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Mechanisms of transport of polymeric and lipidic nanoparticles across the intestinal barrier☆

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ABSTRACT

Unraveling the mechanisms of nanoparticle transport across the intestinal barrier is essential for designing more efficient nanoparticles for oral administration. The physicochemical parameters of the nanoparticles (e.g., size, surface charge, chemical composition) dictate nanoparticle fate across the intestinal barrier. This review aims to address the most important findings regarding polymeric and lipidic nanoparticle transport across the intestinal barrier, including the evaluation of critical physicochemical parameters of nanoparticles that affect nanocarrier interactions with the intestinal barrier.

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Abbreviations: CME, Clathrin-mediated endocytosis; COX, Cyclooxygenase; CPP, Cell-penetrating peptides; CSK, CSKSSDYQC peptide; CvME, Lipid raft/caveolae-mediated endocytosis; Cys, Cysteine; DMEC, Dimethylethyl chitosan; DEMC, Diethylmethyl chitosan; DPP-4, Dipeptidil peptidase-4; EGTA, Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid tetrasodium salt; EIPA, 5-(N-ethyl-N-isopropyl)-amiloride; EMA, European medicine agency; FAE, Follicle-associated epithelium; FDA, Food and drug administration; GLP-1, Glucagon-like peptide-1; HA, Hyaluronic acid; HPMC-AS, Hydroxypropylmethylcellulose acetylsuccinate; IBD, Inflammatory bowel disease; INF-β, Interferon-β; IL-1, Interleukin-1; IRQ, IRQRRRR peptide; LBDDS, Lipid-based drug delivery system; LNC, Lipid nanocapsules; LPS, Lipopolysaccharide; mmePEG$_{750}$P(CL-co-TMC), Monomethylether poly(ethylenglycol)$_{750}$-poly(caprolactone-co-trimethylene carbonate); MβCD, Methyl-β-cyclodextrin; NLC, Nanostructured lipid carriers; NPs, Nanoparticles; PAMAM, Poly(amidoamine); PCL–PEG, Poly-ε-caprolactone-block–polyethyleneglycol; PEG, Polyethylene glycol; PEG$_{2000}$-DSPE, Polyethylene glycol-distearoyl phosphatidylethanolamine; P-gp, P-glycoprotein; PLA–PEG, Poly (lactic acid)-*b*–polyethyleneglycol; PLGA, Poly (lactide-co-glycolide) acid; PLGA–PEG, Poly (lactide-co-glycolic) acid-block–polyethyleneglycol; PSi, Mesoporous silicon; PTK, Protein tyrosine kinase; QCPQ, Quaternary ammonium palmitoyl glycol chitosan; RGD, Arginine–glycine–aspartic; SLN, Solid lipid nanoparticles; SLM, Solid lipid microparticles; TEC, Triethyl chitosan; TEER, Transepithelial electrical resistance; TJ, Tight junction; TMC, Trimethyl-chitosan; TNF-α, Tumor necrosis factor-α; VB$_{12}$, Vitamin B$_{12}$; WGA, Wheat germ agglutinin.

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1. Introduction

In vitro cell models represent a valuable tool to study the oral delivery of drugs, peptides, or vaccines [1–3], especially their oral delivery when included in nanoparticles (NPs) [4]. By mimicking the intestinal barrier, it is possible to evaluate the intracellular trafficking of the NPs and, thus, the passage of the NPs across the epithelial barrier. The permeability of the cargo at the intestinal site can be estimated using Transwell® inserts where the transported amounts of the cargo can be quantified in the basolateral compartment of the inserts. Valuable information about NPs' *in vivo* accomplishment at the intestinal site can be predicted *in vitro* with different cell models.

Recently, special attention has been paid to the mechanism of transport of NPs in different *in vitro* models mimicking different layers of the intestinal epithelium (e.g., enterocyte-like model, mucus-secreting cell model, follicle-associated epithelium model (FAE, containing M cells), as well as to the evaluation of the physicochemical characteristics that drive the NPs across the intestinal barrier [5–10]. The ultimate question is: *Can we dictate and/or anticipate nanoparticle and cargo fate across the intestinal barrier by altering NP physicochemical properties*? [11]. To address this question, researchers have developed a myriad of polymer- and lipid-based NPs differing in surface charge, size, and coating, for example. The different paths of NP interaction with the intestinal cells and NP intracellular trafficking and *in vivo* data based on their physicochemical composition have also been evaluated. The increased interest in NP design for overcoming biological barriers has resulted in the development of tunable nanocarriers for controlled drug delivery [12,13].

