Adapting liposomes for oral drug delivery

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Abstract Liposomes mimic natural cell membranes and have long been investigated as drug carriers due to excellent entrapment capacity, biocompatibility and safety. Despite the success of parenteral liposomes, oral delivery of liposomes is impeded by various barriers such as instability in the gastrointestinal tract, difficulties in crossing biomembranes, and mass production problems. By modulating the compositions of the lipid bilayers and adding polymers or ligands, both the stability and permeability of liposomes can be greatly improved for oral drug delivery. This review provides an overview of the challenges and current approaches toward the oral delivery of liposomes.

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Abbreviations: APC, antigen-presenting cell; AUC, area under curve; BSA, bovine serum albumin; DC, dendritic cells; DMPC, dimyristoyl phosphatidyl choline; DPPC, dipalmitoyl phosphatidylcholine; FAE, follicle-associated epithelia; FITC, fluorescein isothiocyanate; GIT, gastrointestinal tract; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; MRT, mean residence time; MVL, multivesicular liposomes; PC, phosphatidylcholine; PEG, polyethylene glycol; rEGF, recombinant human epithelial growth factor; RES, reticulo-endothelial; SC, sodium cholate; SDC, sodium deoxycholate; SGC, sodium glycocholate; STC, sodium taurocholate; SPC, soy phosphatidylcholine; SUV, small unilamellar vesicles; Tgel, gelling temperature; Tp, phase transition temperature; TPGS, tocopherol polyethylene glycol succinate; UEA 1, ulex europaeus agglutinin 1; WGA, wheat germ agglutinin
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1. Introduction

Since the discovery of liposomes by Bangham and Horne in 1964, the potential of liposomes as drug delivery carriers has been extensively explored via versatile administrative routes such as parenteral, oral, pulmonary, nasal, ocular and transdermal routes. In 1974, AmBisome, a formulation of amphotericin B, became the first injectable liposome product to be licensed. Nevertheless, primitive parenteral liposomes have one severe drawback: they are always cleared from blood very quickly and end up in organs and tissues in the reticulo-endothelial system (RES, e.g., liver, spleen, and lung). The clearing occurs by plasma opsonization and subsequent sequestration from circulation. The successful marketing of Doxil in 1995, a pegylated liposomal doxorubicin product, represents a milestone in the development of parenteral liposomes.

Liposomes consist of enclosed vesicles of concentric self-assembling lipid bilayers composed of phospholipids and cholesterol in common. According to the structure of lipid bilayers and the size of the vesicles, liposomes are commonly classified into large unilamellar vesicles (LUV), small unilamellar vesicles (SU), multilamellar vesicles (MLV) and multivesicular vesicles (MVV). While LUV, SU and MLV are candidate carriers for versatile routes including the oral route, MVV are used for parenteral delivery only. The inner aqueous phase of liposomes is well protected by the lipid bilayers and is able to load hydrophilic entities, whereas the hydrophobic region in the lipid bilayers is able to load hydrophobic entities (Fig. 1). The most remarkable advantages of liposomes are their biocompatibility and safety due to resemblance to biomembranes. Moreover, it is easy to modify the liposomal surfaces by conjugation to polymers and/or ligands so as to endow the vesicles with special properties (Fig. 1). See recent reviews for a better understanding of the history and various application aspects of liposomes.

Oral delivery of liposomes has a long history as well and can be traced to as early as the late 1970s. It is interesting to see that the initial application of oral liposomes was with the delivery of insulin, emphasizing the continual challenge in the field of oral drug delivery. Despite the initial ardor, the efficacy of oral liposomes was not reproducible or predictable. For instance, only 54% of the normal rats and 67% of the diabetic rabbits responded to the treatment of oral liposomal insulin. More negative results added to the disappointment of using liposomes as oral delivery carriers, and there seemed to be a period of quiescence in the 1980s. However, attempts to use liposomes as drug carrier systems for oral delivery resurfaced in recent years, thanks to modern modification technologies to enhance liposomal stability and permeation.

By addition of polymer coatings and modulating liposomal compositions, both the stability of liposomes in the gastrointestinal tract (GIT) and trans-epithelial absorption of active components have been significantly improved. It is worth noting that once again oral delivery of biomacromolecules, especially proteins and peptides, becomes the hot topic of research and discussion. In addition to improved oral bioavailability, the pharmacokinetic and pharmacodynamic profiles are improved as well. In this review, the status quo will be summarized with emphasis on challenges and strategies taken to adapt liposomes for oral delivery.
only 5% of human FAE and 1% of total intestinal epithelial cell population. On the other hand, the rapid secretion and shedding of gastrointestinal mucus significantly restrict the oral absorption of liposomes as well, which are likely trapped in the mucus layers via hydrophobic interaction. There is so far no direct evidence confirming the transport of intact liposomes across intestinal enterocytes.

