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Biological fate of ingested lipid-based nanoparticles: Current understanding and future directions

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Abstract

In recent decades, LN have received considerable attention as nanoscale delivery systems to improve oral bioavailability of poorly absorbed bioactive compounds for health promotion and disease prevention. However, the scientific studies on the biological fate of orally administered LN are very limited and the molecular mechanisms by which they are absorbed through the intestinal lumen into the circulation remains unclear. This paper aims to provide an overview on the biological fate of orally administered LN by reviewing recent studies on both cell and animal models. In general, the biological fate of ingested LN in gastrointestinal tract is primarily determined by their initial physicochemical characteristics (such as particle size, surface property, composition and structure), and their absorption mainly occurs within the small intestine. In particular, depending upon the composition, LN can be either digestible or indigestible, with two distinct biological fates for each type of LN. The detail absorption mechanisms and uptake pathways at molecular, cellular and whole body levels for each type of LN are discussed in detail. Limitations on current research and our vision for future directions to study the biological fate of ingested LN are also provided in this critical review.

Keywords: Oral delivery, lipid nanoparticles, gastrointestinal tract, biological fate, transepithelial transport, absorption

1. Introduction

Nanoscale delivery systems have been proposed as an effective approach to improve pharmacological properties of drugs for decades, particularly through parenteral applications.[1-4] However, oral delivery remains the most preferred drug/nutrient administration route because of its non-invasive nature. Oral route is associated with the greatest degree of patient compliance as it ensures convenience, enables self-administration, and offers great flexibility in dosage regimen.[5, 6] However, the complex and hostile nature of human gastrointestinal (GI) environment significantly hinders the application of oral administration, especially for drugs/nutrients with poor stability in GI tract like proteins, peptides and phytochemicals. Moreover, lots of drugs/nutrients have poor water solubility, chemical instability, and low intestinal permeability and absorption, thus presenting a low oral bioavailability.[7] Thus, many studies have focused on the development of oral delivery systems to enhance the ingested drugs/nutrients. Among the wide variety of oral delivery systems, lipid-based nanoparticles (LN) have received much attention in recent decades.[8, 9] Typical LN formulations include liposomes, emulsions, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) (**Fig. 1**).[10] Liposomes are vesicular structures composed of amphiphilic molecules, such as phospholipids. Emulsions are dispersions of two immiscible liquids (water and oil) stabilized by surfactants or emulsifiers. SLN are nanostructures composed of solid lipids (solid at room temperature) and surfactants/emulsifiers. NLC share similar structures with SLN, except that the solid lipid core is replaced by a mixture of solid lipid and liquid oil. Orally administered LN have numerous benefits, including excellent biocompatibility, efficient permeation enhancement, and enhanced bioavailability.[11] In recent years, many studies have reported that LN delivery systems are able to improve the oral bioavailability of poorly absorbed nutrients and drugs in various animal models, including mice,[12-14] rats,[15-17] guinea pigs,[18] and rabbit.[19] Nevertheless, the in vivo biological fate of LN has not been fully understood yet.

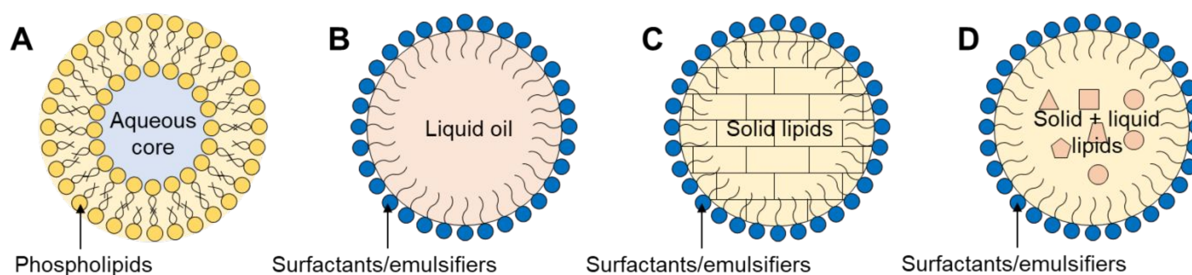

Fig. 1 Structure of liposome (A), nanoemulsion (B), solid lipid nanoparticles (SLN) (C), and nanostructured lipid carriers (NLC) (D).

Orally administered LN are subjected to harsh physiological conditions in the GI tract, such as low pH and enzyme attack, which greatly affect their in vivo biological fate upon ingestion. Some physicochemical characteristics of LN, such as particle size and surface properties, are known to play significant roles in determining their biological behaviors during digestion and absorption in the GI tract. Although there are several review articles that cover this topic, the biological fate of LN is only briefly described and discussed in the previous literature, focusing on the influence of physicochemical aspects of nanoparticles under simulated GI tract conditions.[9, 20-23] In those simulated conditions, most studies employ the static models that usually consist of dilute digestive mixture that mimics pH, minerals, ionic strength, and enzymes in physiological conditions in human GI tract. Unfortunately, information generated from such static models can hardly be related to in vivo conditions, since human GI tract is a complex and dynamic system under physiological conditions. To develop desirable LN for medication or food fortification to improve the oral bioavailability of poorly absorbed drugs/nutrients, it is critical to determine the biological fate of LN through oral administration, which would give us useful guidance for evaluation of delivery efficacy of LN under physiological conditions. In general, there are two major mechanisms of digestion and absorption concerning orally administered LN. Some studies proposed that lipolysis is the principle mechanism of ingested LN. After oral administration, the LN will be digested by lipases together with co-lipases and subsequently transformed into mixed micelles for absorption. The improved oral absorption of encapsulated cargos in LN is mainly due to lypolysates that lead to the prolongation of residence time in GI tract, increase of biliary and pancreatic secretions, and stimulation of lymphatic transport, while the contribution of intact LN is very limited.[24-26] In contrast, other studies found that LN can be taken up and transported across the GI tract as intact nanoparticles.[27, 28] Therefore, this review summarizes the current knowledge about the digestion and absorption of orally administered LN from cell and animal studies, and provides an overview of fundamental mechanisms to comprehensively describe their biological fate under physiological conditions.

2. Structure of GI tract and its function on biological fate of LN

The GI tract (**Fig. 2**), with an average length to approximately 5 m in humans, consists of a hollow muscular tube starting from the buccal cavity, where food enters the mouth, continuing through the pharynx, esophagus, stomach and intestines to the anus, where food residue is

expelled. The primary functions of GI tract are the maintenance of water homeostasis, the digestion, and absorption of macro- and micronutrients and electrolytes, trafficking of the fraction of macromolecular antigens that survive digestion, and the exclusion of pathogens[29, 30]

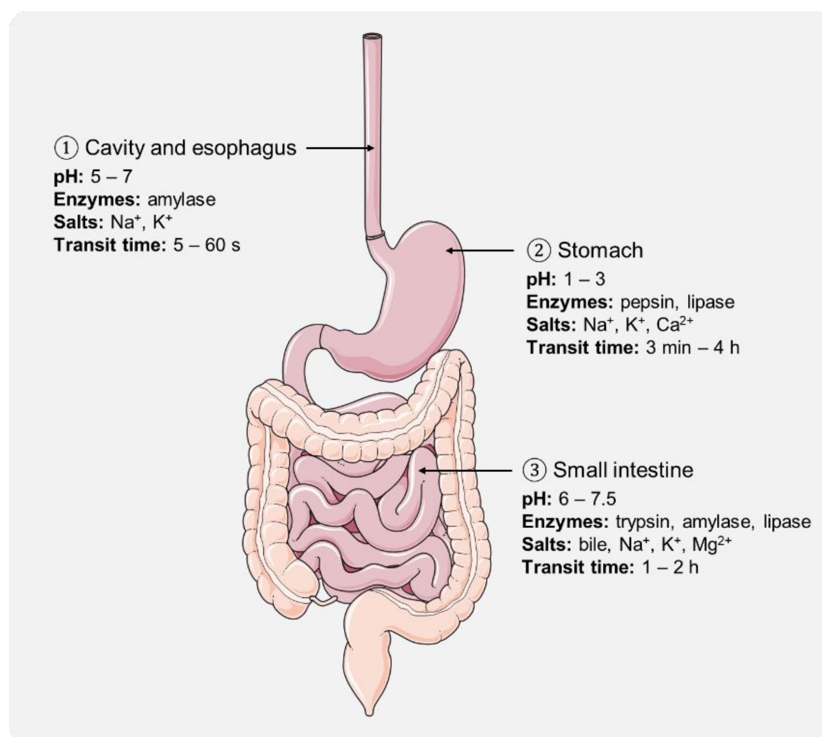

Fig. 2 The segments of GI tract for oral delivery of LN. The diagram of the human digestive tract is adapted from smart.servier.com.