This review addresses the most important findings regarding polymeric and lipidic NPs transport across the intestinal barrier, including the evaluation of critical physicochemical parameters of NPs affecting nanocarrier interactions with the intestinal barrier. We compared both polymeric and lipidic NP transport and provided potential applications (e.g., peptide delivery, enhanced permeability) based on the data obtained through models mimicking the intestinal barrier. Finally, we justified the relevance of mechanistic studies by discussing data where *in vitro* and *in vivo* findings correlate, and presented future perspectives for obtaining more reliable preclinical data using cell models.

2. Transport of nanoparticles across *in vitro* models of the intestinal barrier

Many *in vitro* models of the intestinal epithelium have been developed and used to study intestinal adsorption of bioactive molecules and drug delivery systems. A human enterocyte model using differentiated Caco-2 cells has been extensively used to study adsorption through the human intestine. However, even if the intestinal cell monolayer is mainly composed of enterocytes, several other cell types play an important role in intestinal adsorption. More recently, more complete models of the intestinal epithelium have been developed, such as the mucus-secreting cell model, the FAE model, inflamed intestine models, and, most recently, 3D models.

2.1. Enterocyte-like model

The vast majority of *in vitro* studies of intestinal absorption have been performed using Caco-2 cells. Caco-2 cells are heterogeneous human epithelial colorectal adenocarcinoma cells that spontaneously differentiate into human enterocytes. Caco-2 cells can be seeded directly on plastic for toxicity or biochemical studies or grown on porous inserts (Transwell®) for transport and adsorption studies. Cells are usually seeded at 50,000 cells/cm^2 [14]. The acceptance criteria for a Caco-2 monolayer are usually a transepithelial electrical resistance (TEER) superior to 250 Ω/cm^2 and markers of paracellular transport such as Lucifer Yellow with a P_{app} inferior to 0.4×10^{-6} cm/s [15].

Many efforts have been made to validate this model and to establish a correlation between oral drug adsorption in humans and permeability coefficients that were measured on Caco-2 cells [1,16–18]. Although Caco-2 cells have been used as a model for various applications, in this review, we will focus only on NP transport. Caco-2 cells have been used to test and optimize nanoparticulate formulations designed to cargo fragile or hydrophobic molecules across the intestinal barrier. Recently, Caco-2 cells have been used to evaluate the efficiency and to elucidate cellular mechanisms behind the adsorption of micelles [19], polymeric particles [20–25], lipidic NPs [26], and magnetic NPs [27,28].

Non-human cell lines have also been used to mimic the intestinal epithelium, although for a much more restricted use, including Madin Darby canine kidney (MDCK) cell monolayers [29] and the porcine jejunal cell line (IPEC-J2) [30,31].

2.2. Mucus-secreting cell model

Although a gold standard in oral absorption studies, Caco-2 cell monolayers exhibit certain limitations such as a much higher TEER (up to 500 Ω cm^2) compared to the human intestine (12–69 Ω cm^2) and a lack of mucus at their apical side, which limits the relevance of the Caco-2 model [32]. Consequently, a co-culture model combining human HT29-MTX (goblet-like cells) with Caco-2 cells has been developed. To closely mimic the permeability features of the human intestinal barrier, the co-culture conditions can be adjusted. A Caco-2 cell density of 15,000 cells/cm^2 supplemented with 30,000 HT29-MTX cells/cm^2 at day 3 post-seeding produced an *in vitro* model closer to the human intestinal epithelium in the mucus layer, lower P-gp efflux and paracellular permeability than Caco-2 cells. Modeling NP interactions with the intestinal mucus layer can also be done through an a-cellular *in vitro* model, composed of a pig mucus layer placed at the apical side of a Transwell® insert [33]. Groo et al. tested the impact of lipid nanocapsule (LNC) surface modifications on their diffusion through a 446 μm pig intestinal mucus layer [33].

Although not as widely used, 3D models have also been developed to provide *in vitro* models similar to native intestinal tissues. These usually