2.3. Formulation challenges

Although several liposomal formulations (e.g., Doxil) have been successfully marketed, the production of liposomes is not without challenges. In fact, the mass production of liposomes is largely unsatisfactory due to batch-to-batch variations. Although it may meet the demands for parenteral products, the biggest batch sizes so far are not big enough for oral use, which usually require higher doses and extended courses of treatment. Owing to the instability of liposomes in aqueous dispersion, there is always a need to formulate liposomes into solid dosage forms. Traditionally, freeze-drying is employed to produce solid liposomal formulations with good reconstituting capacities. However, the freeze-drying technology is less efficient and consumes much time and money. More efficient technologies are desired for mass manufacturing of solid liposomal products.

3. Recent advances in modulating liposomes for oral drug delivery

3.1. Stabilization

In view of the poor stability of liposomes during production, storage and transit across GIT, a series of approaches such as modulation of lipid compositions, surface coating and interior thickening have been explored to stabilize liposomes.

3.1.1. Modulation of lipid compositions

Conventional liposomes are commonly comprised of phospholipids and cholesterol, mimicking the physiological compositions of biomembranes. Although liposomes demonstrate certain degree of stability both in vitro and in vivo, they are susceptible to the harsh gastrointestinal environment. Liposomes containing phospholipids with phase transition temperatures (T°) below 37 °C are completely disrupted by bile salts, but this effect is less pronounced for those with T° higher than 37 °C. In early developmental stages, it is an easy option to improve the physical stability of liposomes by optimizing lipid compositions. By incorporating stearylamine, liposomes are charged positively and are capable of suppressing the digestion of insulin by trypsin and enhancing the hypoglycemic effect. Replacing phospholipids or cholesterol with specific lipids or steroids improves the performance of oral liposomes due to enhanced stability in the GIT and pancreatin. Insulin-loaded liposomes prepared with dipalmitoyl phosphatidylcholine (DPPC) and a soybean-derived sterol mixture exhibit a better hypoglycemic effect than conventional liposomes, which was ascribed by the authors to increased rigidity of the lipid bilayers.

As a type of surfactant secreted by hepatocytes, bile salts have been considered to be the main factor for the disruption of liposomes in GIT. Paradoxically, studies revealed that prior incorporation of bile salts into liposomal bilayers stabilized the membranes against the destructive effect of physiological bile salts. It is well accepted that physiological phospholipids and bile salts readily form colloidal mixed micelles, which is the main mechanism for oral absorption of aliphatic acids and glycerides. Bile salts always have a tendency to associate with phospholipids actively, even from lipid bilayers of plain liposomes, thereby compromising the integrity of liposomes. However, the prior incorporation of bile salts in liposomal bilayers offsets the destructive effects of outside bile salts. To date, liposomes containing bile salts, also named as bilosomes, have been widely investigated for both oral immunization and oral delivery of poorly water-soluble drugs and biomacromolecules. Various types of bile salts including sodium glycocholate (SGC), sodium taurocholate (STC) and sodium deoxycholate (SDC) have been incorporated into liposomes to protect enclosed insulin from enzymatic degradation by pepsin, trypsin and α-chymotrypsin. A better protection of insulin is observed for liposomes containing SGC than liposomes containing STC or SDC and conventional liposomes. It is believed that improved stability of liposomes by bile salts contributes at least partly to enhanced oral bioavailability of insulin.

3.1.2. Surface coating

To protect liposomes from the harsh gastrointestinal environment, another workable approach is to coat liposomal surfaces with layers of polymers such as enteric polymers, proteins and chitosans. Enteric coatings are well known to prevent liposomes from disintegration in the stomach thereby improving absorption as more liposomes survive and are exposed in small intestine. Liposomes coated with Eudragit L100 enhance the oral bioavailability of alendronate sodium by 12-fold in rats as compared with the commercial tablets. However, in some cases a layer of coating with enteric polymers such as Eudragit S100 does not protect damage by bile salts. To this end, a design of liposomes-in-microspheres delivery systems comprising chitosan-coated liposomes within Eudragit S100 microspheres was found to be highly effective to resist the attack by bile salts.