After ingestion, the LN will quickly pass through the oral cavity and esophagus. Most of LN could retain their structural integrity in oral cavity and esophagus because of the short residence time, limited lipase secretion, as well as relatively small volume and mucosal area in these two compartments. The surface area for both oral cavity and esophagus is around 0.02 m^2.[31] The characteristics of oral cavity and esophagus determine their function mainly as transport organs. There are limited publications on the digestion and absorption of LN in these two compartments.

The LN will then enter the gastric cavity once they pass through the esophagus. Within the stomach, the LN are exposed to a variety of physicochemical conditions which may greatly alter their structural properties. For example, strong acid condition (pH = 1-3) and high ionic strength (e.g., calcium and sodium salts) within the stomach compartment can affect aggregation status of ingested LN by altering their surface properties, such as surface charge and steric coating, the two major stabilization mechanisms dictating the colloidal stability of LN. Moreover, triglycerides, which are common components of LN, will start to digest in the stomach when

there is sufficient secretion of gastric lipase before LN transit to small intestine. Nevertheless, the LN that surface modified with indigestible coating materials such as polyethylene glycol (PEG)[32] and polysaccharides[33] have been shown to be more stable in the stomach.

The LN leaving the stomach are squirted through pylorus sphincter and enter the small intestine. Small intestine is the longest segment of the GI tract, where the outermost layer is structured with villi and microvilli that project into the lumen, resulting in a very high surface area of 30 m^2 in humans. Once entering the small intestine, the LN will be mixed with alkalic small intestinal fluids containing bile salts, phospholipids, pancreatin, and various salts, resulting in an increase in pH to around neutral. The LN from stomach will be further digested, and finally become fatty acids in small intestine. Then, both lypolysates and encapsulated cargos are transferred via secondary carriers, such as vesicles and cubic nanocarriers, to epithelial surfaces for absorption. While the majority of LN would be broken down and absorbed within stomach and small intestine, the LN with indigestible coatings could still remain intact and direct absorption of these LN through intestinal linings into systematic circulation may take place following different mechanisms which will be discussed later in detail (**Fig. 3**). In some other cases, the indigestible LN may be able to reach the colon due to their specialized surface characteristics. For example, the lecithin-chitosan coated emulsion droplets might restrict the access of pancreatic lipase to the lipids within the droplets, leading to insufficient digestion.[34] These undigested lipids, hence, have a possibility to reach the colon. Moreover, LN with cross-linked dietary fiber shell (e.g. pectin and chitosan) may be able to reach the colon without being absorbed, owing to their resistance to enzymatic digestion in stomach and small intestine.[33, 35-42] In colon, however, dietary fiber shell could be broken down by colonic microflora. The LN might be then digested and the encapsulated cargos can be released and absorbed in the colon.

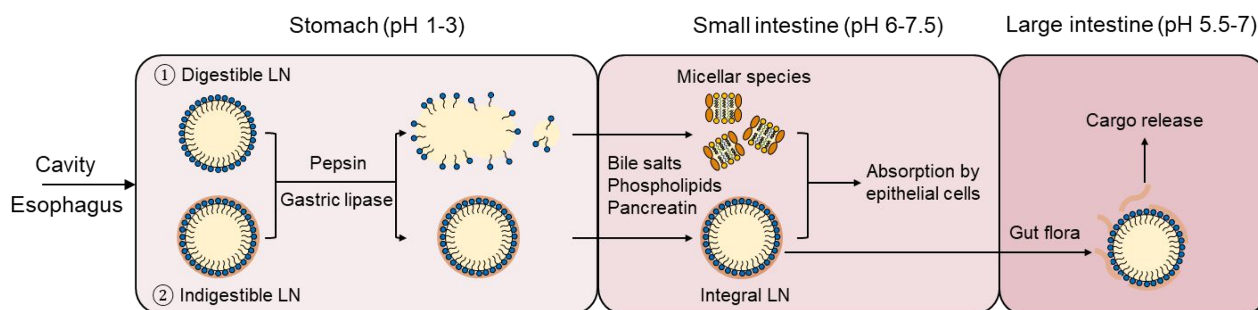

Fig. 3 Gastrointestinal behavior of digestible and indigestible LN after ingestion.

3. Absorption mechanisms of LN in GI tract

Absorption is a process that a substance is transferred from lumen of the GI tract to the underlying epithelial cells and then further transported to the systemic circulation and various tissues. For LN, it is important to determine the region where absorption process occurs within the GI tract. Li et al. conducted a study to investigate oral absorption of quercetin (QT)-loaded SLN (QT-SLN) through different segments of the GIT using Sprague Dawley rats.[43] In this study, QT-SLNs composed of glyceryl monostearate, soya lecithin, Tween 80, and PEG 400 were prepared by emulsification and low-temperature solidification method. The results indicated that the absorption of QT-SLN took place in both stomach and intestine segments, with only 6% absorption from stomach while 82% from intestine region. The surface area of stomach, as well as the permeability of the gastric epithelium to nanoparticles, is relatively low, leading to a minimal passage and absorption of LN in the stomach.[44, 45] In contrast, the large surface area for adhesion and presence of M cells in Peyer's patches in the intestine region are preferable for oral absorption of LN.[43, 45] Particularly, the mucus layer is thinner and there is less interaction between lamellar strings in the small intestine, thus allowing greater access of the luminal contents to the epithelium. Other than absorption sites for LN within the GI tract, it is important to ascertain the state of LN before they are absorbed by epithelium. The LN might undergo various changes during transport in the physiological complex GI tract depending on their initial physicochemical characteristics. For instance, digestible LN consisting of dietary fat and surfactants/emulsifiers could be digested and hydrolyzed and form secondary vesicles when they reach the small intestine. While, indigestible LN composed of mineral oil core and/or indigestible shell could remain intact. Consequently, two absorption mechanisms of LN in small intestine corresponding to the LN with different physicochemical properties (i.e. digestible vs indigestible) will be discussed in the following sections.

3.1. Lipolysis pathway for digestible LN

There are several specialized cell types within the epithelial monolayer of the small intestine. For example, enterocytes are responsible for nutrient absorption, while other types of cell perform functions like secretion of mucus.[45, 46] Once entering the small intestine, LN simulate the gallbladder contractions and biliary and pancreatic secretions. For digestible LN, it is expected that after oral administration, they undergo similar digestion mechanism of ingested dietary lipids. Dietary lipids are the main constituents of the LN that are formulated for oral delivery applications. The esters of lipid core could rapidly be hydrolyzed in the small intestine, and their lipolytic products, mainly 2-monoglycerides and fatty acids will then interact with bile

salts and phospholipids and form different micellar species which may prevent the encapsulated lipophilic cargos from precipitation.[47] Whether those formed micellar species could prevent lipophilic cargos being precipitated depends on the structure of lipids used for LN preparation. Porter et al. compared danazol-loaded microemulsions prepared with medium-chain lipids (C8-10) and long-chain lipids (C18) and found that the use of medium-chain lipids can lead to drug precipitation, and therefore limited absorption of danazol compared with long-chain lipid formulations.[48] At molar equivalent fatty acid content, medium-chain mixed micelles are an order of magnitude less effective at solubilizing danazol than long-chain mixed micelles.[49] Besides, Nanoemulsions containing long-chain lipids incline to increase bioaccessibility of encapsulated lipophilic compounds (e.g. β-carotene and vitamin E) due to greater solubilization capacity of mixed micelle in small intestine than those containing medium-chain lipids.[50, 51] These formed micellar species are subsequently transferred to unstirred water layer and epithelial surfaces for absorption by simple diffusion to enterocytes. Solubilization of lipolytic products and encapsulated cargos in micellar structures can greatly enhance the mass transport of molecules across the unstirred water layer, thus enhancing the absorption of cargos. Transportation of solubilized lipolytic products and cargos into systemic circulation will then follow through either the portal vein or the lymphatic system (**Fig. 4**).

