ANNUAL REVIEWS

Annual Review of Food Science and Technology

A Comprehensive Review of Nanoparticles for Oral Delivery in Food: Biological Fate, Evaluation Models, and Gut Microbiota Influences

Jingyi Xue, Christopher Blesso, and Yangchao Luo

Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut, USA; email: yangchao.luo@uconn.edu

Annu. Rev. Food Sci. Technol. 2023. 14:1–33

First published as a Review in Advance on November 18, 2022

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

https://doi.org/10.1146/annurev-food-060721-025159

Keywords

biological fate, food nanotechnology, gut microbiota, oral delivery, evaluation models

Abstract

Edible nanoparticles are being developed for the oral delivery of nutrients to improve human health and well-being. Because of the extremely demanding conditions foods experience within the gastrointestinal tract, fundamental knowledge about the biological fate of encapsulated nutrients must be constantly revised. In this review, we first provide an overview of the fundamental absorption pathways of ingested foods and then discuss the evaluation models available to test and predict the biological fate of nutrient-loaded nanoparticles. Then, owing to their importance for human health, the impacts of nanoparticles on the gut microbiota are evaluated. Lastly, the limitations of current evaluation methods are highlighted and future research directions on the study and application of edible nanoparticles for the oral delivery of bioactive food compounds are discussed.

1. INTRODUCTION

Nanotechnology can be used to tune material properties at the atomic and molecular levels to design new and innovative products. For biological applications, nanomaterials with unique properties, such as small size, high surface area, high reactivity, and novel functionalization can cross biological barriers and reach specific tissues or cells (Blanco et al. 2015, Chivere et al. 2020, Keiper 2003, Mitchell et al. 2021). The administration of bioactive-loaded nanoparticles is usually via a specific route such as oral, transdermal, intravenous, or inhalational. Among these administration routes, the oral route is the most common and accessible for the administration of bioactive compounds because of its high acceptability and lower costs (Goldberg & Gomez-Orellana 2003). However, unlike the relatively constant physiological environment of the parenteral route, oral administration involves extremely dynamic biological and chemical conditions in terms of pH, ionic strength, enzyme activities, and exposure to intestinal microbiota, which can easily destabilize the structure of nanoparticles and alter their ability to reliably deliver bioactive compounds (Luo 2020, Luo et al. 2020, McClements 2013). To develop desirable nanocarriers for food applications to improve the oral bioavailability of bioactive compounds, it is critical to understand and determine the biological fate of such nanocarriers after oral administration, as this information aids their future development and practical applications (Wang & Luo 2019). In this review, the biological fates of bioactive compounds and nanoparticles are discussed in detail, including a discussion of the interactions between the gut microbiota and the ingested nanoparticles. Furthermore, state-of-the-art evaluation models and techniques commonly used to evaluate interactions among consumed food ingredients, the gut microbiota, and the host are comprehensively summarized.

2. OVERVIEW OF GASTROINTESTINAL FATE OF NUTRIENTS

2.1. Bioactive Ingredients

Bioactive ingredients are compounds that can bring beneficial health effects to the body by regulating physiological or cellular activities. Studies have shown that some food-derived bioactive compounds (e.g., polyphenols, essential oils, carotenoids, vitamins, minerals, bioactive peptides, and probiotics) exhibit various beneficial effects, such as anticancer, anti-inflammatory, and antioxidant, and can be consumed in the daily diet. However, their application is limited by high sensitivity to harsh environments and low bioavailability/bioaccessibility after oral administration. This section discusses the gastrointestinal (GI) fate of bioactive ingredients and describes the digestive process as well as the absorption principles at the molecular level.

2.1.1. Digestion. As illustrated in **Figure 1**, the microenvironments in different locations of the GI tract are appreciably different. Orally administered bioactive compounds must overcome various chemical and physiological challenges in the GI tract before being absorbed into the systemic and lymphatic circulations. The oral cavity is the first challenge that bioactive ingredients experience after ingestion. Typically, ingested foods are mixed with saliva that has a neutral pH and contains salts and amylases. In the stomach, solid foods are physically disintegrated and digested into a thick semifluid mass called chyme, which is then transferred to the intestine for further digestion. During this process, the ingredients encounter a sudden reduction in pH from around 6.8 to 2.0, followed by a transition from a highly acidic to slightly basic environment after reaching the small intestine (Al Rubeaan et al. 2016). The mean transit time in the stomach and the residence time in the small intestine are around 2–3 h and 3–4 h, respectively. Along with the harsh acidic conditions in the stomach, enzymatic degradation of nutrients is another challenge. Because of the presence of gastric pepsin and lipase, some proteins are digested into peptides in the stomach, whereas some lipids are degraded into monoacylglycerols and free fatty acids (Arranz et al.

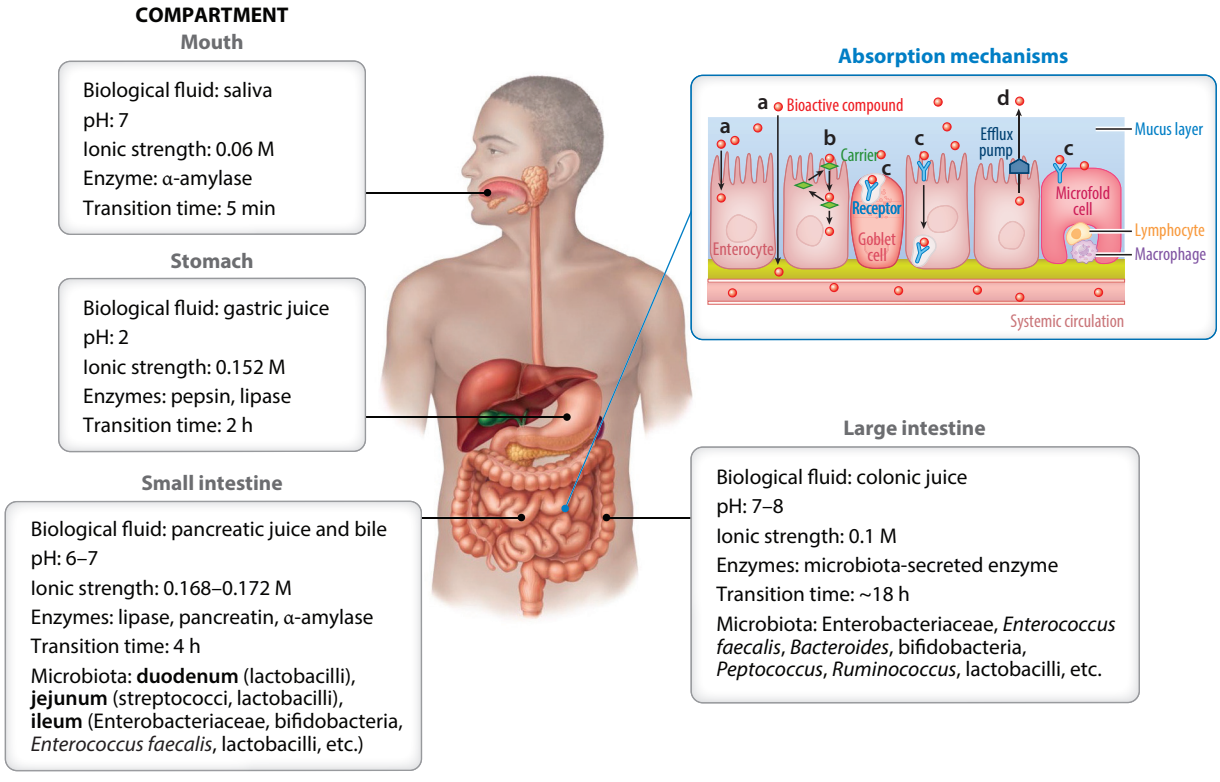

Figure 1

Schematic representation of biochemical compartments to which orally administered nanocarriers are exposed. Schematic representation of different intestinal cells and potential transport pathways: (*a*) passive diffusion, (*b*) carrier-mediated transport, (*c*) receptor-mediated transport, and (*d*) efflux pump mechanism. Diagram of the human digestive tract is adapted from Hutchinson et al. (2007).