Polysaccharides are another kind of functional coating materials used to stabilize liposomes in the GIT. Arabinoside-loaded liposomes coated with O-palmitoyll pullulitan (OPP), a polysaccharide derivative, are able to withstand the damage caused by sodium cholate (SC) at a concentration up to 16 mmol/L at pH 5.6 or pH 7.4. Moreover, OPP-coated liposomes showed a reduced release rate at pH 2.0 and 5.6 at 37 °C as compared with uncoated liposomes. Polysaccharide-coated liposomes loading bovine serum albumin (BSA) are capable of producing higher levels of serum IgA and IgG in comparison with naked liposomes, indirectly verifying improved stability of the model drug. In addition to OPP, O-palmitoylcurdlan sulfate and O-palmitoyl-scleroglucan have been utilized to protect liposomes from SC and pancreatin. Well-known as a gelling agent, pectin has also been studied as a stabilizer for liposomal drug delivery systems. Low- and high-methoxylated pectins show improved liposomal stability upon storage without disturbing membrane permeability. Among various polysaccharides, chitosan may be the choice of coating materials because it is positively charged and readily interacts with the negatively charged liposomal surfaces to ensure firm coating. On the contrary, positive charges should be introduced onto liposomal surfaces to achieve firm coating with negatively charged polymers such as pectins via electrostatic interaction. In vitro studies show that chitosan-coated liposomes achieve better protection of liposomes as well as the protein payloads in artificial gastrointestinal media. Further
observation of enhanced oral bioavailability confirms the effectiveness of coating with chitosan\(^1\). Moreover, the stability of chitosan-coated liposomes can be strengthened by subsequent cross-linking using \(\beta\)-glycerophosphate\(^2\).

Pegylation, a technique originally developed for extending drug half-life in blood\(^3\), has also found applications in the oral delivery of liposomes\(^4\). Pegylation of DPPC and PC liposomes significantly enhances the oral bioavailability of recombinant human epitelial growth factor (rhEGF), which was ascribed by the authors to suppression of enzymatic degradation by coating with PEG\(^5\). Liposomes coated with PEG 2000 or mucin are able to withstand bile salts and improve the stability of encapsulated insulin in GIT\(^6\).

In addition to the coating materials mentioned above, there are many other compounds available for chemical modifications of liposomes. For instance, polyelectrolytes perform well to stabilize liposomes loading doxorubicin\(^7\) or paclitaxel\(^8\) by layer-by-layer (LBL) coating in artificial gastrointestinal fluids with enhanced oral bioavailability by 4–6 folds vs. conventional liposomes. Inorganic materials such as silica\(^9\) and silica nanoparticles\(^10\) are among other stabilizers for oral delivery of liposomes. The formation of layers of protective coatings, as a result of surface adsorption of silica particles, is believed to contribute to enhanced liposomal stability\(^9\)\(^1\)\(^2\)\(^1\)\(^3\).

### 3.1.3. Interior thickening

The physical stability of liposomes can also be improved by thickening the interior aqueous phases. Normally, interior thickening is initiated by increasing the viscosity of the interior aqueous phases, or alternatively by reconstitution of lipid bilayers to enclose hydrogel beads upon mixing the beads with liposomes\(^10\)\(^2\). The so-called Supermolecular Biovector (SMBV\(^\text{TM}\)), which consists of charged, cross-linked polysaccharides cores surrounded by lipid membranes, was found to be an amiable carrier for proteins\(^10\)\(^3\)\(^,\)\(^4\). Another group reported a kind of lipobeads prepared by self-assembling of lipid bilayers around hydrogel beads initiated by acrylamine-functionalized lipids tethered to the bead surfaces\(^10\)\(^5\). In vitro evaluation indicated enhanced stability of lipid bilayers even at temperatures below \(T_g\)\(^10\)\(^6\). Interior thickening can also be attained via \textit{in situ} gelling after formation of liposomes in response to physical stimuli. UV-induced polymerization within liposomes has been utilized to prepare lipobeads with increased mechanical strength and enhanced stability\(^10\)\(^7\)\(^,\)\(^10\)\(^8\). By incorporating reverse-phase thermosensitive in \textit{in situ} gel into the aqueous phase of liposomes, interior thickening was achieved when liposomes were heated to a temperature above the gelling temperature (\(T_g\))\(^10\)\(^9\)\(^,\)\(^10\)\(^1\). \(T_g\) can be tailored within the range of room temperature and physiological temperature (37 °C) through adjusting the ratio of the thermosensitive gel (poloxamer 407/poloxamer 188)\(^10\). Therefore, the liposomes were prepared under conditions similar to conventional liposomes at ambient temperature\(^10\)\(^9\)\(^,\)\(^10\)\(^1\). After administration, the liposomal interior gelates in response to increased temperature. Further study showed that interior gelling improves physical stability and protects the lipid bilayers against membrane destabilizers (Fig. 2)\(^10\)\(^1\). Significantly prolonged elimination time after intravenous injection suggests enhanced liposomal performance in vivo\(^10\)\(^9\). Interior thickening improves some of the physicochemical properties of liposomes such as increased rigidity of the lipid bilayers, modified shape, improved physical stability and sustained release of the payloads. However, the utility of these liposome formulations for oral delivery of liposomes awaits experimental validation.

**Figure 2** Rationale of interior thickening of liposomes with thermosensitive poloxamer 407/188 \textit{in situ} gels. Adapted from Ref. [109] with permission.