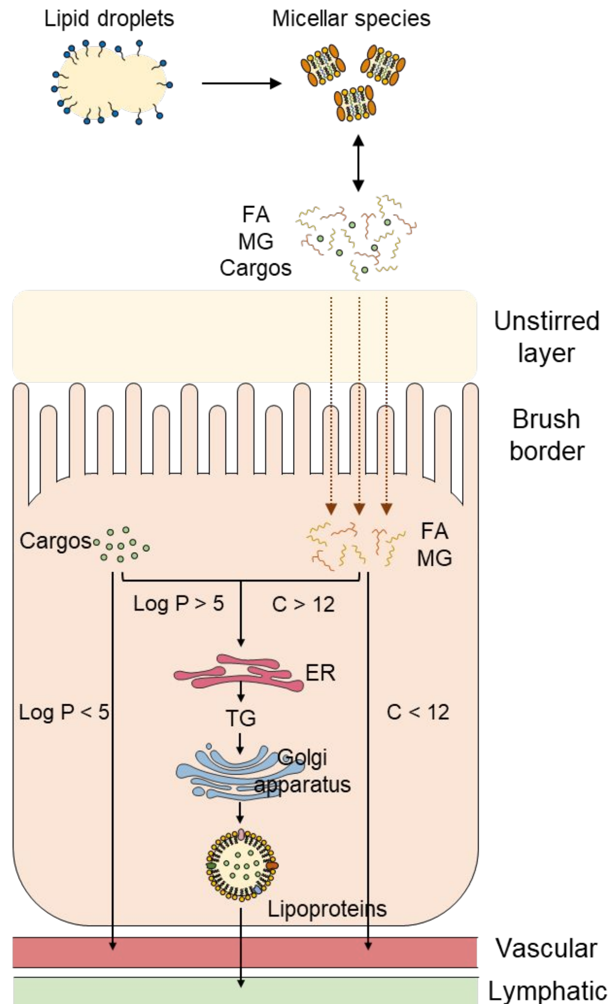

Fig. 4 Transport of digestible LN and encapsulated cargos to systemic circulation. FA: fatty acid, MG: monoglyceride, TG: triglyceride, ER: the endoplasmic reticulum.

The mechanisms by which lipophilic cargos access the lymphatics over the portal vein are still largely unknown, and it is reported that the critical step is whether the encapsulated cargos could associate with intestinal lipoproteins produced in enterocytes.[52, 53] In case of the LN prepared with long-chain triglycerides, the major lipolytic products are fatty acids and monoglycerides with carbon chain longer than 12, which could be massively reconstituted to form triglycerides in endoplasmic reticulum (ER) mainly via the consecutive actions of monoacylglycerol and diacylglycerol acyltransferases.[54, 55] From the ER to the Golgi apparatus, these triglycerides are packaged with cholesterol, phospholipid, and apolipoprotein into intestinal lipoproteins. The chylomicrons are the major lipoproteins produced by the enterocytes.[56] For those highly lipophilic cargos with log P value greater than 5 and solubility

higher than 50 mg/g in long-chain triglyceride lipid, they tend to associate with intestinal lipoproteins, specifically within the core of lipoproteins.[57, 58] Several studies investigated whether the lipophilic cargos were transported in the form of lipoproteinated nanocapsules by the use of a chylomicron flow blocker, cycloheximide. It has been reported that cycloheximide can inhibit the lymphatic transport pathway without damaging other passive and active absorption pathways.[59] Fang et al. prepared docetaxel-loaded NLC and investigated the absorption of NLC in rats treated with cycloheximide. The peak concentration (C_{max}) of docetaxel was lower and the AUC_{0-12} was significantly decreased by about 57% compared with control group (without cycloheximide treatment). Zhang et al. found similar results using candesartan cilexetil-loaded SLN and cycloheximide-treated rats.[60] When SLNs were orally administered to rats pretreated with cycloheximide, the C_{max} of cycloheximide was reduced by 40% and the AUC_{0-24} was decreased by about 30% significantly. The formed chylomicrons are relatively large in size (75 to 600 nm), thus it is difficult for them to permeate to the endothelium of portal vein.[61] While, the more open structure of the lymphatic endothelial barrier allows the passage of lipoproteins, together with lipoprotein-associated cargos, to the lymphatic system. After the chylomicrons are exocytosed from the small intestine, they enter lymphatic vessels and transport lipids from the small intestine to peripheral tissues including muscle, adipose tissues, and heart. Triglycerides in chylomicrons are hydrolyzed by lipoprotein lipases at the inner surface of capillaries, allowing the released free fatty acids and encapsulated cargos to be absorbed by the peripheral tissues.[62, 63] These chylomicron remnants are then taken up by liver cells via interacting with specific receptors in the liver.[64] This pathway could avoid the liver and first-pass metabolism. On the other hand, due to their amphiphilicity, the digestion products of short- or medium-chain triglycerides (fatty acids and monoglycerides with carbon chain shorter than 12), as well as cargos with low log P (i.e. < 5), are mainly absorbed through portal vein. Finally, lipolytic products and cargos will enter the portal vein, pass through the liver and undergo first-pass metabolism before they could reach the systemic system.

In summary, digestible LN undergo similar digestion pathway of ingested dietary lipids. During digestion, lipolytic products of LN and encapsulated cargos might transport and absorb through either the intestinal lymphatic system or the portal vein, depending upon physical structure of lipids in LN, as well as the lipophilicity of encapsulated cargos. Generally, long chain triglyceride lipids are preferentially resynthesized in the enterocyte, assembled into intestinal lipoproteins, and secreted into the mesenteric lymph, whereas shorter-chain triglycerides are primarily absorbed directly into the portal blood. Many previous studies compared the digestion and absorption of LN made from long-chain and medium/short-chain lipids. Caliph et al.

demonstrated that the lymphatic transport of halofantrine in lipid vehicles was enhanced by an increase in the fatty acid chain length of the coadministered lipids and concluded that short- or medium chain lipids are poor simulators of intestinal lymphatic transport.[58] Another study further investigated intestinal absorption route, rate, and form of intragastrically administered radioactive fatty acid emulsions using lymph- and portal vein-cannulated rats.[65] The results showed that most (85%) of long-chain fatty acids ([I-^{14}C] oleic acids) were transported through lymphatic system and radioactivity was presented as triglycerides. In contrast, about 94-98% of absorbed short-chain fatty acids ([I-^{14}C]- caprylic and 2-[^{14}C] ethyl-n-caproic acids) was transported via the portal system and 96-102% of the radioactivity was present as free fatty acids. Simultaneously, encapsulated cargos will be transported into systematic circulation together with lipolytic products of LN. It is generally considered that highly lipophilic encapsulated cargos (log $P > 5$) have a tendency to bind with intestinal lipoproteins and transport through lymphatic system, while amphiphilic encapsulated cargos will reach the systemic system through portal vein. Although the digestible LN follow the same digestion and absorption processes as common dietary lipids, which may increase the oral bioavailability of encapsulated cargos, especially those with high lipophilicity, the premature release of encapsulated cargos during digestion of LN, as well as the possibility of cargo precipitation when transforming to mixed micelles may lower the efficiency of delivery.

3.2. Uptake pathway of indigestible or intact LN

The indigestible LN are usually fabricated with mineral oil core and/or indigestible shell, which are resistant to digestion under GI environment. Thus, the original nanostructures of these LN are maintained when they reach the small intestine epithelium for absorption. Compared with digestible LN, the indigestible LN protect the encapsulated cargos until being absorbed into the circulation. Furthermore, as these LN will be absorbed as intact nanoparticles, their surface is often functionalized to increase the delivery accuracy and efficiency.[66-68] However, the epithelium penetration efficiency of indigestible LN is relatively low and the elimination mechanism of such LN from the circulation is not fully understood.

There are several potential uptake pathways that the indigestible LN might undergo during passing through the epithelium and entering the systematic circulation, i.e. paracellular transport, transcellular transport, and persorption. Ideally, the LN that can reach the small intestine as intact nanoparticles only relate to indigestible LN. However, to unravel each specific uptake pathway of intact LN, many studies have been conducted using different in vitro cellular models

by simply assuming that the prepared LN would reach the epithelium as intact nanoparticles. In some studies discussed in this section, even though the LN were prepared from digestible ingredients they were still studied as “intact” nanoparticles in cellular models, without any prior digestion treatment. While these studies may not reveal the “real” in vivo biological fate of indigestible LN in the GI tract, they still provide scientific information to understand the potential uptake pathways of intact LN.

3.2.1. Mucus penetration

The intact LN may be absorbed at the epithelial lining only if they can successfully permeate through the mucus that covers the epithelium. Mucus, secreted by intestinal goblet cells, mainly consists of mucin glycoproteins sheets resulting in an adherent unstirred layer coating the epithelium of small intestine.[69] As the first barrier to absorption process in the GI tract, mucin molecules are entangled and cross-linked adhesively to create a filter that can block particulates that are too large to permeate the mucus mesh spacing (~200 nm).[46, 70, 71] Thus, particle size plays a central role in penetration of LN through mucus layer. Several previous studies demonstrated that transportation rate of nanoparticles through intestinal mucus layer decreased with increasing particle size.[72, 73] Besides, due to negative charge of glycosylated groups in mucins and hydrophobic region of non-glycosylated protein chain, the mucus can tightly bind nanoparticles through hydrophobic and electrostatic interactions.[74-76] Therefore, hydrophilicity and surface charge of LN are the two key factors affecting the permeation of LN across the small intestine epithelium.