2016). The degradation continues in the duodenum and ileum by pancreatic enzymes, including lipase, trypsin, elastase, and carboxypeptidases A and B. Digestible polysaccharides (like rapidly or slowly digested starch) are hydrolyzed into oligosaccharides and glucoses by amylases, whereas the majority of tri- and di-acylglycerols are digested into free fatty acids and monoacylglycerols for further absorption and metabolism by epithelial cells (Langguth et al. 1997, McClements 2013). Although the food ingredients are mostly digested and absorbed in the upper part of the GI tract, some components, such as dietary fibers and mineral oil, are nondigestible due to their unique compositions and structures. These components pass to the colon and are broken down there by various microbiota (Arranz et al. 2016).

2.1.2. Absorption. The absorption of ingested food ingredients and nutraceuticals mainly occurs in the small intestine, which consists of two parts: the mucus barrier and the intestinal epithelial cell layers. The physical structure of the mucus layer is a hydrogel that consists of negatively charged glycoproteins (mucins) and highly branched polysaccharides. Ingested ingredients must permeate across the mucus barriers prior to absorption by epithelial cells. The diffusion of nutrients through the mucus layer depends on their physicochemical properties, as these determine their molecular interactions with the mucus polymers, such as hydrogen bonding, electrostatic, and hydrophobic interactions, as well as polymer entanglement. The pore size and interactions

of the mucus layer impact the penetration of nutrients to the epithelial cells. Substances that are larger than the mucus pore size, which is a few hundred nanometers, cannot easily pass through the mucus layer (Boegh et al. 2014, Yu et al. 2016). The digested components then contact the intestinal cell layer, which mainly consists of three different types of cells (**Figure 1**), where absorption occurs. First, enterocytes, the most abundant cells in the small intestine, have microvilli on the lumen side and are responsible for nutrient absorption through both passive diffusion and active transport. Second, the goblet cells scattered among the intestinal cells are responsible for the secretion of mucus. Third, the microfold (M) cells distributed in Peyer's patches have high transcytosis capacity and participate in immune responses (Cone 2009, de Sousa et al. 2015, Gullberg et al. 2006, Tyrer et al. 2006).

2.1.3. Transportation. As illustrated in **Figure 1**, there are four main transport mechanisms for orally ingested ingredients that pass through the intestinal cell layer. The first mechanism is passive diffusion, which is an energy-independent pathway that occurs via the paracellular or transcellular pathway because of osmotic pressure. Hydrophobic small molecules can be fused to the cell membrane and then transferred into the cell by transcellular diffusion, whereas hydrophilic small molecules are transported by paracellular diffusion through tight junctions located between intestinal cells (Burton et al. 1991, Cao et al. 2022, Liu et al. 2021). The second mechanism is a receptor-mediated transport, an energy-dependent pathway triggered by the specific binding of ligands within the nutrient to their corresponding receptors located on the surface of the intestinal cells (Swaan 1998). The third mechanism is carrier-mediated transport, which allows compounds to enter or exit the cells utilizing the cellular protein transporters, including both energy-dependent active transport and facilitated diffusion (Pawar et al. 2014). The final mechanism is via efflux pump, an energy-dependent pathway that is responsible for resistance to multiple drugs, as through these efflux pumps, absorbed bioactive compounds can be pumped out to the luminal side of the intestine, resulting in relatively low bioavailability (Abuznait et al. 2011, Lowrence et al. 2019).

2.2. Nanoparticles

Recently, the modernization of the entire food chain from production to processing, storage, and consumption has been accelerated by nanotechnology (Ndlovu et al. 2020). Nanoparticles play an important role in improving the oral bioavailability of bioactive compounds, as they can increase the solubility, stability, and bioaccessibility of nutrients and may help the nutrients pass through the various barriers in the human body and deliver their cargos to the designated organs in a targeted manner (Arshad et al. 2021). Most nanotechnology-based products for in vivo applications are composed of "soft" nanostructures, prepared mainly from organic compounds, such as proteins, polysaccharides, phospholipids, and lipids (Chen et al. 2020; Hu et al. 2020, 2021; Veneranda et al. 2018; Wang et al. 2017a; Wang & Luo 2021; Wusigale et al. 2020; Zhou et al. 2016b). On the other hand, "hard" nanostructures, which are primarily made from inorganic compounds such as metallic or metal oxide materials (e.g., gold, silver, iron oxide, and titanium oxide), are commonly developed for medical imaging and theragnostic purposes (Keiper 2003, Pugazhendhi et al. 2018, Yang et al. 2021). The size, structure, and composition of nanoparticles determine their ability to encapsulate, protect, and deliver nutrients. In general, the biological fate of nanoparticles depends on their initial physicochemical characteristics (such as composition, structure, dimensions, interfacial properties, and physical state), as well as any changes in these properties as they pass through the different regions of the GI tract. As summarized in **Table 1**, the physicochemical properties of nanoparticles affect their absorption and distribution after oral administration, as nanoparticles may be small enough to pass through narrow gaps in the mucus layer and epithelial cells (tight

Table 1 Impact of characteristics of nanoparticles on their absorption and distribution

Absorption				Circulation and distribution	
Mucus		Enterocytes/M cells			
Size	**Rank**	**Size**	**Rank**	**Size**	**Rank**
<100 nm	High absorption	<50 nm	High absorption	<100 nm	Longest circulation
200 nm	Moderate absorption	100–400 nm	Moderate absorption	>200 nm	Rapid clearance
300–500 nm	Low absorption	500–5,000 nm	Low absorption	NA	NA
>500 nm	No absorption	NA	NA	NA	NA
Charge					
No charge	High absorption	No charge	Moderate absorption	No charge	Longest circulation
Positive	Adheres to mucus	Positive	High absorption	Positive	Fastest clearance
Negative	Low absorption	Negative	Moderate absorption	Negative	Rapid clearance
Water affinity					
Hydrophobic	Low absorption	Hydrophobic	High absorption	Hydrophobic	Faster clearance
Hydrophilic	High absorption	Hydrophilic	Low absorption	Hydrophilic	Longest circulation

Abbreviation: NA, not available. Adapted from Beloqui et al. (2016) and Borel & Sabliov (2014).

junctions) or they possess specific surface properties to achieve high absorption rates. Generally, nanoparticles enter the cells through active transport mechanisms (endocytosis) similar to those of food ingredients, including phagocytosis and micropinocytosis, which are mediated by various carriers and receptors (Beloqui et al. 2016).

2.3. Gut Microbiota

The human body may be routinely exposed to nanoparticle-containing consumer products such as pharmaceutics, packaging materials, personal care products, and some food products. Despite the benefits provided by nanotechnology, the potential alteration of the gut microbiota and their potential side effects on the host should be considered (Bergin & Witzmann 2013). In humans, the gut microbiota consists of up to 5,000 species, with the majority belonging to the phyla Bacteroidetes and Firmicutes, which can be modulated by a variety of factors such as diet, lifestyle, host genome, and drugs. Feces are most commonly sampled as a representative source of the gut microbiome and are used to examine microbiota composition as well as for metagenomic and metabolomic analyses of host health, as the majority of the microbiome exists in the ileum and colon, as shown in **Figure 1** (Lamichhane et al. 2014). The intestinal microbiota exceeds the total number of nucleated cells in the human body by several fold (Sender et al. 2016). Some researchers have proposed it to be an endocrine organ because the homeostasis of the intestinal microbiota maintains not only the healthy state of the gut but also that of the host (Monda et al. 2017). Through their co-evolution, the microbiota and host have developed a relationship based on mutual benefits. The microbiota can help with the extraction of nutrients from foods, and the host provides a warm and nutrient-rich environment, which is essential for establishing a relatively stable ecosystem for the microbiota. Dysbiosis is an imbalance in the microbial community of the gut deviating from that seen in a healthy host, which can lead to several disorders such as obesity, diabetes, cancer, and mental disorders such as anxiety and depression (Dahiya et al. 2017). For ingested nanoparticles, even if they are not absorbed, they may induce toxic effects and cause alterations to the normal microbiota in the host. For instance, researchers have demonstrated that soluble fibers, such as inulin, pectin, and fructo-oligosaccharides, which can