#### 3.1.4. Other strategies

In addition to the methods mentioned above, other strategies have also been utilized to improve the stability of liposomes. For example, novel double liposomes, prepared by filtering preformed inner liposomes through a glass filter painted with lipid bilayers, demonstrate even more improved stability\(^11\). The outer bilayers serve as protective coatings against the destruction by intestinal enzymes; as a result, significantly enhanced hypoglycemic effect (insulin)\(^11\)\(^1\) or hypocalcemia effect (salmon calcitonin)\(^11\)\(^2\) was achieved. In another study, liposomes were embedded into gelatin matrices to stabilize the lipid bilayers and attained controlled release of the vesicles\(^11\)\(^3\), although no \textit{in vivo} data were provided.

### 3.2. Absorption enhancement

#### 3.2.1. Enhanced absorption due to mucoadhesion

Mucoadhesion endows liposomes with prolonged GIT residence, allowing prolonged contact of liposomes and/or the payloads with intestinal epithelia and subsequently enhancing opportunities for oral absorption of either liposomal vesicles or the payloads. Enhancement of mucoadhesion is attainable through coating with polymers or modulating surface charges. Positively charged liposomes gain not only mucoadhesion but also resistance to enzyme destruction\(^12\), and thus improve oral bioavailability of the payloads\(^2\). Coating liposomes with mucoadhesive polymers such as polysaccharides seems to be one of the most promising approaches to achieve mucoadhesion\(^4\)\(^1\)\(^,\)\(^11\)\(^4\)\(^–\)\(^11\)\(^6\). Pectins are one class of preferable polysaccharides commonly used\(^11\)\(^5\)\(^,\)\(^11\)\(^7\). Pectin-coated liposomes show adhesion to mucin with high-methoxylated pectin-coated liposomes performing the best\(^11\)\(^5\). In another study, mucoadhesive pectin-liposome nanocomplexes (PLNs) gave better intestinal absorption of calcitonin than uncoated liposomes\(^11\)\(^3\). High density of fluorescently labeled PLNs, observed by confocal laser scanning microscopy, were found adhering to intestinal epithelia and remained for a prolonged duration, suggesting strong mucoadhesion\(^11\)\(^4\).

As a natural cationic polysaccharide derived from chitin via deacetylation, chitosan represents one of the most popular coating materials for oral liposomes due to low toxicity, biocompatibility, biodegradability and mucoadhesion. Various chitosan derivatives are reported to improve mucoadhesive properties of liposomes by either chemical coupling\(^11\)\(^8\) or physical coating\(^11\)\(^9\)\(^–\)\(^11\)\(^2\). Insulin-loaded liposomes coated with mucoadhesive polymers such as chitosan, polyvinyl alcohol and poly (acrylic acid) show better and more prolonged hypoglycemic effect than uncoated ones\(^11\)\(^2\).
type of chitosans also influences the degree of mucoadhesion and thereby the in vivo behaviors; low-molecular-weight chitosans show stronger mucoadhesion. A comparison of different materials on mucoadhesion confirms that chitosan is the best coating materials for liposomes following the order of chitosan-coated liposomes ≥ carbopol-coated liposomes > positively charged non-coated liposomes > negatively charged non-coated liposomes. Combinatory use of chitosan with other mucoadhesive materials such as tocopherol polyethylene glycol succinate (TPGS) reinforces mucoadhesiveness.

Apart from polysaccharides, many other mucoadhesive polymers are also used to coat liposomes. Coating with PEG and mucin not only improves the stability of liposomes but also extends the residence time in GIT, which altogether contribute to the hypoglycemic effect of insulin. In contrast to the mechanism of prolonged residence of pegylated nanocarriers in circulation following intravenous administration, the extended residence of PEG-coated liposomes in the GIT is due to deep penetration of the PEG chains into the mucus layers lining the GIT wall and interweaving with mucin. The extended retention in the GIT thus strengthens the uptake of the vesicles by M cells and subsequent efficacy of oral immunization.

3.2.2. Enhancer-facilitated absorption
Various absorption enhancers have been studied to facilitate the oral absorption of liposomal payloads. TPGS 400, cetylpyridinium chloride and cholylsarcosine, in combination with stearylamine, were confirmed to enhance the oral absorption of liposomal fluorescein isothiocyanate (FITC)-dextran, a hydrophilic macromolecule. Tween-80, a surfactant commonly used as a solubilizer, enhances the oral bioactivity of insulin when it is incorporated into liposomes at a level of 1%. In a comparative study, cetylpyridinium chloride performed better on enhancement of oral bioavailability of human growth hormone than a few other absorption enhancers including α,ω-TPGS 400, phenylpiperazine, sodium caprate and octadecanol.