Previous studies demonstrated that positively charged LN (e.g. chitosan-coated SLN) showed a higher affinity to mucus layer and stronger mucoadhesive property, since the intestinal mucus appears to carry more negative charge.[33] However, the positively charged nanoparticles can be trapped in mucus due to strong electrostatic interactions, which might result in low permeation efficiency of these nanoparticles. Another strategy to develop lipid-based nanocarriers which can effectively penetrate the mucus layer was inspired by capsid viruses. Olmsted et al. has shown that capsid viruses could diffuse through mucus since they have a net neutral surface charge. Besides, they have few exposed hydrophobic regions which might be able to make polyvalent bonds with the hydrophobic domains within the mucins, thus there is low possibility that capsid viruses would be trapped in the mucus layer.[77] This property is now being explored in many studies to fabricate mucus penetrating particles by mimicking the essential surface properties of virus that allow them to avoid mucoadhesion. For example,

surface functionalization of LN with PEG to make their surface neutral and hydrophilic has been reported to enable such LN to diffuse through mucus by overcoming hydrophobic and electrostatic interactions. Zhang et al. fabricated PEGylated fenofibrate-loaded SLN using solvent-diffusion technique and investigated the effect of PEGylation on the interactions between the nanoparticles and mucins by incubating SLN with mucin-rich ex vivo intestinal fluids prepared from Sprague–Dawley rats.[78] The results indicated that the particle size of conventional SLN increased by 8.9-fold after incubation, indicating considerable adsorption of mucins onto the SLN. Meanwhile, the particle size of PEGylated SLN only slightly raised from 339.8 nm to 409.2 nm, demonstrating that PEGylated SLN can effectively mask the binding of free mucins. Another study conducted by Yuan et al. compared the mucus penetrating ability of PEGylated SLN formulations with different weight percentage (5%, 10%, and 20%) of polyethylene glycol monostearate (PEG_{2000}-SA) using Caco-2/HT29 co-culture cell monolayer and everted gut sac system prepared from rats by measuring permeability coefficient (P_{app}) of encapsulated doxorubicin (DOX).[79] The P_{app} is defined as the flux rate of mass transport across the monolayers. The P_{app} of DOX through Caco-2/HT29 (75:25) was about 5.8, 8.25, and 6 (cm/s, 10^{-6}) for PEGylated SLN with 5%, 10%, and 20% PEG_{2000}-SA respectively. In this study, the P_{app} was not positively correlated with the PEGylation degree, indicating that appropriate PEGylation is critical to improve mucus-penetrating properties of LN for oral delivery across gastrointestinal mucus. In addition to PEGylation, some other surface modifications such as dextran-protamine[80] and Pluronic® F-127[81] have also been exploited to lower the mucoadhesion of LN for improved penetration through mucus.

3.2.2. Transport across intestinal epithelia cells

Once the indigestible or intact LN have penetrated through the mucus layer as the intact nanoparticles, they will then transport through the epithelium cells mainly via three mechanisms: paracellular transport (i.e., between adjacent epithelial cells), transcellular transport (i.e., through an epithelial cell), and persorption (i.e. through dead or dying extruded enterocytes).[82] The direct uptake of indigestible or intact LN is illustrated in **Fig. 5**.

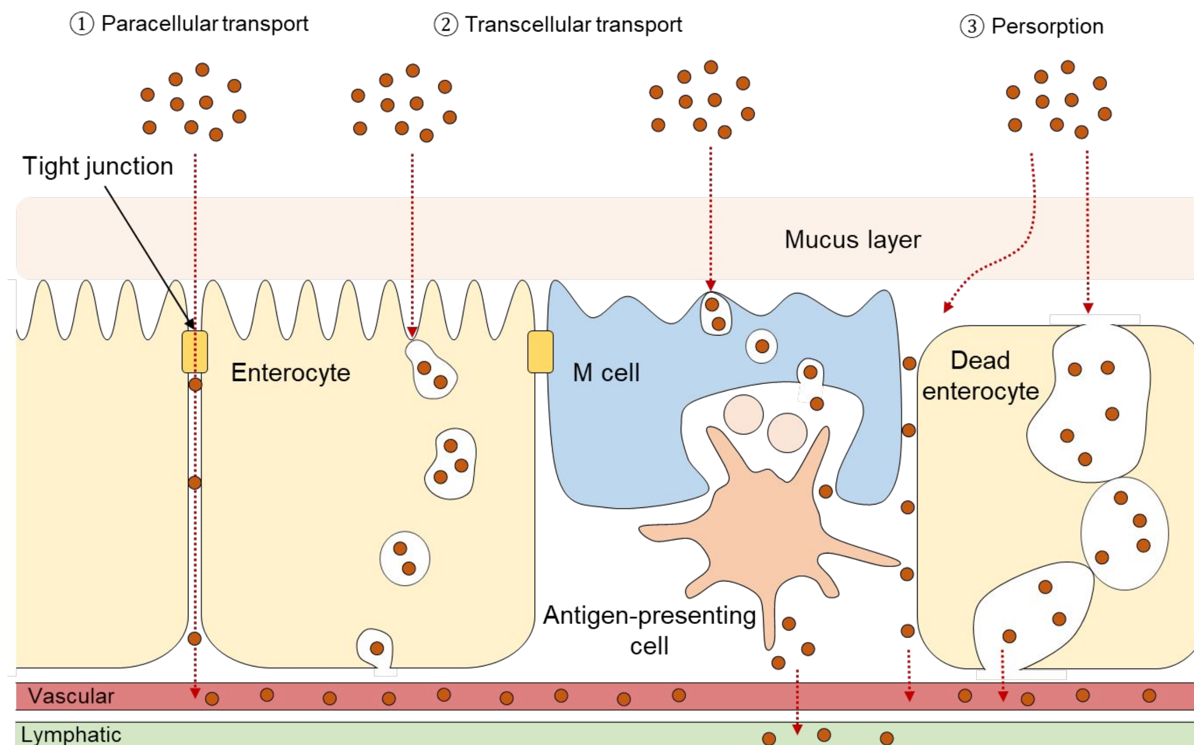

Fig. 5 Schematic of indigestible or intact LN transport across the intestinal epithelium.

3.2.2.1. Paracellular transport

Paracellular transport refers to the transfer of particles across an epithelium by passing through an intercellular space between the cells (i.e. tight junctions). The paracellular uptake pathway is considered to be inefficient because the dimension of tight junctions is about only 3 to 4 nm in size under normal conditions, which is too small for most LN to permeate. Besides, tight junctions only account for about 0.1% of the total absorption surface area of the intestine.[83] However, penetration enhancers such as surfactants (nonionic, anionic, and cationic surfactants), ethylenediaminetetraacetic acid (EDTA), fatty acids, and chitosan could modulate the tight junctions and may increase the gap dimension and permeability to allow trafficking of LN with larger dimension.[84, 85] Among these enhancers, chitosan is the most commonly used for LN formulation. Garcia-Fuentes et al. prepared chitosan-coated SLN containing fluorescent marker Texas Red®-dextran and investigated the paracellular permeability of Caco-2 cells to the prepared SLN by measuring the transepithelial electrical resistance (TEER) and P_{app} at predetermined time points.[86] The measurement of TEER is a common way to determine paracellular permeability of Caco-2 cells to ions or large molecules and changes of monolayer

upon exposure to a permeation enhancer. The results indicated that incubation with chitosan-coated SLN induced a dose-dependent reduction in TEER of Caco-2 monolayers of up to 45%, thus simultaneously an enhanced transport of encapsulated cargos (higher P_{app}) compared with control group (PEG-coated SLN) exhibiting only about 10% reduction in TEER. Similar results were found in another study conducted by Fonte et al., in which they demonstrated chitosan-coated SLN were able to enhance the transport of insulin across Caco-2/HT29 co-cultures compared with non-coated SLN.[87] Nevertheless, the effect of chitosan coating to increase paracellular permeability of nanoemulsions in Caco-2 cells has been shown to be highly concentration-dependent, especially to nanoemulsion type of LN.[88] The extent of TEER values decrease was remarkable only for high concentrations of chitosan-coated nanomeulsion. When the treatment dose of nanoemulsion was 80 μg/cm^2, the TEER value was slightly reduced by 10%. While a 35% decrease in TEER was observed for a high dose (204.5 μg/cm^2) of nanomeulsion. After paracellular transportation, the LN will subsequently enter the circulatory system via portal vein.