be fermented by gut bacteria into the short-chain fatty acids (SCFAs) acetate, butyrate, and propionate in the colon, can induce icteric hepatocellular carcinoma (HCC) in multiple strains of dysbiosis mice, but this microbiota-dependent HCC did not develop in germ-free or antibiotics-treated mice (Singh et al. 2018). Furthermore, consumption of a soluble fiber–enriched high-fat diet induced both dysbiosis and HCC in the mice, but this disease could be prevented either by pharmacologic inhibition of fermentation or through the depletion of fermenting bacteria. Meanwhile, the alteration of microbiota status could also affect nanomaterial absorption. For instance, lipopolysaccharides (LPS) produced by all Gram-negative bacteria in the colon provide extra adherence for nanoparticles. This may result in the enhanced absorption of nanomedicine by the increase of the retention time (Cattani et al. 2010). Meanwhile, potential interference with nutrient absorption may lead to pathological changes in gut microbial metabolism, such as the antibiotic activity of some nanocarriers. Therefore, it is imperative to assess and investigate both the potentially toxic and beneficial effects of nano-delivery systems on the intestinal microbiota, especially when designing and developing oral delivery vehicles.

2.4. Evaluation of Biological Fate

As discussed above, the harsh conditions in the GI tract often lead to destabilization and degradation of nanoparticles. Hence, good GI stability of nanoparticles across a wide pH range is crucial to protect the cargos from chemical and enzymatic degradation throughout the GI transit. Studies have shown that the dissociation or disassembly of nanostructures may occur due to the deprotonation or protonation of nanoparticle components, resulting in a possible loss of efficacy and/or increased toxicity of the encapsulated cargos (Olbrich & Müller 1999, Zhou et al. 2016a). Variations in particle dimensions, composition, interfacial properties, and shape influence the stability of nanoparticles in different simulated GI fluids. Also, to accurately simulate human digestion, the impact of host microbiota, immune response, and hormonal regulation should be considered (Guerra et al. 2012). Moreover, cellular uptake, lymphatic transport, and peripheral circulation also need to be simulated to ensure precise prediction of the in vivo behavior of nanoparticles. However, given the time-consuming, expensive, and inaccessible nature of in vivo experiments, a series of in vitro models with various levels of complexity and number of compartments have been developed to reveal the behavior of nanoparticles after oral administration and investigate the associated effects on human digestive and circulatory systems (Zhou & McClements 2022). Furthermore, various in vitro cellular models have been established to predict interactions between the nanoparticles and the GI tract, providing information about digestion and absorption at the molecular level (Etheridge et al. 2013, Le Feunteun et al. 2021). Sometimes, in vitro models fail to offer sufficient information on the biological fate of nanomaterials in the host organisms, and further ex vivo, in situ, and in vivo studies are required to derive a more comprehensive picture. In the following sections, currently available in vitro, ex vivo, in situ, in vivo, and microbiota-based models that have been exploited to assess the efficacy and toxicity of orally administered nanoparticles to understand their biological fate are discussed.

3. STATIC IN VITRO EVALUATION MODELS

3.1. Simulating Gastrointestinal Digestion

The most basic in vitro digestion model, often used to evaluate the oral performance of edible nanoparticles, is the use of synthetic media to simulate enzymatic digestion to mimic the physiological properties of the GI fluids, including digestive enzymes and pH conditions. As mentioned above and illustrated in **Figure 1**, oral administration leads to the exposure of nano-based

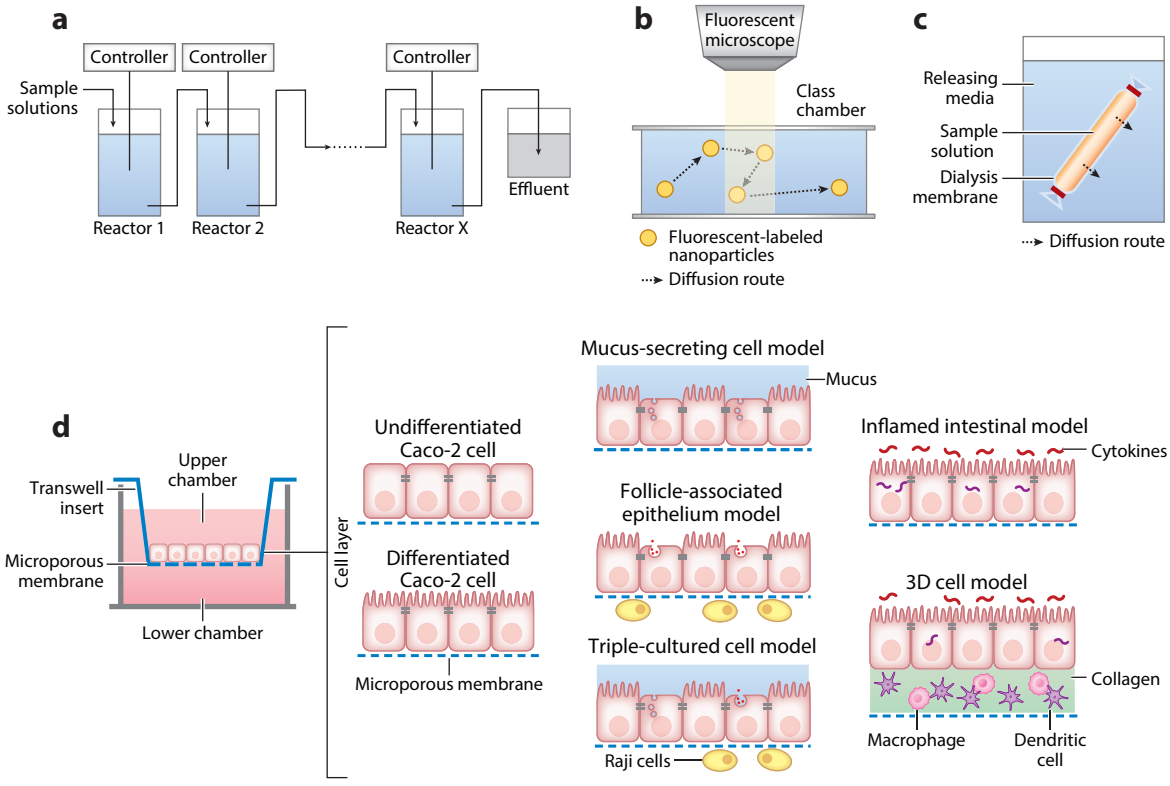

Figure 2

Schematic illustration of the static in vitro evaluation models: (*a*) simulated gastrointestinal (GI) fluid, (*b*) mucus-penetration and mucoadhesive model, (*c*) control release model, and (*d*) cell models.

formulations to different compartments that are characterized by well-defined biochemical conditions (e.g., various pH ranges, enzymes, salts, etc.) before entering the body, posing a challenge to the stability of nano-based formulations. Several in vitro digestion models with different complexities have been proposed to meet various demands, all based on simulated digestive fluids with chemical and enzymatic compositions close to those secreted in vivo by the GI tract (Bao et al. 2019). In the early stages, the simplest experimental model consisted of only one compartment that mimicked intestinal digestion and was considered to be an adequate representation of the digestive process (Dahan & Hoffman 2007, Fatouros et al. 2007, Kaukonen et al. 2004, Porter et al. 2004). However, this simple intestinal digestion model is insufficient to predict the impacts of gastric digestion, as well as gastric emptying and sudden pH changes in the stomach (Carrière 2016). For this reason, multiple-stage models have been developed that allow investigation of the impact of gastric and intestinal conditions on food properties (Christophersen et al. 2014, Klitgaard et al. 2017, Van de Wiele et al. 2015). The basic procedure is shown in **Figure 2*a***, where each reactor represents one compartment in the GI tract and each controller contains different acid/base solutions, salts, enzymes, and other GI components (e.g., mucin in the small intestine and microbiota in the colon), whereas the gastric-emptying pattern and intestinal movement can be achieved by changing the flow rate of simulated fluids (Klitgaard et al. 2017). The testing nanoparticle solution is injected into the reactor chamber and incubated at 37°C for predetermined amounts of time. At each time point, samples are withdrawn, followed by the evaluation

