugated with LMWC through disulfide bonds                 | Exendin-4                         | N/A                                   | Size: 101 ± 41 nm; ZP: 44.36 mV                                                               | 400 µg/kg       | 6.39                        | 33                           |
| CS/Fe³⁺-γ-PGA NPs                                            | Exendin-4                         | Paracellular way                       | Size: 260.6 ± 26.4 nm; EE: 60.92%                                                             | 300 µg/kg       | 14.0 ± 1.8                  | 34                           |
| CS/TTP                                                      | Exendin-4                         | Paracellular way                       | Size: 303.1 ± 10.36 nm; ZP: 18.37 ± 1.15 mV; EE: 38.02%                                       | N/A            | N/A                         | N/A                          |
| PLGA/CS-CPP (PSI)/CS-CPP (GLP-1coloaded with DPP4 inhibitor) | N/A                               | N/A                                   | Size: 277.2 ± 3.8 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                         | 39,40                        |
| CS/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 | Size: 184.5 ± 13.5 nm; ZP: 24.70 ± 2.45 mV; EE: 83.51 ± 4.24%                                   | 75 IU/kg        | 7.55 ± 1.32                 | 60% decrease of PGL in 12 h | 83                           |
| PGA-γ-DA micelles with CSK peptide conjugated TMC            | Ins                               | Clathrin-dependent and caveolar-dependent endocytosis | Size: ~150 nm; ZP: 20.0 ± 3.4 mV; EE: > 95%                                                    | 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                               | TJ opening                            | 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                          |

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| Formulation composition                        | Model drug | Main transport mechanisms                           | Characterization (size, ZP, EE) | PK Dose | PK F (%) | PD | Ref.        |
|------------------------------------------------|------------|-----------------------------------------------------|-------------------------------|---------|----------|----|-------------|
| CS/γ-PGA-EGTA NPs                              | Ins        | Paracellular way                                     | Size: 328.6 ± 2.3 nm; ZP: 38.7 ± 0.2 mV; EE: 78.7 ± 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: 5.41 ± 1.9% | 50 IU/kg | 8.56      | 36% decrease of PGL in 4 h  | 131 |
| PSA coated protamine NCs                       | Ins        | Paracelluar way and caveolae (predominantly) and Clathrin mediated endocytosis | Size: 301 ± 84 nm; ZP: −4 ± 1 mV; EE: 51 % | 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/albunin-coated alginate/dextran sulfate nanoparticles | Ins        | Clathrin-mediated endocytosis.                      | Size: 300.8 ± 3.8 nm; ZP: 28.9 ± 0.9 mV; EE: 30.7 ± 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 ± 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 ± 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                                                  | Size: N/A; ZP: N/A; EE: N/A | 50 IU/kg | ~4.43     | ~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, glycy-γ-glutamic acid conjugated nanoparticles; TMC-CM-AA, alanyl-alanine conjugated nanoparticles; TJs, tight junctions; INS, insulin; TPP, sodium tripolyphosphate; CS, chitosan; NCs, nanocomplexs; γ-PGA, 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-mercaptopicolinic acid; TGA, thioglyclic acid; PAA, polyacrylamide; S-chitosan, succinylo 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 ± 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.123. 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.63 evaluated the CS-based NCs modified by L-valine and phenylboronic acid (a glucose-responsive unit). Results showed a corresponding bioavailability of 7.55 ± 1.32% after oral administration. CSKSDYQC (CSK) peptide has been identified to specifically recognize goblet cells, the second large cell population on epithelium. Zhang et al.64 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-folder than that of unmodified NPs and the total decrease of PGL was 1.9-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. Li 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-folder 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-folder 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 release.123. 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).125. 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.126. 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 alanyllalanine (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-mercaptopicolinic acid (MNA) is a thiolated CS with strong mucoadhesive properties and a pH-independent reactivity. Millottl et al.23 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 pH.30. The protection of thiol groups on the thiomers prevents an early oxidation before contacting with mucosa. Dünnaueit et al.29 prepared sulfhydryl-protected thiolated CS for oral delivery of antide. They utilized thiglycopic 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%. Sukamran et al.30 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 physiochemical properties of N-(2-hydroxypropyl) methacrylamide copolymer (pHPMA) can be easily tuned by manipulating the monomers. Liu et al.31 designed NPs composed of Ins-loaded TMC-based polyelectrolyte complex core, and a dissociable coating of poly(2-hydroxypropyl) methacrylamide copolymer (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 Byetta™. 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.127 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 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-jejunally to healthy rats resulted in a moderate reduction of PGL (20% reduction) and lasted for 4 h.