3.2.2.2. Transcellular transport

Transcellular transport of LN occurs by transcytosis, which begins with an endocytic process that takes place at the cell apical membrane. Then, particles are transported through the cells and released at the basolateral side. There are two main types of epithelium cells in small intestine where particle absorption through transcellular transport may take place: enterocytes and microfold cells (M cells). Enterocytes are the most abundant type epithelium cell lining the small intestine (up to 80% of epithelium cells). They are columnar cells with microvilli on the apical surface, which greatly increase the surface area for digestion and transport of nanoparticles from the intestinal lumen. The first step of the transcytotic route is endocytosis. Endocytosis is a cellular process in which extracellular materials are transport into the cell through membrane vesicles. In general, cellular uptake of particles increases with decreasing particle size[80, 89] And the particles in the lumen could be endocytosed into enterocytes through four types of pathways: clathrin-mediated endocytosis, caveolae, micropinocytosis, and phagocytosis.[90]. Both clathrin- and caveolin-dependent endocytosis involve receptor binding process and subsequent internalization. Such receptor-mediated endocytosis begins when the LN in the intestinal lumen bind to clathrin or caveolin on the exterior of the apical cell membrane, initiating endocytosis. The clathrin-mediated endocytosis shows an upper size limit for internalization of approximately 200 nm.[91, 92] While, studies on caveolae-mediated internalization pathway showed controversial results on different cell types.[89, 93] In general, it has been

reported that the size of caveosomes that involved in caveolae-mediated endocytosis was ranged from 60-100 nm in Caco-2 cells, thus only particles with small diameter could be transported.[93, 94] Macropinocytosis is a highly active and non-selective mechanisms of endocytosis, through which large volumes of fluid can be internalized.[95] During macropinocytosis, large particles with diameter greater than 0.2 μm could be internalized into cells.[95, 96] The resulting intracellular vesicles fuse with lysosomes for enzymatic degradation of the contents. Phagocytosis involves the internalization of larger particles (up to several μm in size) such as pathogens.[97]

Many studies have been conducted to investigate the molecular mechanism and route of LN transport crossing simulative epithelial enterocytes (Caco-2 cell monolayers) through transcytosis using fluorescence markers as indicators (**Table 1**). Caco-2 cells, which are derived from human colorectal adenocarcinoma cells, are typically used to represent the predominant cell type in the gut and mimic the epithelial barrier of the intestines, since good correlations have been well-established between data on oral absorption in humans and the results from Caco-2 model.[98] These in vitro experiments are usually performed in the presence of transport inhibitors in order to understand the molecular mechanism of endocytosis involved in uptake of LN by enterocytes. For instance, chlorpromazine inhibits clathrin-mediated endocytosis, methyl-β-cyclodextrin (MβCD) and filipin could prevent caveolae-mediated endocytosis, and 5-(N-Ethyl-N-isopropyl) amiloride (EIPA) and cytochalasin D attenuate macropinocytosis.

Chai et al. prepared octadecylamine-fluorescein isothiocyanate (ODA-FITC)-labeled SLN with glycerol monostearate and poloxamer 188 and investigated their endocytosis pathways in Caco-2 cells by applying different transport inhibitors.[27] Transport inhibitors including sodium azide, chloropromazine, MβCD, dynasore, and EIPA were applied to Caco-2 cells to inhibit the energy-dependent procedure and different endocytosis processes. The results indicated that uptake of LN by enterocytes was an energy-dependent and vesicle-mediated process, which was mainly mediated by clathrin- and caveolae-related routes and macropinocytosis. Nevertheless, Neves et al. investigated cellular uptake and internalization pathway of LN through Caco-2 cell monolayers and found that the internalization of both SLN and NLC occurred mainly through a clathrin-mediated endocytosis.[99] Although caveolae-mediated pathway was also likely to take place with SLN, such procedure did not play a predominant role in SLN internalization, with only 15% reduction in the uptake of SLN in filipin (caveolae-mediated pathway inhibitor) treated Caco-2 cells. Compared with the results from study conducted by Chai et al., the different endocytosis mechanism might be caused by a significant

larger diameter of LN in this study (**Table 1**). As mentioned earlier, clatherin-coated vesicles are able to transport particles with a diameter between 100 and 200 nm, while caveosomes could only bring in particles with size smaller than 100 nm. In this study, SLN and NLC present a mean diameter around 180 nm, it is plausible that the clathrin pathway, not caveolae, was mainly involved in their internalization. From these two studies, it is reasonable to assume that mechanism of endocytosis in Caco-2 cells is dependent on size of LN. Subsequently, Fan et al. demonstrated that the endocytosis of lipid-based emulsions in Caco-2 cells was size-dependent using corn oil-caseinate emulsions with different size (170 nm, 265 nm, and 556 nm).[100] The results showed that caveolae-dependent endocytosis and macropinocytosis played a critical role in the internalization of emulsions with relatively small (170 nm) and large (556 nm) diameter, respectively. Surprisingly, different from study conducted by Neves et al., in which clathrin-related mechanism was the predominant pathway for internalization of LN with 180 nm in diameter, in this study, clathrin-related pathway was inefficient for 170 nm emulsion compared with caveolae-dependent endocytosis. Another study conducted by Beloqui et al. reported that not only the size but also the surface hydrophobicity of NLC formulation could influence the endocytosis in Caco-2 cells.[80] They prepared saquinavir-loaded NLC with different surfactants to confer various surface hydrophobicity and found that the cellular uptake in Caco-2 cells is positively correlated with surface hydrophobicity of NLC. However, there was no significant difference in the presence of clathrin- or caveolae-mediated endocytosis inhibitors regardless of the nanoparticle formulation.

Based on previous studies, clathrin- and caveolae-dependent mechanisms are two major pathways that involved in LN endocytosis (**Table 1**). Previous studies have indicated that mechanism of cellular uptake and transport of encapsulated compounds are linked to particle size. For instance, caveolae-dependent endocytosis and micropinocytosis is major transportation mechanism for LN with small and large particle size, respectively. Other than particle size, factors including emulsifiers, lipid matrix, or surface properties represent significant confounding effects.[101, 102]

Table 1 Examples of studies investigating the cellular uptake and transcytosis mechanism of intact LN.

Delivery system	Lipid core	Surfactant	Particulate characteristics	Study design	Transport inhibitor	Transcytosis mechanism
SLN [27]	Glycerol monostearate	poloxamer 188 (0.1%, w/v)	Particle size: 86.7 nm	In vitro study (Caco-2 cells)	Sodium azide, chloropromazine, Methyl-β-Cyclodextrins (MβCD), dynasore, EIPA	Energy-dependent
			PDI: 0.128			Macropinocytosis, clathrin- and caveolae-related
			Zeta potential: -28.78			

			mV			routes
NLC [80]	Precirol ATO®5, Miglyol 812	Tween 80 (1-2%, w/v), Poloxamer 188 (0.5-1%, w/v)	Particle size: 165–1090 nm PDI: 0.16-0.6 Zeta potential: -33–-21 mV	In vitro study (Caco-2 cells)	Chlorpromazine, nystatin, MβCD+lovastatin	Clathrin-related routes, caveolae-related routes
SLN and NLC [99]	SLN: Cetyl palmitate; NLC: Cetyl palmitate, Miglyol 812	Tween 60 (2%, w/v)	Particle size: 189.2 nm (SLN), 172.9 nm (NLC) PDI: 0.205 (SLN), 0.203 (NLC) Zeta potential: -31 mV (SLN), -30 mV (NLC)	In vitro study (Caco-2 cells)	Sucrose, chlorpromazine, filipin, cytochalasin D, ammonium chloride	Energy-dependent Clathrin- and caveolae-related routes
Nanoemulsion [28]	Captex® 8000	Lipoid® S75-3 (0.6%, w/v), Solutol® HS15 (4.4-14%, w/v)	Particle size: 25–130 nm PDI: 0.034–0.136 Zeta potential: -2.8–-6.4 mV	In vitro study (Caco-2 cells)	MβCD+lovastatin, filipin, chloropromazine	Caveolae-related routes
Nanoemulsion [103]	Miglyol 812, oleic acid	Solutol® HS15, Tween 20, lecithin	Particle size: 48.9–68.3 nm PDI: NA Zeta potential: -30 mV	In situ study (intestinal perfusion)	Chlorpromazine, nystatin	Clathrin- and caveolae-related routes
Nanoemulsion [100]	Corn oil	Sodium caseinate	Particle size: 170, 265, 556 nm PDI: 0.198, 0.150, 0.113 Zeta potential: -40.5, -41.2, -42.3 mV	In vitro study (Caco-2 cells)	Sodium azide, phenylarsine oxide, EIPA, nystatin	Energy-dependent Macropinocytosis, clathrin- and caveolae-related routes
SLN [104]	Glyceryl monostearate	Glyceryl monostearate, D-a-tocopheryl polyethylene glycol 1000 succinate	Particle size: 114.3 nm PDI: 0.188 Zeta potential: -12.9 mV	In vitro study (Caco-2 cells)	Sodium azide, chlorpromazine, nystatin	Energy-dependent Macropinocytosis, clathrin- and caveolae-related routes