Bile salts are physiological surfactants that play a very important role in lipid absorption. By incorporating bile salts into the lipid bilayers of liposomes, the oral bioavailability of a variety of hydrophilic and lipophilic drugs has been significantly enhanced. Owing to their structural resemblance to cholesterol, bile salts can be easily incorporated into liposomal membranes to form bilosomes. Among the bile salt family, SC, STC, SDC and SGC are popular candidates used in bilosomes for enhancement of oral absorption. The oral bioavailability of cyclosporine A was significantly enhanced by bilosomes in comparison with conventional liposomes. The enhancement is probably due to facilitated absorption by SDC rather than improved release because drug release from liposomes is very slow.

Non-ionic surfactants are also used as absorption enhancers. Tween 80-reinforced liposomes composed of SPC and choles- terol significantly enhanced the absorption of (+)-catechin following oral administration with increased area under the curve (AUC) and prolonged mean residence time (MRT) as compared to the solution control. Enzyme inhibitors are always used in combination with enhancers to improve the absorption of liposomal biomacromolecules. This was demonstrated by the significantly enhanced hypoglycemic effect of calcitonin when chitosan conjugated with an inhibitor aprotinin was used to coat liposomes.

3.2.3. Polymer-facilitated absorption
Besides enhancement of liposomal stability and mucoadhesion, polymers also enhance intestinal permeability. By opening tight junctions, N-trimethyl chitosan has become a preferable polymer to coat liposomes for oral delivery of various ingredi- ents. Another chitosan derivative, methylated N-(4-N, N-dimethylaminobenzyl) chitosan, was applied to coat FITC-conjugated liposomes to enhance the permeability of a model protein BSA across Caco-2 cell monolayers. The combined use of cell-penetrating peptide such as oligoarginine further enhanced the efficacy of chitosans. It should be noted that opening epithelial junctions with these agents can have both positive and negative effects. The latter may include risks of concurrent entry of toxins as well as payload. The loss and gain of using chitosans are still awaiting systemic evaluation.

The trapping capacity and fast turnover of mucus are known factors which impede the permeability of liposomes across mucus layers. Recently, mucus-penetrating polymers were used to coat liposomes to facilitate permeation. For instance, liposomes coated with chitosan-thioglycolic acid 6-mercaptopropanolamine-conjugate (an S-protected thiomer chitosan with mucus-penetrating capabilities) achieved 8.2-fold enhancement of physiological bioavailability (areas above curves of the blood calcium levels) of calcitonin following oral administration in rats. Pluronic F127-coated liposomes were reported to enhance oral absorption of lipophilic ingredients due to the intestinal mucus-penetrating properties. A 1.84-fold enhancement of AUC<sub>口服</sub> of cyclosporine A-loaded pluronic F127-coated liposomes was seen following oral administration vs. chitosan-coated liposomes. Additionally, polymers with polyethylene oxide tags such as pluronic 85 and PEGylated G5 PAMAM dendrimer inhibit the P-glycoprotein efflux system and enhance overall oral bioavailability when used as coating materials for liposomes.

3.2.4. Ligand-mediated targeting to epithelial cells
To overcome the poor permeability of conventional liposomes, ligands have been investigated to enhance intestinal uptake by epithelial cells via receptor-mediated endocytosis. Since most cell proteins and lipids in cell membranes of the GIT are glycosylated, lectins have been widely utilized to modify liposomes for oral immunization or oral drug delivery. This is possible due to the specific recognition and binding by lectins to glycans. Wheat germ agglutinin (WGA)-modified liposomes containing insulin achieved superior control of blood glucose as compared with alex europaeus agglutinin 1 (UEA 1)-modified ones. However, the results are not consistent with the findings obtained by another group, who reported that UEA 1 performed better than WGA. By taking advantage of the interaction between lectins and glycans, mannose derivatives were applied to modify liposomes to target mannosyl receptors expressed in antigen-presenting cells (APCs). Antibodies were attached to liposomes to enhance gastrointestinal permeability as well. In this case, IgA-coated liposomes containing ferritin showed enhanced immune responses. The authors ascribed the enhancement to increased uptake via M cells, but did not mention the relevant receptors. In a recent work, Fe fragments were used as ligands to modify liposomes for active targeting to neonatal Fe receptors. Results with these liposomes showed significantly improved hypoglycemic effects of insulin. In view of the instability of peptide ligands in the GIT, non-peptide ligands such as folic acid (FA) and biotin are preferred for liposomal surface
modification. FA-modified polymer-stabilized multilayer liposomes gave an approximately 20% relative bioavailability of insulin following oral administration vs. results from subcutaneous administration. Similarly, functionalization of bilosomes with glycomannan improved liposomal targeting and stability. In addition to the ligands mentioned above, ligands employed in the oral delivery of other types of nanoparticles can also be utilized to modify liposomes.

### 3.3. Mass production

The practice of developing liposomes as oral drug delivery systems has motivated investigations on the mass production of liposomes on an industrial scale. On a laboratory scale, liposomes can be prepared using a variety of methods such as thin-film dispersion, reversed-phase evaporation, detergent dialysis, solvent injection and a few other methods. So far, these methods are only successful for small scale production of liposomes. Problems encountered with scale-up include poor size distribution, poor batch-to-batch reproducibility, physicochemical instability, residues of organic solvent and high production costs.