Table 2 Bile salt concentrations and enzyme activities in suggested compositions for simulated gastric and intestinal digestion media for in vitro lipolysis studies

Simulated model	Human adult	Human infants[a]	Rat
Gastric fluid			
Bile salts	0.08 mM	NA	0.08 mM
Phospholipids	0.02 mM	NA	0.02 mM
Gastric lipase	50 TBU/mL	17 TBU/mL	NA
Pepsin	450 U/mL	126 U/mL	NA
pH	2.5	6.4	4.0
Intestinal fluid			
Bile salts	2.95 mM	1 mM	50 mM
Phospholipids	0.26 mM	0.2 mM	3.7 mM
Porcine pancreatic extract	600 USPU/mL	50 TBU/mL	179 USPU/mL
pH	6.5	6.5	6.5

Abbreviations: NA, not available; TBU, tributyrin unit; U, enzyme unit; USPU, United States Pharmacopeia Unit. Adapted from Berthelsen et al. (2019).
[a]Age 0–2 months, average weight 2.0 kg.

of their physicochemical properties and morphologies (such as their particle size, polydispersity, charge, and microstructure) to determine the stability and behavior of the nanoparticles. The diversity and richness of the gut microbiota are also measured to determine the influence of the nanoparticles on the human body. This in vitro simulation model provides a useful tool for investigating nano-based formulations in high-throughput mode. By varying synthetic digestive fluid composition, different compartments and physiological conditions (fed versus fasted) can be simulated, greatly expanding model flexibility (Wang et al. 2017b, Xue et al. 2018).

Currently, most in vitro models simulate the digestive system of healthy human adults. However, this may mislead predictions of the biological performance of nanoparticles in special populations (e.g., infants and the elderly), where the physiological GI environment differs appreciably. **Table 2** summarizes the digestion media compositions in human adults (Mosgaard et al. 2015), human infants (Kamstrup et al. 2017), and rats (Jørgensen et al. 2018) at different states. The composition and properties of the GI tract differ markedly between human adults and infants. Compared with adults, infants have underdeveloped pancreatic lipase and relatively low gastric enzyme activities, gastric fluid volume, and bile salt concentration (Guimarães et al. 2019). Meanwhile, in contrast to adults, infants have a relatively high gastric pH due to the frequent feeding process of breast milk or infant formula (Fredrikzon & Olivecrona 1978). In addition to the influence of different growth stages, the impact of species difference should be included. Usually, to predict the behavior of nanoparticles in humans, the selected formulations should be tested in vivo in animals as preclinical assessments prior to clinical studies (Anby et al. 2014). As illustrated in **Table 2** and reviewed in the literature (Sjögren et al. 2014), the physiology and composition of the GI tract of experimental animals differ significantly from that of humans. A recent study demonstrated that an in vitro study simulating the rat GI fluids produced a better correlation with in vivo *pK* data in rats, whereas the authors were unable to correlate the data obtained from the simulated human intestinal fluids with the data obtained from in vivo rat experiments (Jørgensen et al. 2018).

In summary, the design of population- and species-specific digestion models that can improve the accuracy of in vitro–in vivo correlations is gaining increasing attention, and we envision this will be a major focus of in vitro studies in the future.

3.2. Mucus-Penetration and Mucoadhesive Models

As mentioned earlier, the luminal surface of the GI tract is protected by a layer of highly viscoelastic and adhesive mucus, which is responsible for the removal and elimination of foreign particles or pathogens. Ingested nanoparticles can be trapped in the mucus layers because of steric hindrance and adhesion, resulting in delayed absorption (Dünnhaupt et al. 2015). High-resolution multiple particle tracking (MPT) has been used to test the diffusion of nanoparticles labeled with fluorescence dyes. MPT can be used to measure the percentage of mucus-trapped nanoparticles at predetermined times and generate quantitative measurements of the extent of particle hindrance to free diffusion at both individual and ensemble-average levels, which provides important feedback for the development of nanoparticles for oral delivery. The basic setup of the MPT method is shown in **Figure 2*b***. Mucus is placed in a glass chamber and then fluorescently labeled nanoparticles are added. By tracking and recording the movement of the nanoparticles using a fluorescence microscope, the mean square displacements (MSDs) and effective diffusivities are determined and used to represent the mucus-penetration rate. Researchers have used the MPT method to study the diffusivity of amino- or carboxyl-modified polystyrene particles (diameter 100–500 nm) through mucus and showed that the modified nanoparticles moved at least 300-fold slower in fresh human sputum than in water (Dawson et al. 2003). Researchers compared the diffusivity of hydrophobic polystyrene particles and hydrophilic modified poly(lactic-co-glycolic) acid nanoparticles using mucus obtained from the pig GI tract and showed that the transport rate of these hydrophilic nanoparticles was at least 10 times higher than the hydrophobic counterparts (Dawson et al. 2004). Furthermore, in a recent study, cationic liposomes were used as carriers for oral insulin delivery and the impact of surface modification was investigated (Wang et al. 2019). The results showed that the MSD of bovine serum albumin–coated liposome was 21-fold higher than that of the uncoated liposome and exhibited the ability to penetrate the mucus layer and pass through the epithelial cells rather than remain trapped within the mucus layer, indicating the importance of investigating the permeability of nanoparticles.

3.3. Drug Release Models

The dialysis membrane model is another traditional approach to determining the transport properties of bioactive compounds from nanoparticles into intestinal fluids. As shown in **Figure 2*c***, nanoparticle dispersions are placed in a dialysis bag or tube with the desired pore size and then suspended in a releasing media. To analyze the drug release properties, aliquots of the exterior membrane buffer are removed at predetermined time intervals and the drug or nutrient content is measured using appropriate methods, such as spectroscopy or chromatography (Ahmad et al. 2018; Chang et al. 2017; Hu et al. 2019; Rodriguez et al. 2019; Wang et al. 2017b, 2018; Zhou et al. 2018). Subsequently, release profiles can be obtained by fitting an appropriate mathematical model, and specific release behavior can also be achieved by predicting formulation parameters (Malekjani & Jafari 2021). By changing the composition in the releasing media, this model can be customized to fit different experimental needs. For example, a phosphate buffer solution (PBS) at pH 7.4 has been used as a releasing media to mimic the physiological environment when studying drug release from nanoparticles (Ahmad et al. 2018). To simulate GI conditions, the use of a buffer with various pH and high ionic strength allows the model to represent gastric and intestinal fluids more accurately (Wang et al. 2018). Furthermore, in the cases of drugs released from physiological stimulus-responsive nanoparticles, PBS with a range of pHs, ionic strengths, and temperatures can be used to determine the releasing profile under different conditions (Ma et al. 2022, Pan et al. 2019).

3.4. Cell Models

In vitro models of the intestinal epithelium are used to study and predict the efficacy and safety of encapsulated bioactive compounds and nanoparticles. Caco-2 cells, which resemble human enterocytes, are widely used as the basis for cell culture models (Behrens et al. 2001). However, enterocytes are not the only cell type present in the intestinal epithelium; secretory cells such as goblet cells, Paneth cells, and M cells are all involved in the transportation and adsorption of extraneous components from the intestine. In the following sections, recently developed in vitro cell models with different complexities and specialties (e.g., mucus-secreting cell models, follicle-associated models, inflamed intestinal models, and 3D cell models) are discussed.