Alginates (ALG) extracted from brown seaweed are natural water-soluble linear polysaccharides composed of α-1-β-D-mannuronic acids and β-1-α-L-guluronic acids. They are anionic compounds featured by the capability to form hydrogels in the presence of divalent cations such as Ca2+. They have significant advantages in oral delivery for their pH sensitivity and low cost. Lee et al.136 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/v 200:40) releases 90.8 ± 1.2% of encapsulated SOD at pH 7.4 in 2 h, while only 11.4 ± 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 8.47 ± 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 HPMC. Initially, they attuned pH-sensitive property of HPMC 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 (HPMCP, pKa 5.2) as a pH-sensitive polymer. Fluorescently-labeled CS/HPMCP NPs showed 2–4 times improvement in the intestinal mucosal adhesion and penetration compared to CS/TPP NPs. Following oral administration, CS/HPMCP NPs with a relative bioavailability of 8.47 ± 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 HPMC. Initially, they attuned pH-sensitive property of HPMC 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 (HPMCP, pKa 5.2) 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 matrix.

4. Inorganic particles

Some of the inorganic NPs have been successfully applied in oral delivery of therapeutic peptides/proteins, gold NPs (Au NPs)38,42,146, selenium NPs (Se NPs)39, silica NPs (Si NPs)32,147,148, and 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 Dose | PD F (%) | Ref. |
|-------------------------|------------|---------------------------|---------------------------------|--------|----------|------|
| NiMOS-loaded HNTs       | Albumin    | N/A                       | Size: 215.3±2.5 nm; EE: 63.16±4.66% | N/A    | N/A      | 25   |
| Si NPs–PEG              | Ins        | N/A                       | Size: 409.7±89.10 nm; ZP: −15.2±0.0 mV; EE: 85.4% | N/A    | N/A      | 43   |
| A bubble carrier system loading DTPA, SBC, SDS and Ins | Ins | Transcellular 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±3.42% | N/A    | N/A      | 87   |
| Montmorillonite coated with TiO2 | Ins |         | Size: 50 nm; ZP: −54.5 mV | 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      | 156  |
| Ins/ZrP coated with TiO2 | Ins |         | Size: 364.2±53.9 nm; ZP: 27.3±2.4 mV | N/A    | N/A      | 159  |

ZP, zeta potential; EE, encapsulation efficiency; F, relative bioavailability; PGL, plasma glucose levels; NiMOS, nanotubes-in-micrgel 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 shown 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 research61. 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–PEG20,000 showed a faster diffusion followed by Si NPs–PEG40,000 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 nancapsule 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.151 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 release. 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 to 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. Inchaurraga and coworkers 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 PEG10,000 and lower for excessive densities of coated PEG. However, a dilemma occurs when selecting proper materials between high mucus permeation and high epithelial transmigration. LMWP (VSSRRRRGGRRRR), composed of 10 arginine residues, could function as a CPP to achieve effective intracellular transport. Shan et al. demonstrated that the NPs co-loaded with PEG as a core and then coating dissolvable pHMA could successfully solve the dilemma. The NPs exhibited 20-fold higher absorption than free Ins on epithelial cells that secret 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. developed monomeric Ins/LMW conjugates (w/w, 1:1) by using succinimidyl-[(N-maleimidompropionamido)-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 Zn\(^{2+}\) improves the thermal stability of cargos by forming Zn\(^{2+}\)-imidazole coordination complexes via modulating Zn\(^{2+}\) at the active site of proteins. Zhang et al. used PEG–PLGA as the forming polymer to orally deliver LMWP–exenatide–Zn\(^{2+}\) complex. The relative bioavailability of LMWP–exenatide–Zn\(^{2+}\) NPs was augmented by 1.74-fold compared to LMWP free NPs. Additionally, the AUC of exenatide–Zn\(^{2+}\) NPs was 3.27-fold higher than NPs contained no Zn\(^{2+}\), which confirmed the function of Zn\(^{2+}\) in improving the biological activity of exenatide.