Later, in order to investigate and elucidate the destinations and fate of SLN in enterocytes after endocytosis, Chai et al. explored the effects of labelled different organelles including lysosomes, transferrin (Tfn)-related endosomes (the well-known clathrin-mediated route), the ER, and the Golgi apparatus using fluorescence markers.[27] The results indicated that SLN were co-localized with lysosomes, Tfn-related endosomes, the ER, and the Golgi apparatus. Furthermore, several inhibitors including brefeldin A, monensin, nocodazole, and bafilomycin A1 were used to inhibit endocellular transport process of LN after internalization of SLN. Brefeldin A, monensin, nocodazole, and bafilomycin A1 have been reported to inhibit the delivery between ER and Golgi apparatus, delivery between Golgi apparatus and cell membranes, formation of microtubules, and the maturation process of lysosomes, respectively. The results showed that the amount of SLN remaining in Caco-2 cells significantly increased with the addition of these four inhibitors, indicating that ER, Golgi apparatus, and microtubules were involved in

endocellular transport process and further delivery of SLN out of Caco-2 cells. It is worth mentioning that suppression of lysosomes maturation process with bafilomycin A1 increased the amount of endocellular SLN remaining. Thus, it is reasonable to conclude that a significant proportion of SLN have been degraded and digested in lysosomes during transportation in enterocytes.

In addition, some other studies investigated transcytosis mechanism of LN using Transwell plate with Caco-2 cell monolayer inserts. To evaluate the transcytosis of LN in enterocytes, Beloqui et al. incubated the prepared NLC in the Caco-2 cell monolayers along with the clathrin- and caveolae-mediated inhibitors, chlorpromazine and nystatin, respectively, and measured the P_{app} of encapsulated saquinavir.[80] The results indicated that the caveolae-mediated internalization of NLC was involved in the transcellular transport of the encapsulated saquinavir across Caco-2 cells. However, this study did not provide information about the fate of nanoparticles inside of the cell. Furthermore, they did not evaluate the presence of the nanoparticles in the basolateral compartment, and thus no conclusion can be drawn from the study on whether integral nanoparticles could be transported from apical side to basolateral side of enterocytes. In contrast, in study conducted by Roger et al.,[28] they demonstrated that clathrin- and caveolae-related routes were implicated in transcytosis mechanisms. Moreover, they observed the basolateral medium by transmission electron microscopy (TEM) after transcytosis study and found integral particles at the basolateral compartment, even though these particles are about 80 nm larger than particles in apical side. The transportation of intact LN across enterocytes was also evidenced by comparing the morphology of SLN in the apical and the basolateral side using TEM in the study conducted by Chai et al.[27] The whole transcytosis process of SLN occurred via the macropinocytosis pathway and clathrin- and caveolae-related pathways. The particle size of SLN observed in basolateral side was similar to SLN in apical side. However, the concentration of nanoparticles in basolateral side was significantly lower, indicating low transcytosis efficacy (**Fig. 6**). They later proved that such low transcytosis efficacy might be caused by lysosome degradation, as well as delivery back of large proportion of SLN to apical side.

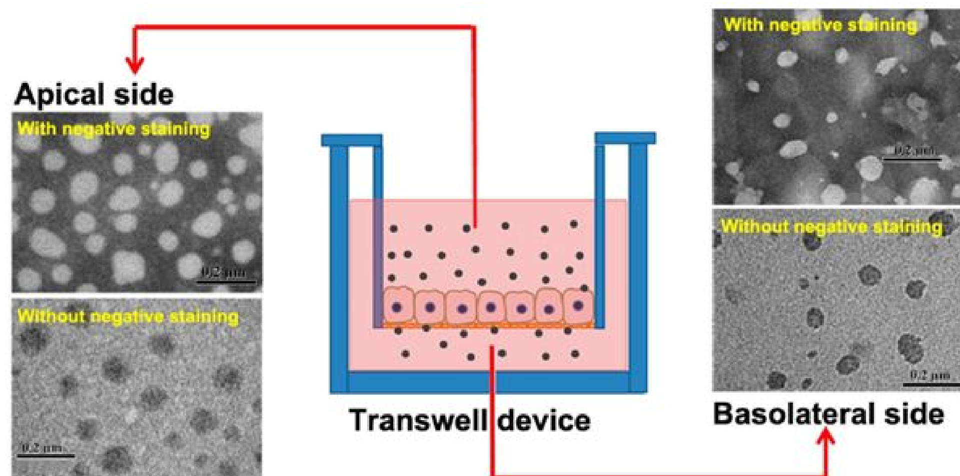

Fig. 6 TEM results of Au nanoparticle-loaded SLNs in the apical or the basolateral side under the condition of the Caco-2 cell monolayers incubated with ODA-FITC-labeled SLNs for 4 h. The specimens are negatively stained or without negative staining for TEM viewing. Scale bar in TEM is 0.2 μm. This figure has been reprinted from ref.[27] with permission from American Chemical Society.

Based on these previous studies, the transport pathway of intact LN across Caco-2 cell monolayers is briefly illustrated in **Fig. 7**. The transport vesicles containing the LN that bud into the apical cell membrane are first fused with apical early endosome (AEE). Then, there will be three possible pathways in the delivery of LN through the AEE. 1) They can be directly recycled back to the cell membrane of apical side, with or without LN. The LN bound to the internalized receptor can be released into cytoplasm, while the receptor is delivered back to the cell membrane. The released LN may diffuse slowly through the cytoplasm and degrade during the transportation to basolateral membrane. 2) The AEE may deliver LN to late endosomes and finally to the lysosomes. Lysosomes route is a degradative pathway, since lysosomes contains a wide range of hydrolases in their lumen and can degrade almost all kind of cellular components such as proteins, lipids, carbohydrates and even organelles.[105] The LN transported into lysosomes may be degraded and some LN may further be transported to both membrane sides. 3) The AEE may be delivered to apical recycling endosomes (ARE), from where the LN could be either delivered back to apical side or transported to common recycling compartment (CRC). Subsequently, the LN in CRC will be delivered to the ER, Golgi apparatus, or directly to basolateral early endosome (BEE) for transcytosis. Finally, these LN will be transported to peripheral circulation.

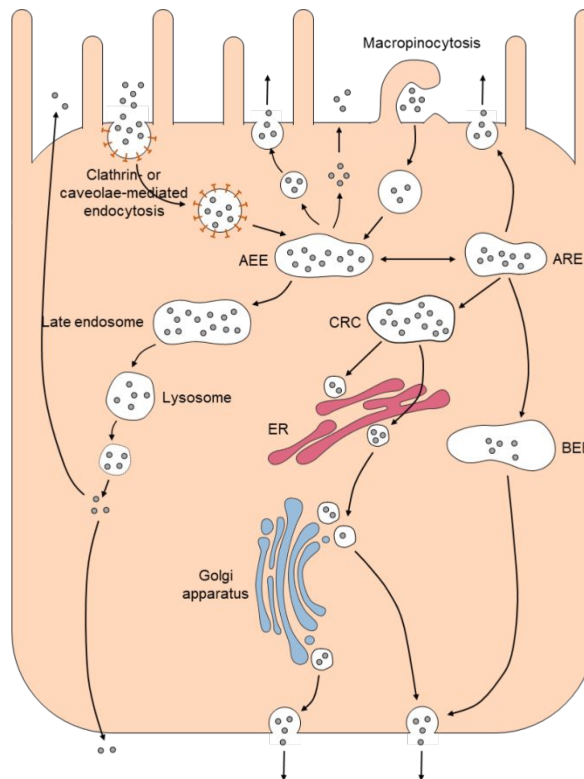

Fig. 7 The intracellular itinerary of indigestible or intact LN in enterocytes. AEE: apical early endosome, ARE: apical recycling endosome, CRC: common recycling compartment, BEE: basolateral early endosome, ER: the endoplasmic reticulum.

Nevertheless, there are two major limitations that should be taken into account when evaluating transcellular transport of LN through enterocytes. First, during apical-to-basolateral transcytosis, apical exocytosis back to the lumen greatly weakens the delivery efficiency into blood circulation.[27] Second, the lysosomes in enterocytes could degrade and digest LN during transcytosis process, leading to loss of nanoparticles.[27, 106] Therefore, even though enterocytes are the most abundant type of cell lining the small intestine, they are not particularly efficient at absorbing and transporting LN.