3.4.1. Caco-2 monolayer. As illustrated in **Figure 2*d***, both undifferentiated and differentiated Caco-2 cells are widely used to access the toxicity of encapsulated bioactive compounds and nanoparticles because of their high similarities to the human intestinal epithelium. These cells are originally derived from a colon carcinoma. In cytotoxicity experiments, Caco-2 cells are commonly seeded directly onto a single cell culture well plate at approximately 50,000 cells/cm^2 with a transepithelial electrical resistance (TEER) greater than 250 Ω/cm^3 (Wang et al. 2008). Undifferentiated Caco-2 cells are usually more sensitive to external stimuli. For example, the concentration causing a 50% reduction in cell viability (TC 50) was lower in undifferentiated Caco-2 cells than in differentiated ones after being treated with silver, silica, and zinc nanoparticles, thereby suggesting that the differentiation state influences the cytotoxic response of Caco-2 cells (Böhmert et al. 2014, Gerloff et al. 2013). According to the literature, the oxidative stress–induced cell apoptosis may be suppressed due to the upregulation of extracellular antioxidant selenoprotein P in differentiated Caco-2 cell monolayers, resulting in a relatively higher TC 50 compared with that found in undifferentiated cells (Speckmann et al. 2010).

Differentiated Caco-2 cells displaying a polarized morphology with apical brush borders and tight junctions between adjacent cells and expressing hydrolases (isomaltase, aminopeptidase N, and dipeptidylpeptidase IV) and microvillar transporters (P-glycoprotein) are commonly used to study the adsorption and transportation of nanoparticles throughout the human intestine (Shahbazi & Santos 2013). In the Transwell® model (**Figure 2*d***), differentiated Caco-2 cells are grown on an inserted microporous membrane until the TEER value reaches 250 Ω/cm^3, followed by the addition of test compounds in the upper chamber of the monolayer. In a comparison study, the permeability of various hydrophobic and hydrophilic compounds, as well as compound-loaded nanocarriers, was tested on both differentiated Caco-2 monolayers in vitro and human jejunum in vivo (Lennernäs et al. 1996). The results showed that although the use of Caco-2 monolayers is a good model for predicting the passive transport of hydrophobic compounds in humans, it showed a low permeability for hydrophilic compounds and nanocarriers, which may be caused by the low paracellular permeability associated with the colonic origin of the cell line and under expression of active transporters, leading to saturation of compounds at low concentrations. Although paracellular transport rate is lower in Caco-2 monolayers compared with actual in vivo circumstances, its high-throughput selectivity is still useful (Artursson et al. 2001). Thus, this model is still widely used to evaluate the transport efficiency of nanoparticles and elucidate cellular mechanisms behind the adsorption of various nanoparticles (Chen & Yao 2017, Durán-Lobato et al. 2016, Ke et al. 2015, Zhang et al. 2020).

3.4.2. Mucus-secreting cell model. Even if the intestinal cell monolayer is mainly composed of enterocytes, several other cell types, such as goblet cells involved in mucin and antimicrobial molecule secretion, also play important synergistic roles in intestinal absorption. Furthermore, the drawbacks of using Caco-2 cell layers need to be considered, such as the high TEER, which is

250–500 Ω/cm^3 in Caco-2 cell monolayers but only 12–69 Ω/cm^3 in human intestinal cells (Shahbazi & Santos 2013). To better mimic the small intestine and overcome its limitations, Caco-2 cells and mucin-secreting HT29 cells (a human adenocarcinoma cell) are combined to provide a more accurate model of the human intestine (Béduneau et al. 2014). The presence of HT29 cells, which differentiate into mature goblet cells under the influence of methotrexate (MTX), in the coculture system with Caco-2 cells decreases the tight-junction resistance and subsequently enhances the paracellular permeability and P-glycoprotein activity (Shahbazi & Santos 2013). Therefore, when the percentage of HT29-MTX increases, the expected TEER value decreases. It was shown that for a cell monolayer that contained 25% of cells as HT29-MTX cells, the TEER value was increased to 790 Ω/cm^3 after 23 days of incubation, whereas this value was decreased to 310 Ω/cm^3 for a cell monolayer containing 75% of HT29-MTX cells (Walter et al. 1996). Researchers found that a Caco-2 cell density of 15,000 cells/cm^2, supplemented with 30,000 cells/cm^2 HT29-MTX on day three post-seeding, produced an in vitro model more closely resembling human intestinal epithelium cells than Caco-2 cells alone (Béduneau et al. 2014). In another experiment, HT29 subclones with various depths of mucus layer were generated, ranging from approximately 50 to 142 μm, and these HT29 subclones could be used to imitate different segments in the GI tract (Behrens et al. 2001). Compared to Caco-2 monolayers, the mucus-secreting cell model permits the study of both carrier-mediated and paracellular transport mechanisms, facilitating the investigation of the impact of the mucus layer on the oral delivery systems.

3.4.3. Follicle-associated epithelium model. In the intestine, the lymphoid follicles, such as Peyer's patch, are part of the mucosal immune system and the main site of intestinal antigen sampling, separated from the lumen by the follicle-associated epithelium (FAE), which contains enterocytes, goblet cells, and some M cells (des Rieux et al. 2007). M cells are in direct contact with immune cells in the lamina propria to prevent the occurrence of mucosal inflammation and have a high transcytosis ability, showing a potential portal for oral delivery of bioactive compounds and particulates from the gut lumen to the underlying mucosal immune system (des Rieux et al. 2005, Kucharzik et al. 2000). The formation of M cells in the Transwell® system involves the use of mouse lymphocytes. In this model, Caco-2 cells are first seeded on the basolateral side of the insert, followed by the addition of isolated mouse lymphocytes in the upper chamber facing the basolateral side of Caco-2 cells. The conversion of Caco-2 cells to M cells was evidenced by the transport of *Vibrio cholerae* bacteria, which are uniquely taken up and transported by M cells (Kernéis et al. 1997). Subsequently, to avoid the use of biomaterials for other species, an alternative transformation method was developed that utilized human Burkitt's lymphoma Raji B cells. In this model, Caco-2 cells were seeded on the apical side of the insert, whereas Raji cells were grown at the basolateral side, as shown in **Figure 2*d*** (Gullberg et al. 2000). The conversion of M cells could be monitored by reduced TEER values and characterized by the absence of microvilli, lower number of lysosomes, reduced alkaline phosphatase activity, a reduced glycocalyx, and the presence of microfolds (Kucharzik et al. 2000). More recently, several triple-cultured cell models have been generated to provide more accurate models, such as the combination of Caco-2–HT29-MTX and Raji cells (Schimpel et al. 2014) or Caco-2–Raji cells and J774A.1 cells, which are mouse reticulum sarcoma cells acting as immune cells beneath the M cells (Xie et al. 2016). Researchers have studied insulin transport using Caco-2, Caco-2–HT29-MTX, and Caco-2–HT29-MTX–Raji models. Compared to monolayer or mucus-secreting models, the triple-culture model was shown to be more efficient for insulin transport, as this process was influenced by the presence of transporters on the surface of M cells (Araujo et al. 2016). Lozoya-Agullo and co-workers investigated the permeability of 12 model compounds using monolayer, coculture, and triple-coculture models (Lozoya-Agullo et al. 2017a). The results showed that Caco-2 monoculture was better for studying passive diffusion than were coculture models because the Caco-2 monolayer was a simpler

and less laborious model for the screening stages of nanoparticle development. However, to thoroughly study the transport mechanism of a specific bioactive compound or nanocarriers, coculture and triple-culture models were more suitable because of their higher in vitro–in vivo correlation (Lozoya-Agullo et al. 2017a). In short, triple-culture models are more reliable for conducting permeability tests than are traditional monoculture models.