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. 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. synthesized Ins-loaded PLGA–PEG copolymer NPs using the double-emulsion method (w/o/w), in which PEG\(_{2000}\) showed higher EE (62.3 ± 5.6%) than PEG\(_{4000}\). Although PEG has been widely used as a hydrophilic segment of diblock copolymers, it might lead a protein-repelling effect. García-Díaz et al. incorporated premixing amphiphilic lipids–Ins into PLGA NPs, resulting in a significantly enhanced bioavailability 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. 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. 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. 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. applied a three-layer colonic system to prepare polyelectlyone imine (PEI) coated Ins-DEX NPs. The three-layer release technology platform was consisting of a flexible film composed of a neutral polymethacrylate Eudragit\(^R\) NE and sodium starch glycolate Explotab\(^R\), 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 substractures 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. 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. BRO modified NPs exhibited higher permeating abilities by altering the structure of mucosal layer compared to PAP conjugated ones. Koetting et al. synthesized pH-responsive hydrogels composed of itaconic acid (IA) copolymerized with N-vinylpyrrolidione (NVP) to orally deliver therapeutic proteins with high pH. 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. 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 functionlization PEG (Bu-PEG) NPs,
| Formulation composition | Model drug | Main transport mechanisms | Characterization (size, ZP, EE) | PK Dose | PD F (%) | Ref. |
|-------------------------|------------|---------------------------|---------------------------------|---------|----------|------|
| mPEG-g-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<sub>b</sub>-PLGA<sub>11000</sub> 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                       | Size: 13.4 ± 5.8 µm; ZP: 80.2 ± 1.3% | 50 IU/kg | 7.08 | The BGL dropped considerably by 70% in 10 h | 45 |
| PEA-COOH/Arg-PEA microspheres | 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 |
| CS/pluronic F127-lipid vesicles/PEO core shell corona nanolipoparticles | Ins | N/A                       | Size: 195.3 ± 136-143 nm; ZP: 81%–85% | 120 IU/kg | 1.2 | 74% decrease of PGL in 30 h | 53 |
| 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; ZP: ~11.89 ± 0.11 mV; EE: 57.4 ± 0.03% | 50 IU/kg | 9.28 | 58.8% decrease of PGL after 4 h | 86 |
| Bu-PEG NPs              | Ins        | MCT1-mediated endocytosis | Size: 90.8 ± 1.73 nm; ZP: ~9.89 ± 0.11 mV; EE: 57.4 ± 0.03% | 20 IU/kg | 17.98 ± 5.61 | 50% decrease of PGL in 9 h | 121 |
| Ins-LMWP conjugates loaded TMC-coated PLGA nanoparticles | Ins | Paracellular way and clathrin-dependent endocytosis and adsorptive endocytosis. | Size: 2.53 ± 6.4 nm; ZP: 47.5 ± 5.4 mV; EE: 49.3 ± 2.1% | 20 IU/kg | 3.02 ± 0.66 | 50% decrease of PGL in 10 h | 163 |
| Insulin/CPP NCs coated pHMA | Ins | Paracellular way | Size: 177.3 ± 15.2 nm; ZP: ~10 mV; EE: 94.9 ± 1.1% | 75 IU/kg | N/A | 60% decrease of PGL in 20 h | 167 |
| ConA–PEG-PLGA           | Ins        | Lectin-receptor mediated transport | Size: 196.3 ± 4.5 nm; ZP: ~25 ± 6.18 mV; EE: 44.6 ± 3.5% | 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); pHMA, N-(2-hydroxypropyl) methacrylamide copolymer; PGA-g-DA, dodecylamine-graft-g-polyglutamic acid; FEO, polyethylene oxide; PGL, plasm glucose level; Bu-PEG, butyrate-conjugated PEG; MCT1, monocarboxylate transporter 1; mPEG-g-AA, mPEG grafted algic acid; Con A, concanavalin A; PEA, poly(ester amide); ARG, arginine; DOCA, deoxycholic acid; TMC, N-trimethyl chitosan.
established by Wu et al. 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. demonstrated that the mPEG grafted alginic acid micelles with size of 72 nm could significantly improve (P<0.001) the oral absorption of secretin 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. The established system showed a delayed response (2–4 h) in the reduction of PGL within an acceptable range. Samstein e al. hypothesized that bile salts could be used to enhance the bioavailability of PLGA NPs by protection and elevated absorption. Oral administration of Insl 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 PLs, 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” NPs 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 overstated. 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 bioavailability. 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 destinations. Whether these interactions of NPs with organelles are beneficial to the oral delivery of peptides/proteins remains to be investigated. 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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