Considerable effort has been made in recent decades to overcome these problems. A continuous high-pressure extrusion apparatus was developed to prepare liposomes with uniform size on a one-liter scale. The leakage of drugs upon extrusion is seen as a drawback of this method. A high-speed dispersion method has been developed to prepare liposomes with high physical stability and encapsulation efficiency. One concern with this technique may be the production of smaller-sized liposomes, ranging from 280–350 nm. High-pressure homogenization/extrusion has been applied to downsize large liposomes containing plasmid DNA with commercially available instrumentation. Although this methodology has the capability for large-scale continuous (1–1000 L/h) production but drug leakage and high production costs of this complex process restrict its industrial application.

The ethanol injection technique is probably the most suitable present method for implementation at industrial scale due to its simplicity and safety. Regarding this method, size distribution is controllable by modulating the aqueous phase temperature in large-scale production. A novel ethanol injection method using a microengineered nickel membrane was recently developed. Depending on the size of the membrane, this technique can be easily scaled up to a very large scale. Moreover, the size and size distribution of liposomes can be controlled via the oscillating membrane system during scale-up. Another scalable production technology based on ethanol injection produces liposomes regardless of production scale under fully sterile conditions. Economical evaluation of liposome production by ethanol injection suggests economic feasibility for a plant with a daily production of 288 L of liposomal suspension.

Owing to the physical instability of liposomes in aqueous media, the storage problems must also be considered. Therefore, there has been consistent effort to prepare liposomes as solid dosage formulations. Spray-drying and freeze-drying are commonly used to address this problem. However, factors such as high cost of freeze-drying and heat liability of the payloads in spray-drying limit industrial applications. In contrast, proliposomes are an alternative for mass production and storage of liposomes due to the solid state formulations and simplicity in production. Preliminary evaluation of proliposomes containing amphotericin B demonstrated promising features for large-scale industrial applications. More importantly, the final dosage forms of proliposomes resemble conventional oral solid dosage forms and can be easily adapted to conventional manufacturing facilities and processes. This was demonstrated by BSA proliposome tablets coated with Eudragit L100 that could be completely reconstituted into liposomes.

In spite of the progress in mass production of liposomes, only a few parenteral, and no oral liposomal products are successfully marketed. There are significant remaining impediments to successfully developing liposomes as oral drug delivery carriers.

### 4. Mechanisms of oral absorption of liposomes

Despite the advances outlined above, the mechanisms of oral delivery of liposomes have yet to be elucidated. To begin this topic, it is important to outline the general fate of liposomes as well as the embedded drug payloads following oral administration (Fig. 3). Orally administered liposomes are partially destroyed following exposure to gastric acid. Although some of the payload drug is released, other liposomes and their cargo survive. While free drugs follow their own fate, surviving liposomes are emptied from stomach and transit into small intestine, where another fraction is destroyed by intestinal surfactants and enzymes. Liposomes surviving this step penetrate the mucus layers and make close contact with intestinal epithelia. There is still the possibility of destruction of liposomes at this stage as well as release of embedded drugs. However, the fractions of liposomes that survive the whole digestion process are able to be absorbed as integral vesicles via the M cell-to-lymph pathway. Liposomes may be taken up by enterocytes as well, but their fate following this step is unknown. Several mechanisms are proposed as follows.

#### 4.1. Enhanced gastrointestinal stability

As mentioned above, liposomes are prone to degradation in response to the combined effects of gastric acids, bile salts and pancreatic lipases. Degradation of liposomes leads to the leakage of the payloads, which further leads to inactivation or degradation of labile drugs (e.g., peptides and proteins). Leakage also causes precipitation of lipophilic ingredients, thus decreasing the total fraction of oral absorption. Many studies show that enhancing the
stability of liposomes or their payloads significantly improves oral bioavailability. In a sense, improving the stability means to enhance the surviving rate of liposomes and thereby enhance the opportunities to be taken up by intestinal epithelia.

Several strategies have been applied to enhance the stability of liposomes, and the underlying mechanisms have been partly elucidated. For example, phospholipids with a higher \( T_m \) endow liposomes with rigid membranes at physiological temperature, and thus help to resist the gastrointestinal destabilizing factors. Incorporation with bile salts improves the flexibility of the lipid biomembranes and helps to withstand the detrimental effects of bile acids in the GIT. Imaging evidence show that bilosomes surviving the gastrointestinal environment can be absorbed as intact vesicles. By exterior coating, the liposomal membranes are separated from the harsh environment in the GIT due to steric hindrance induced by polymers or polymer-formed water layers, protecting the membranes from the influence of gastric acid, bile salts and pancreatic lipases. Moreover, enzyme inhibitors can stabilize the proteins released from liposomes by inhibiting various enzymes in the GIT.