3.4.4. Inflamed intestinal model. Various in vitro cellular models mimicking certain disease conditions have been developed by exposing Caco-2 cells to proinflammatory cytokines to test the effect of the new formulations on the inflamed intestinal epithelium (**Figure 2*d***). For instance, after 9 hours of exposure to 100 ng/mL of TNF-α, Caco-2 cells exhibited an intestinal condition like that of Crohn's disease (Ranaldi et al. 2013). Furthermore, inflammation induced by LPS or the combination of LPS with other cytokines such as IL-1β and IFN-γ can also represent the intestinal conditions of a diseased GI tract (Beguin et al. 2013, Wang et al. 2015). In addition to cytokine-induced inflamed intestinal models, RAW 246.4 macrophages and activated human neutrophils can be used to induce inflammation in Caco-2 cells (Frontela-Saseta et al. 2013, Nielsen et al. 2017, Vermeer et al. 2004). Because this model needs to resemble the intestinal inflammatory processes as closely as possible and should not show substantial or permanent destruction or disruption of the cell barrier, several criteria were applied to define a good inflamed model (Kämpfer et al. 2017). First, a TEER reduction of 20–25% compared to the Caco-2 monoculture is expected due to the opening of tight junctions caused by the downregulation of several tight-junction proteins and the reduction should persist for at least 24 h. Second, after coculture, the concentration of released cytokines needs to be significantly higher than that observed in the uninflamed cell model. Third, Caco-2 cells must be able to restore the TEER to at least 90% of its original value after 48 h of coculture because the system needs to recover by itself and resolve the inflammation-like process without additional manipulation of the culture. More recently, an inflamed intestinal model that cocultured differentiated THP-1 cells, which are pre-exposed to LPS and IFN-γ, with IFN-γ-primed Caco-2 cells has been developed and its responses to silver nanoparticles (AgNPs) and silver nitrate have been investigated. The results showed that after treatment with nontoxic concentrations of AgNPs and silver nitrate, the death of necrotic and apoptotic cells was detected in both uninflamed and inflamed models, with more pronounced expression in the latter case (Kämpfer et al. 2020). These results demonstrated the importance of considering intestinal health status in the safety and toxicity assessment of nanomaterials.

In addition to traditional inflamed models, several 3D cell models have been developed to better understand interactions between nanoparticles and the intestinal immune system (Ghadimi et al. 2012, Leonard et al. 2010). For instance, human macrophages (differentiated THP-1) and human dendritic cells (MUTZ-3) were embedded in a collagen scaffold and seeded on the apical side of Transwell® inserts (**Figure 2*d***). The Caco-2 cells were then seeded on the top of this layer to form a 3D structure, followed by two days of incubation with 10 ng/mL IL-1β to induce inflammation (Susewind et al. 2016). The TEER value of this 3D model was 20% lower than that of the Caco-2 monolayer, whereas the concentration of released IL-8 was significantly increased. After treatment with gold nanoparticles (AuNPs), the slight increase in IL-8 gene expression implied that a coculture system with macrophages and dendritic cells might exhibit an amplified inflammatory response when in contact with nanoparticles. Meanwhile, a less time-consuming and variable model was established using THP-1 and MUTZ-3 cell lines instead of primary immune cells collected from the blood samples of different patients (Susewind et al. 2016).

3.4.5. Other cell models. Like M cells and goblet cells, intestinal dendritic cells are also found close to Peyer's patches and are responsible for the protective effects of probiotics against

pathogenic bacteria such as *Salmonella typhi* (Bermudez-Brito et al. 2015). An in vitro cell model for studying oral tolerance was successfully established by coculturing Caco-2 and monocyte-derived dendritic cells for 24 h. In this model, Caco-2 cells, dendritic cells (differentiated by IL-4), and granulocyte-macrophage colony-stimulating factor were cocultured in Transwell® inserts for 24 h. The cocultured cells showed decreased sensitivity against bacterial ligands, owing to the increased production of TGFβ, which could downregulate the expression of the receptor of the bacterial ligand (Butler et al. 2006). Furthermore, another study demonstrated that a *Ganoderma atrum* polysaccharide, isolated from food and traditional Chinese herbal medicines, could promote the maturation of dendritic cells in a coculture model and regulate their immune reaction in the intestine, a key factor in modulating intestinal homeostasis and enhancing the body's immunity (Ding et al. 2020).

Mast cells, which regulate the function and integrity of intestinal epithelial cells and modulate the immunity of innate and adaptive mucosa, are another fundamental element of intestinal epithelial barriers (Albert-Bayo et al. 2019). Researchers found that a model obtained by coculturing Caco-2 cells (apical component) and BL-2H3 cells (basolateral component), isolated from a rat basophilic leukemia cell line, was able to mimic the phenotype of the intestinal mast cell, thus providing an in vitro model that could evaluate the antiallergic effects and investigate the allergic mechanisms for bioactive compounds (Yamashita et al. 2016). In short, as technology evolves, more accurate models can be built to suit a variety of needs and conditions.

3.5. Assessment of Transport Pathways

Phagocytosis and pinocytosis are the two main endocytic mechanisms for nanoparticle transport, which are mediated through receptors located on the surfaces of the intestinal epithelial cells. To identify the specific mechanisms behind the transport of nanoparticles across intestinal epithelial cells, colocalization of the fluorescence-labeled nanoparticles with specific endocytosis inhibitors (**Table 3**) is often evaluated by confocal laser scanning microscopy. For instance, researchers have examined the effects of the surface charge of nanoparticles on the internalization pathways using Caco-2 monolayers (Bannunah et al. 2014). The results showed that after being treated with an inhibitor, the clathrin-mediated internalization and transport rates of positively charged

Table 3 Most commonly used chemicals as transport mechanism inhibitors across intestinal cell lines for nanoparticle transport evaluation

Function	Inhibitor	Concentration
Inhibitor of clathrin-mediated endocytosis	Chlorpromazine	30 μM, 62.7 μM
	Phenylarsine oxide	20 μM
Inhibition of caveolae-mediated endocytosis	Nystatin	30 μM
	Filipin (III)	8 μM, 15.3 μM
	MβCD	5 mM, 10 mM
	Genistein	100 μM
Inhibition of pinocytosis	EGTA	20, 50, 100 μM
	Cytochalasin D	0.1 μg/mL, 20 mM
Inhibition of P-gp	Verapamil	100 μM
	Vinblastine	100 μM
Tight-junction opening	EGTA	3 mM

Abbreviations: EGTA, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrasodium salt; MβCD, methyl-β-cyclodextrin; P-gp, P-glycoprotein. Adapted from Beloqui et al. (2016).

nanoparticles (100 nm) were significantly reduced, whereas the caveolae-mediated transport remained unaffected after incubation with genistein (caveolae inhibitor). In contrast, for negatively charged nanoparticles (100 nm), significant reductions in internalization and transport rate were observed in the presence of caveolae inhibitor, whereas the clathrin-mediated internalization remained unaffected. This study showed that the transport mechanisms were appreciably influenced by the surface characteristic of nanoparticles.

Other studies suggest that cell type also plays a role in determining the transport pathways of nanoparticles. Researchers have used different inhibitors of major endocytic pathways to test the transport mechanisms of carboxylated polystyrene nanoparticles (40–200 nm) in different cell lines. The results showed that nanoparticle transport through 1321N1 cells (brain astrocytoma) was significantly affected by chlorpromazine treatment, but a stronger reduction of the cellular uptake by A549 cells (lung carcinoma) was noted after genistein treatment, compared with other cell types (Dos Santos et al. 2011). Thus, different nanoparticles may exploit different transport mechanisms to enter different types of cells.