In the meantime, the M cells are far less abundant than enterocytes, however they have a higher capacity for transcytosis of particles.[107] The M cells could be mainly found in the gut-associated lymphoid tissue (GALT) of the specialized regions called "Peyer's patches". They also can be found in mucosa-associated lymphoid tissue (MALT) in other parts of the GI tract.[21, 108] The M cells are able to take up the antigens and microorganisms in lumen and deliver them to the underlying immune system of mucosa.[109, 110] After transcytosis cross the follicle-

associated epithelia of Peyer's patches, the antigens move to the intraepithelial pocket containing lymphocytes, macrophages, and dendritic cells on the basolateral side of M cell.[45] Compared with enterocytes, the M cells have unique morphological features suited for efficient endocytosis and/or transcytosis. The luminal surfaces of M cells are characterized by poorly developed glycocalyx, undeveloped microvilli, and a much thinner mucus layer, which altogether allow the cells to have easy access to lumen contents.[111] Furthermore, M cells have fewer lysosomes than other intestinal epithelial cells and present low lysosomal enzyme activity.[112, 113] In addition, the basolateral side of M cells is juxtaposed to the apical side at intervals of a few microns, making it easy for transcytotic vesicles to translocate through the epithelial barrier. Ma et al. have described that the trans-monolayers transport of LN was significantly enhanced after insertion of Raji cells into Caco-2 cells monolayers, which was to mimic the transporting functions of M cells.[114] They conjugated a poorly soluble drug silybin and lipids with different chain lengths (6C, 12C, 18C) to form lipid-silybin conjugates and formulated the conjugates into SLN. The results indicated that insertion of Raji cells into Caco-2 cell monolayer could increase the trans-monolayer transport of silybin by around 2, 1.8, and 8 times for lipid-silybin conjugates with 18C, 12C, and 6C, respectively. Although the M cells pathway might be a very important route for oral absorption of integral LN, the exact mechanisms of the transcytosis process of LN via M cells are not completely understood. After adherence to the M cell apical membranes and transport across the thin apical cytoplasmic rim, LN are delivered to the underlying inductive mucosa-associated lymphoid tissue (MALT) sites and may subsequently disseminate via the lymphatics.

3.2.2.3. Persorption

Other than paracellular and transcellular transport, the entrance of microparticles through the intestinal lumen into the systematic circulation could be achieved by a process called persorption, Persorption is transportation of intact particles through holes formed in the epithelium caused by extrusion of dead enterocytes from epithelial layer.[115] Previous studies have demonstrated GI uptake of various of large particles by persorption, such as pollen and starch.[116, 117] For persorption of nanoparticles, persorption were only observed during GI uptake of gold nanoparticles (4, 10, 28, and 58 nm) under TEM.[115] However, the mechanism under persorption of nanoparticles remains unclear. Although no previous studies about persorption of LN have been conducted, such phenomena might be a possible route for GI uptake of LN.

4. Biological fate of LN in animal models

Although the Caco-2 cells in vitro model allows the screening of specific mechanisms of LN transcytosis in Caco-2 cells and integral nanoparticles have been found in basolateral side of Caco-2 cell monolayers in some studies, whether integral LN can be taken up and transported across the intestinal epithelium remains controversial. The in vitro models cannot fully recapitulate important aspects of human intestinal physiology such as topography of the intestinal villi. Furthermore, in vitro models cannot provide information of digestion and absorption of LN in a continuous and conclusive manner. Therefore, researchers attempted to investigate whether integral LN can be transported across the intestinal epithelium using in vivo models. However, it is difficult to develop an appropriate in vivo model due to the complexity of body physiology and the small size of LN makes the detection very difficult. In a study conducted by Yuan et al., ODA-FITC was synthesized and used as a fluorescence probe to label the stearic acid SLN prepared by solvent diffusion method.[118] They found that the transport efficiency of integral SLN by oral administration was about 30%, and most of SLN (approximately 77.9%) were transported into systematic circulation via lymph. However, the fluorescence probe (ODA-FITC) used in this study can still emit fluorescence even after releasing from nanoparticles, thus it is not accurate to use the fluorescence signal as an indicator of integral nanoparticles. Later, Chen et al. prepared a mixed lipid shell and an aqueous core containing FITC-E4-loaded micelles and labelled the lipid shell with rhodamine-phospholipid.[119] In other words, they labeled the encapsulated cargo and lipid shell separately with different fluorescence probes. Images from fluorescence microscope suggested that FITC-E4 could be transported deep into the villi or even the capillaries, while most of the solid lipid shell of the nanoparticles remained in the epithelial cells, indicating that the integral nanoparticles could not be taken up by intestinal epithelia. In another study, Hu and coworkers developed a novel environment-responsive near-infrared fluorescent probe (P2), which had water-quenching properties upon contact with water, to monitor the in vivo fate of SLN.[120] The rationale of detection was that the fluorescence signal can be quenched when the probes were released from hydrophobic lipid core to hydrophilic external environment. The results indicated that the chance of absorption of intact SLN through oral delivery seem to be little, since no fluorescence signals were detected in various organs and tissues (blood, liver, spleen, lung, kidney, brain, etc.) except the GI tract (**Fig. 8**). However, in another study conducted by the same group, they challenged previous conclusions by elevating the total dose of SLN as well as total fluorescence dose and found that fluorescence signals could be tracked in blood, lymph and liver (**Fig. 9**).[114] Although SLN can be transported by the M cells as integral particles into

the circulation and ended up in the liver, only a very small amount of SLN could be absorbed through this route. Most of the SLN were degraded and released the encapsulated cargos after entering the GI tract. The released cargos would be absorbed through passive diffusion or be incorporated into mixed micelles for further absorption. However, the stability and integrity of Tween 80 emulsified SLN in the two above-mentioned studies cannot be guaranteed during transportation in GI tract. Thus, only a small amount of integral SLN remained when reaching the intestinal epithelia for absorption. Whether indigestible LN can be taken up and transported across the intestinal epithelium as integral nanoparticles still requires experimental verification.

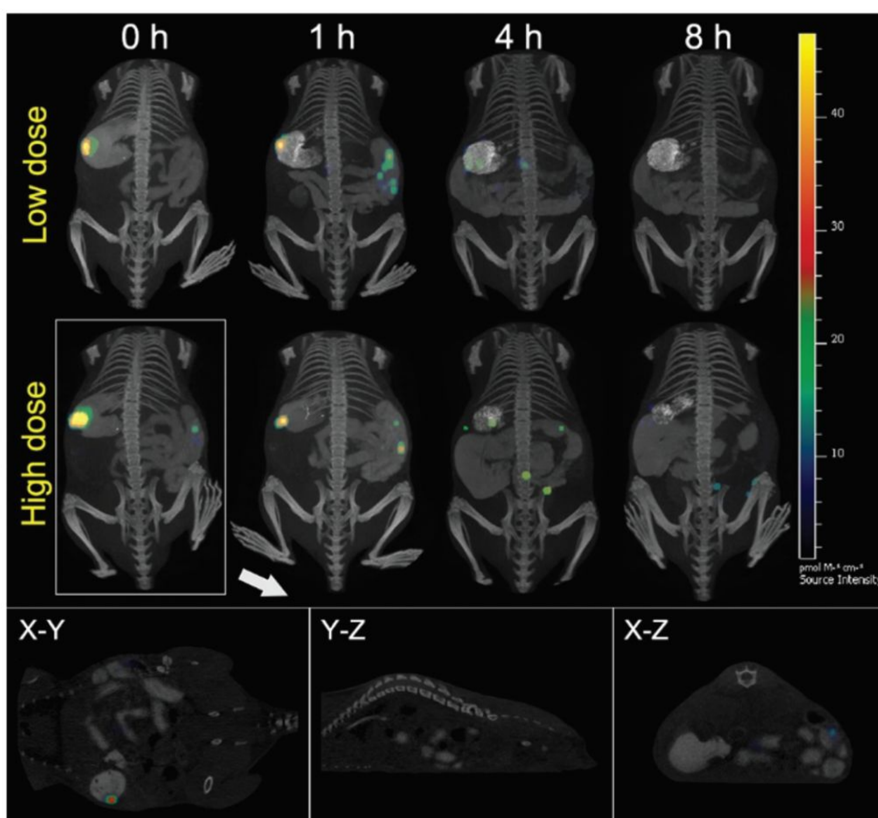

Fig. 8 In vivo 3D fluorescence plus CT living imaging of ICR mouse after gavage administration of different doses of P2-SLN in a supine position. The lower part shows the representative X–Y, Y–Z and X–Z position cross-section imaging. This figure has been reprinted from ref.[120] with permission from Royal Society of Chemistry.