There is currently a disagreement about whether the payloads are released first before absorption or the liposomes are absorbed as integral vesicles. In the first case, the payloads such as proteins are released in the gastrointestinal lumen and inhibitors must be used together to suppress enzymatic degradation. Secondly, uptake of intact liposomes via clathrin-dependent endocytosis, caveolae-dependent endocytosis, macropinocytosis or fusion may be alternative routes for oral absorption of liposomes. Abundant evidence indicates that free insulin without concomitant use of enzyme inhibitors elicits no hypoglycemic efficacy. Our previous work that validates the transcellular transit of bilosomes shows that some bioenhancers incorporated in liposomes may act as integral vesicles and facilitate uptake across intestinal epithelia as well as enhance passive permeation across intestinal epithelia. A mechanism was proposed for insulin- or calcitonin-loaded chitosan-coated liposomes suggesting that the drugs are released in the mucus layers upon interaction with mucin and degradation of the liposomes, and subsequently absorbed without enzymatic degradation. Other studies ascribe enhanced oral absorption to adherence of the polymers to the mucus layers and prolonged retention therein, facilitating penetration of liposomes and payloads across intestinal epithelial cells.

### 4.3. Facilitated translocation across the mucus layers

The intestinal permeability of liposomes is known to be restricted by the trapping and fast turnover of the mucus layers. The turnover time of the mucus layers are supposed to be a limiting factor that determines the transit time of mucadhesive liposomes. Considering the intestinal mucus turnover time is between 50 and 270 min, mucadhesive liposomes are not expected to adhere to the mucus for more than 4–5 h, a factor that greatly limits the efficacy of mucadhesive polymer-coated liposomes. Therefore, facilitating mucus penetration potentially enhances residence time of liposomes in mucus, thereby increasing the oral absorption of liposomes and their payloads. A series of polymers possessing mucadhesive properties have been utilized to coat liposomes to render them mucus-penetrating instead of mucus entrapped. Phytolip F127 has very good mucus-penetrating ability and has been used to modify liposomes for oral drug delivery. It is reported that facilitated penetration in the mucus layers promotes direct contact of liposomes with epithelia, and thus improves liposomal uptake by caveolae- or clathrin-mediated endocytosis. The mucus-penetrating ability is thought to be attributable to the PEG chains of Phytolip F127 on the surface of liposomes that ease hydrophobic and electrostatic interaction of liposomes with mucins. Besides liposomes, PEG modification has also been used for mucus-penetrating polymeric nanoparticles.

### 4.4. Enhanced permeation across the enteric epithelia

The oral bioavailability of liposomes is limited by poor intestinal permeability of both the vesicles and the payloads, especially biomacromolecules. Incorporation of absorption enhancers along with polymer coatings has been shown to efficiently enhance permeation across enteric epithelia. As for small molecular weight drugs, the effects and mechanisms of absorption enhancers are clear. However, enhancers for absorption of integral liposomes may have different mechanisms. Carrier-mediated transmembrane absorption and penetration through intercellular regions are proposed for enhancing oral absorption of deformable liposomes containing surfactants. Another in vitro study using Caco-2 cell models shows that some bioenhancers incorporated in liposomes may act by interfering with cellular lipid bilayer structure, which leads to facilitated uptake of payloads or higher fusion affinity of liposomes with cell membranes. The opening tight junctions that facilitate paracellular absorption of drugs is another potential mechanism. Furthermore, some absorption enhancers also enhance the oral bioavailability of liposomal payloads by forming lipophilic ion-pair complexes with various organic cations, which increase permeability of the cations across biological membranes. It is worth noting that many absorption enhancers such as bile salts act via multiple rather than a single mechanisms. Chitosans and derivatives are unique types of polymers widely investigated to coat liposomes for oral delivery. The interaction of chitosan with cellular membranes...
is reported to initiate a structural re-organization of tight junction-associated proteins, thus facilitating paracellular transport of hydrophilic macromolecules\[^{176,177}\]. However, a majority of mechanistic studies with chitosan-coated liposomes are carried out in Caco-2 models\[^{91,133}\]. Moreover, P-gp, a multidrug transporter, is responsible for the efflux of various drug substrates, and P-gp inhibition represents another potential mechanism for enhancement of oral absorption of liposomal payloads\[^{36,124,138,178,179}\].