3.6. Assessment of Toxicity

The small size of nanomaterials means that they may cause toxicity after oral administration. Although standard protocols for testing nanomaterial toxicity are still lacking, Caco-2 cell models (undifferentiated and differentiated) have been considered suitable models for studying the potentially adverse effects of nanocarriers after oral administration. Several toxicological endpoints, such as cell mortality and biochemical changes, can be used to characterize the toxicity of nanocarriers, as it can induce many detrimental effects on cells, such as DNA damage, abnormal protein expression, cellular organelle alterations, chronic inflammation, and apoptosis. The most common in vitro assays that are used to evaluate cellular viability and mortality as well as investigate nanomaterial modes of action such as the promotion of oxidative stress and genotoxicity are summarized in **Table 4** (Ciappellano et al. 2016).

4. DYNAMIC IN VITRO EVALUATION MODELS

Most in vitro digestion models used to evaluate the GI fate of nanoparticles use simple static models, where incubation times, pH values, enzyme activities, mineral compositions, and other factors are kept constant in each GI compartment. To more accurately simulate the dynamic nature of the GI tract, more complex models have been developed such as the dynamic gastric model (DGM), human gastric simulator, and TNO's (Nederlandse Organisatie Voor Toegepast Natuurwetenschappelijk Onderzoek; an organization based in the Netherlands) GI model (TIM).

4.1. Dynamic Gastric Model

To better mimic the different parts of the human stomach, the DGM has two separate sections, the main body and antrum (**Figure 3*a***). The main body simulates the fundic part of the stomach, where the digestive enzymes and HCl are secreted, and serves as a storage reservoir for the food matrix. A cone-shaped elastic membrane is used to ensure that the heat in the water bath is transferred over a short period of time, and the gentle changes in external pressure ensure the production of constant agitation (Thuenemann et al. 2015, Wickham et al. 2012). The lower part of the stomach is simulated by the antrum part of the DGM, which consists of a barrel and a moving piston, which applies shear forces to the food matrix so that the mixing and grinding that occur in the human gastric area can be mimicked (Mercuri et al. 2011, Vardakou et al. 2011). However, the DGM only simulates gastric digestion; thus, the secreted chyme needs to be transferred to

Table 4 Summary of the most common in vitro assays to assess toxicological endpoints

Endpoint	Cellular process	Assays
Cell viability	Metabolic activity	MTT, MTS, XTT, WST-1, Alamar blue®
	ATP content	ATP assay
Cell permeability/tight-junction functionality	Membrane integrity	TEER
	Apparent permeability	TEER, Lucifer yellow, mannitol, dextran
Cell death	Apoptosis	Caspase-3/7 activation, annexin V, FACS, ELISA, immunoblotting
	Necrosis	LDH, trypan blue, neutral red, propidium iodide by FACS, ELISA, and immunoblotting
	Autophagy	Electron microscopy, optical microscopy, immunoblotting, immunoprecipitation, immunofluorescence
Oxidative stress	ROS generation	ROS assay, FACS
	Lipid peroxidation	TBA assay for malondialdehyde, HPLC, spectrofluorimetry, gas chromatography–mass spectrometry, and F_2 isoprostanes
	Protein carbonylation	DNPH assay, immunoblotting, mass spectrometry
	GSH depletion	GSH/GSSG ratio
	Superoxide dismutase activity and expression	Nitro blue tetrazolium, immunoblotting, PCR
Genotoxicity	DNA damage	Comet assay, micronuclei presence, TUNEL assay
Immunogenicity	Alteration of immune system	ELISA or real-time PCR, FACS, microarray, CFU-GM and CFU-E, whole blood cultures, hemolysis test, thrombogenicity assays (activated partial thromboplastic time assay, thrombin generation assay, blood-clotting time assay, calibrated thrombin generation assay), phagocytosis assay, DC maturation

Abbreviations: ATP, adenosine triphosphate; CFU-E, colony forming unit-erythroid; CFU-GM, colony forming unit-granulocyte macrophage progenitor; DC, dendritic cell; DNPH, 2,4-dinitrophenylhydrazine; ELISA, enzyme-linked immunosorbent assay; FACS, flow cytometry; GSH, glutathione; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; LDH, lactate dehydrogenase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PCR, polymerase chain reaction; ROS, reactive oxygen species; TBA, thiobarbituric acid; TEER, transepithelial electrical resistance; TUNEL, transferase dUTP nick end labeling assay; WST, water-soluble tetrazolium salts; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide. Adapted from Ciappellano et al. (2016).

a different intestinal model to evaluate the effects of intestinal digestion (Wickham et al. 2012). The key advantage of the DGM is the simulation of the mechanical effects occurring within the human stomach.

4.2. TNO's Gastrointestinal Model

TNO's GI model (TIM) simulates the upper part of the human GI tract and consists of four sequential compartments from the stomach through to the small intestine: stomach, duodenum, jejunum, and ileum. As shown in **Figure 3*b***, the temperature of each compartment is controlled by a water jacket, whereas peristalsis is simulated by two sections with alternating contractions. Pumps and valves controlled by a computer ensure physiologically relevant transit times and secretion of gastric, duodenal, jejunal, and ileal fluids, containing bile salts, electrolytes, and different digestion enzymes (Dickinson et al. 2012). However, because this model does not mimic absorption steps, such as active transport, efflux, and gut wall metabolism, the bioaccessibility of filtrates obtained from the intestinal compartments can only be evaluated after being transferred to an additional intestinal absorption model, such as a Caco-2 monolayer. Furthermore, the complexity of the TIM model makes it expensive, laborious, and time-consuming to use. Researchers also found that

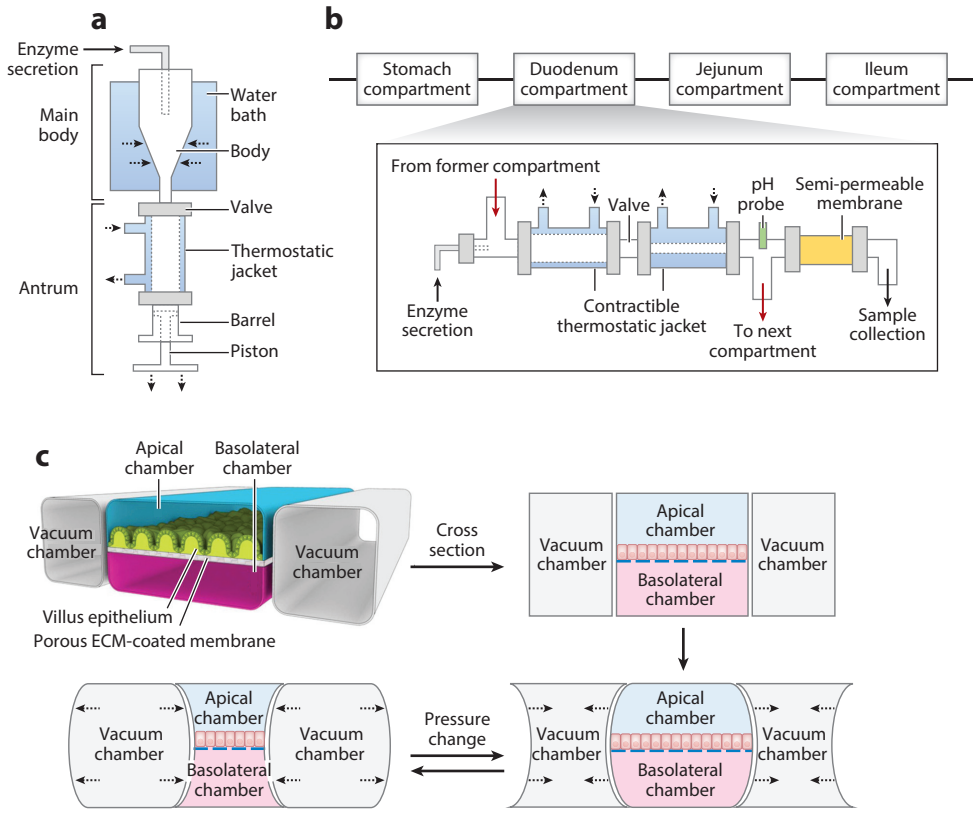

Figure 3

Schematic illustration of the dynamic in vitro evaluation models: (*a*) dynamic gastric model, (*b*) TNO's gastrointestinal model 1, and (*c*) gut-on-chip. 3D model adapted from Villenave et al. (2017), copyright 2017 Public Library of Science. Abbreviation: ECM, extracellular matrix.

lipophilic bioactive compounds can be absorbed on the surface of plastic compartments, resulting in a relatively low recovery rate (Deat et al. 2009). However, this model can still provide accurate predictions for the biological behavior of edible nanoparticles, as earlier studies revealed that a dynamic in vitro model can produce a good correlation to the in vivo data on pH, GI transit, and enzymatic composition (Minekus et al. 1995).