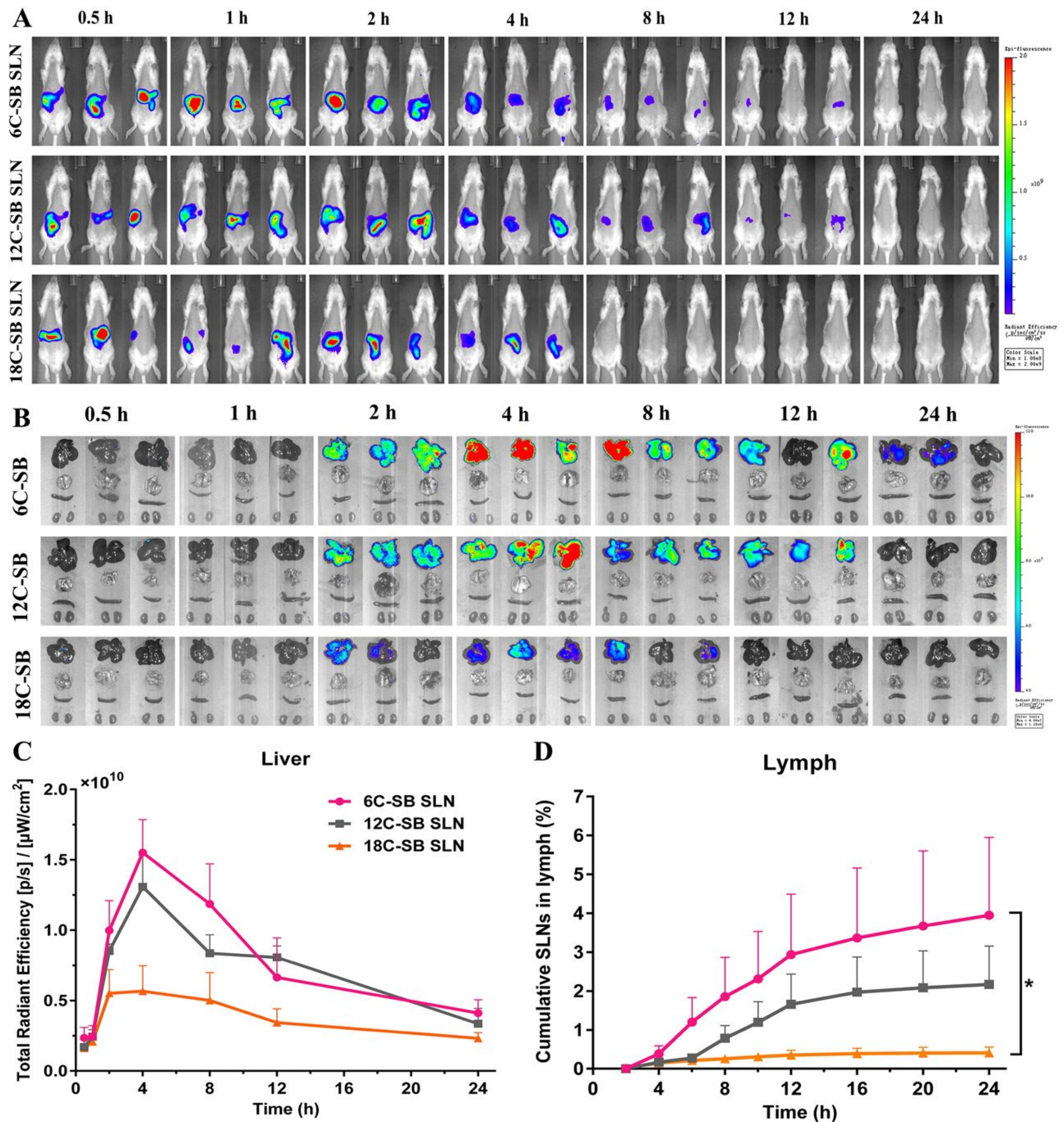

Fig. 9 In vivo live imaging of the digestion of SLNs in rats after gavage administration (A); ex vivo imaging of biodistribution in organs and tissues (B); quantification of liver-borne fluorescence (C); cumulative transport of integral SLNs recovered from mesentery lymph in a lymphatic cannulation model (D). *P<0.05. This figure has been reprinted from ref.[114] with permission from Elsevier.

5. Concluding remarks

In summary, LN have been widely used to encapsulate, protect, and deliver lipophilic drugs/nutrients. Although existing studies have demonstrated that LN could improve oral bioavailability of encapsulated cargos, the understanding of their in vivo digestion and absorption is an impediment to their further development and practical application. Taking together plenty of studies related to this topic, the in vivo biological fate of ingested LN and the underlying mechanisms and pathways can be classified into two categories based on whether or not the LN are digestible in the GI tract. For digestible LN, first of all, the ingested LN will be subjected to digestion by lipases and hydrolysis throughout the GI tract, and the lipid digestion products containing fatty acids and monoglyceride will be transformed into mixed micelles, together with encapsulated cargos, bile salts and phospholipids. After absorption by enterocytes, the lipid digestion products and encapsulated cargos enter the systemic system through either portal vein or lymphatic system, depending on the structure of lipids in LN formulation and hydrophobicity of encapsulated cargos. In contrast, for indigestible LN, they can maintain integral and possibly penetrate through the mucus layer to contact with epithelia. After penetration, these LN can be transported through tight junction, or taken up by enterocytes or transported by M cells, to enter the circulation. Even though the transportation mechanisms of integral LN through intestinal epithelia have been well studied using in vitro models (mainly Caco-2 cell monolayers and Caco-2/HT29/Raji cells co-culture), such in vitro results were only parts of the whole picture. In recent years, several animal studies revealed that most of digestible LN were degraded in the GI tract via lipolysis and hydrolysis and only a small fraction of LN that survived the GI environment were absorbed by enterocytes and/or M cells. In general, the lipolysis and subsequent stimulation of micellular species production seem to be the major mechanisms for enhancing absorption of lipophilic compounds delivered by LN. However, the LN formulation used in many studies are way too simple to ensure the integrality and stability of LN under physiological GI conditions before the absorption by epithelia could occur. Therefore, the transportation of indigestible LN across the intestinal epithelium still requires experimental verification, especially for GI-stable LN with complicated structure (e.g. lipid-polysaccharide hybrid nanoparticles).

In future studies, GI-stable LN formulations and well-designed in vivo models are required to present a more complete understanding of the biological fate of orally administered LN. Before such an approach could be pushed forward, it will be crucial to develop and validate the animal models and analytical methods available for assessing LN digestion and uptake. For instance, the fluorescence probes/markers should be able to act as an effective indicator for nanoparticles. Since some of the fluorescence probes, such as FITC, can still emit fluorescence after leakage,

the fluorescence signals could not be used as indicators for intact nanoparticles. Another important aspect is the dosing methodology in animal studies. During in vivo study, the tested LN are orally administered either via gastric gavage or in food/drinking water. While dosing in food/drinking water is more realistic, the experimental procedure might be less accurate compared with dosing by gavage. For this consideration, the gavage may still be preferred due to higher accuracy and smaller individual variation, though there are some limitations such as lack of buccal cavity exposure. In previous research, fluorescent probes and fluorescence microscopy techniques are the most commonly used method to detect the location, accumulation, and concentration of LN and encapsulated cargos. However, more detailed information should be revealed to get a comprehensive understanding of in vivo biological fate of LN and the encapsulated cargos, such as their specific metabolic products after absorbing into the circulation. In particular, metabolomics has emerged as an important tool to systematically identify and quantify the metabolic products of a biological system (e.g. cell, tissue, organ, and biological fluid) in nanoparticles-treated animals. It has been successfully employed in safety assessment of orally administrated titanium dioxide nanoparticles in rats through biomarkers in urine and serum.[121] Thus, we envision that in the near future, the research on the metabolomic responses to the ingested LN in animal models will provide a bigger picture on their biological fate than regular animal studies focusing on GI behavior alone. Furthermore, although animal models are widely used to predict human response to either LN or encapsulated cargos after oral ingestion, most of the findings from animal studies may be hard to reproduce in human trials (clinical studies). Thus, in order to bridge the translational gaps between animal models and humans, it is necessary to perform clinical studies to fully understand the in vivo biological fate of ingested LN and encapsulated cargos. In addition, future work may also focus on incorporation of nutrient-loaded LN into food matrix to prepare health supplements for enhanced absorption and bioavailability for disease prevention or even potential treatment, which may provide useful guidance with respect to LN formulation design and commercialization as nutraceuticals and pharmaceutics.

6. Conflict of interest

There are no conflicts to declare.

7. Acknowledgements

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Graphical abstract

This review summarizes current knowledge on digestion and absorption of ingested lipid-based nanoparticles at molecular, cellular and whole body levels.