### 4.5. Ligand-mediated endocytosis

Inspired by the fact that some nutrients are absorbed via active absorption, liposomes can be modified with nutritional ligands to achieve active targeting to specific receptors in the enteric epithelia. Ligands are able to further enhance the cellular uptake and trans-epithelial transport of liposomes and thus improve oral absorption. The ligand-receptor interaction probably brings about two aspects of functions: i.e., receptor-mediated transport and accumulation of liposomes at the sites of absorption. The former is comprised of the mechanisms of pinocytosis and phagocytosis, mainly restricted to M cells\[^{180}\]. The latter refers to the ligand-receptor interaction that achieves adherence and accumulation of liposomes at the site of absorption, thus facilitating absorption of the payloads if they are meant to be released there. In general, receptor-mediated pinocytosis occurs by clathrin-mediated endocytosis (CME) or caveolae-mediated endocytosis (CvME)\[^{180}\]. Compared to CME, CvME is not concerned with lysosomal biodegradation. Therefore, the use of liposomes exploiting CvME may be advantageous for oral delivery of enzyme-sensitive drugs. Size seems to be an important factor that determines the patterns of cellular internalization via either CME or CvME\[^{181}\].

Significantly enhanced oral absorption has been reported for liposomes modified with FA\[^{147,148}\], biotin\[^{149,182}\], lectins\[^{140,168}\] and mannose\[^{143,144}\]. FA and biotin interact with their own receptors, (both of which are expressed widely by intestinal epithelia) to improve liposomal uptake via receptor-mediated endocytosis\[^{147–149}\]. Moreover, CME rather than CvME may be an important route for endocytosis, as confirmed by utilizing endocytosis inhibitors (Fig. 4)\[^{149}\]. Lectins interact with the specific glycosylation patterns expressed in M cells or absorptive cells or both to enhance liposomal uptake\[^{139,140}\]. APCs in the GIT, including the macrophages and dendritic cells (DC) (the major APCs present in the vicinity of Peyer's patches), abundantly express the mannose receptors (also called C-type lectin) and thus can be utilized as targeting cells for oral liposomes\[^{143,183}\]. It is worth mentioning that phagocytosis plays an important role in receptor-mediated endocytosis in M cells and APC targeting. In spite of its high efficacy, receptor-mediated endocytosis may not be the sole mechanism for enhanced oral absorption of ligand-modified liposomes\[^{150}\]. Accumulation of liposomes at the sites of absorption and sustained release of payloads prior to absorption contribute to enhanced oral absorption as well.

### 4.6. Uptake by M cells

M cells are specialized epithelial cells located in the FAE of Peyer's patches. They are able to transport a broad range of particles, such as bacteria, viruses and antigens, from the intestinal lumen to the underlying lymphoid tissues\[^{184}\]. Despite the small population of M cells, liposomal absorption through the M cell pathway has many advantages, including less glycocalyx, reduced levels of membrane hydrolases, few lysosomes and high

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**Figure 4** Schematic presentation of enhanced oral absorption of biotin-decorated liposomes via ligand-mediated endocytosis following active targeting to intestinal epithelia. Adapted from Ref. [148] with permission.
endocytosis capabilities\textsuperscript{150}. Furthermore, M cells are the least protected cells by mucus in enteric epithelia and the most exposed to chyme because M cells do not secrete mucus. Therefore, M cells are easily accessible for liposomes via mechanisms of adsorptive endocytosis, fluid phase endocytosis and phagocytosis\textsuperscript{185}. It was shown that the M cell pathway contributes to total oral absorption of liposomes\textsuperscript{187,188}, and the liposomal surface charges influenced the efficiency\textsuperscript{187}. In addition, prolonged residence of liposomes in GIT increases the opportunity of uptake by M cells\textsuperscript{188,189}, which partly explains the contribution of stabilization of liposomes to enhanced oral absorption\textsuperscript{69,95}. Polymer-coated liposomes can be transcystosed by M cells as well due to prolonged contact with intestinal epithelia\textsuperscript{189}. To further increase oral absorption, ligands such as lectins\textsuperscript{41,168} have been utilized to modify liposomes to target M cells as mentioned above. In conclusion, the M cell pathway has been shown to be an important route for the oral absorption of liposomes.

5. Conclusions and perspectives

Despite the growing number of investigations on the oral delivery of liposomes, essential breakthroughs are still needed to develop and market these products for clinical use. The bottleneck to development of oral liposomes lies in the poor understanding of the absorption mechanisms. Following the transit of liposomes from the stomach to small intestine, liposomes are gradually broken down. The drug payloads can be released immediately into the gastrointestinal lumen or be transferred into secondary carriers like mixed micelles and transported to the intestinal epithelia for absorption. This represents the first mode of drug absorption. As for labile biomacromolecules, released fractions are degraded quickly and will not be absorbed; only liposomes that survive the gastrointestinal environment and manage to penetrate the mucus layers can reach the intestinal epithelia and be absorbed together with the payloads. To enhance the oral absorption of liposomes as well as the payloads, the initial challenge is to maintain the integrity of liposomes and prolong gastrointestinal residence, thereby enhancing penetration of the mucus layers. Recent advances are focused on modulating the compositions of the lipid bilayers or modifying the liposomal surfaces with polymers or ligands to modulate the \textit{in vivo} fate of liposomes after oral administration.

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