4.3. Organs-on-a-Chip Model

Recently, microfabrication devices capable of manipulating small amounts of liquids and controlling fluid flows have been used to replicate key processes within the GI tract. These microdevices such as gut-on-chip (GOC) combine a microfluidic technique with a 3D cell model to better reproduce the intestinal microenvironment, chemical gradients, and tissue–tissue interfaces (Ahadian et al. 2020, Giampetruzzi et al. 2018, Sontheimer-Phelps et al. 2019). As illustrated in **Figure 3*c***, a GOC device has two independent chambers, apical and basolateral, separated by a microporous membrane where small intestinal model cells are cultured. The fluidic platform is equipped with electrodes for TEER monitoring during the cell culture experiment, and the flow rate and fluid ingredients in each chamber, such as electrolytes, nutrients, and gases, are controlled by a computer through separate micropumps (Kimura et al. 2008). The addition of vacuum

chambers on both sides of the microchannel can provide intestinal peristalsis by circulating strain under computer control (Kim et al. 2012). Owing to their relatively low cost, high speed, and small sample requirements, these microfluidic devices can be used in a high-throughput screening mode for the evaluation of bioactive compounds and nanoparticles. Incubation of polystyrene nanoparticles within a Caco-2–HT29-MTX GOC device showed that less than 10% of nanoparticles crossed the cell membrane after 24 h and the nanoparticles on the apical side were mostly clustered together (Esch et al. 2014). Researchers have treated a Caco-2 GOC device with a virus and found a gradient of cytopathic effects that are consistent with the direction of flow, which cannot be revealed by traditional cell models (Villenave et al. 2017). GOC models also allow for the investigation of the interactions between intestinal epithelium cells and the microbiota normally present inside the intestinal lumen. *Lactobacillus rhamnosus* isolated from the human gut has been cultured on top of a Caco-2 monolayer under intestinal peristalsis-type motion and flow for at least 1 week without reducing cell viability (Kim et al. 2012). The same research group further investigated the influence of intestinal mechanical movement on the composition of Caco-2 cells and found that when cultured in a mechanically active microenvironment that mimicked the living intestine, Caco-2 cells could form all types of intestinal cells, including absorptive, mucus-secretory, enteroendocrine, and Paneth cells, and produce biochemical activities (Kim & Ingber 2013). Furthermore, they also found that bacterial overgrowth, similar to that observed in patients with ileus and inflammatory bowel disease, could be triggered by the termination of peristalsis-like motions, providing an in vitro disease model for drug and nanoparticle development (Kim et al. 2016). These models have also been used to investigate the interactions between different organs (Ahn et al. 2018), such as skin (Sriram et al. 2018), liver (Kang et al. 2015), lymph node (Rosa et al. 2016), spleen (Rigat-Brugarolas et al. 2014), bone marrow (Chou et al. 2020, Sieber et al. 2018, Torisawa et al. 2016), and even tumors (Ekert et al. 2014, Sung et al. 2014). The accurate reproduction of the in vivo environments controlled by the microfluidics parameters via computerized devices helps to reduce the costs and time spent by researchers and may eventually replace animal testing.

5. EX VIVO EVALUATION MODELS

5.1. Ussing Chamber

The Ussing chamber (**Figure 4*a***) was first designed by Hans Henriksen Ussing to study water excretion through toad skin in the middle of the twentieth century. It has since been used for the study of transport properties, permeability, and physiology of intestinal epithelial tissues.

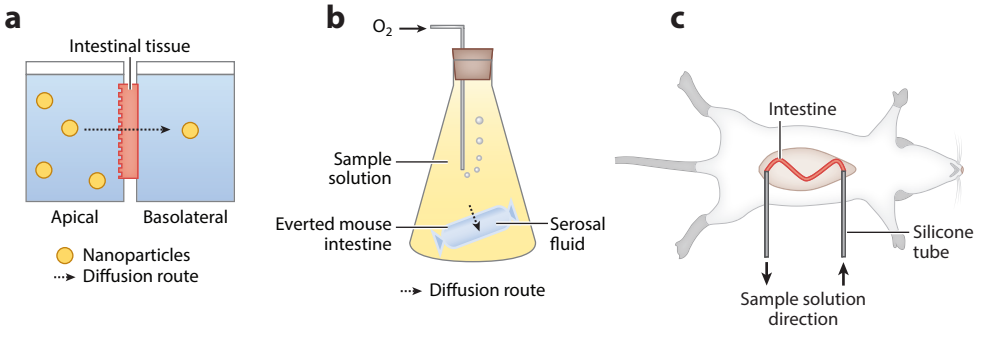

Figure 4

Schematic illustration of ex vitro and in situ evaluation models: (*a*) Ussing chamber model, (*b*) everted intestinal sac, and (*c*) single-pass perfusion model.

The chamber is set up to isolate the apical and basolateral sides. Through electrodes, the voltage and short-circuit current can be measured to determine the permeability and transportation of bioactive molecules or nanoparticles across human intestinal tissue from the colon or ileum, often donated by cancer patients. Nanoparticles are added to the chamber on the apical (mucosal) side of the tissue, and their passage through the epithelium to the basolateral (serosal) chamber is monitored. The transportation experiments are conducted under well-defined conditions, and the duration of experiments should be less than 30–60 min to avoid decreased tissue viability and integrity (Lundquist & Artursson 2016).

5.2. Everted Intestinal Sac

The everted intestinal sac is another ex vivo model to measure the intestinal permeability, transportation, and cellular uptake of different nanoparticles such as lipid-based nanoparticles (Masiiwa & Gadaga 2018, Yang et al. 2017), polysaccharide-based nanoparticles (Zare et al. 2018), and protein-based nanoparticles (Madan et al. 2020). As shown in **Figure 4*b***, this method involves isolating a small segment of the intestine of laboratory animals such as rats, frogs, or chickens. The everted intestinal segment is gently washed and tied up after being filled with drug-free buffer solution. The entire segment is then immersed in a flask filled with a large volume of oxygenated buffer solution containing nanoparticles. At predetermined time intervals, the segment is removed and the concentration of nanoparticles in the serosal fluid is determined by the appropriate analytical method such as spectroscopy or chromatography (Bothiraja et al. 2016).

6. IN SITU EVALUATION MODELS

Two in situ models are commonly used to investigate the permeability of bioactive compounds or nanoparticles: the single-pass perfusion model (Fairstein et al. 2013, Zur et al. 2014) and the closed-loop intestine model (Lozoya-Agullo et al. 2015). As shown in **Figure 4*c***, in a typical single-pass perfusion model, rats are anesthetized and placed on a controlled temperature heating pad to avoid hypothermia, after which a 3-cm incision is made in the midline of the abdomen. The desired intestinal segments such as the jejunum or colon are carefully exposed and cannulated on two ends with silicone tubing, and then rinsed with blank perfusion to remove food components and feces. The test solutions are subsequently perfused through the intestinal segment at a flow rate of 0.2 mL/min for 1 h to ensure a steady state is reached. Samples are then withdrawn in predetermined time intervals for approximately 2 h and then immediately analyzed by an appropriate analytical method, such as chromatography (Beig et al. 2012).

In a closed-loop intestine model, rats are anesthetized and placed on a heated surface. After incision, an isolated compartment in the intestine is created from the beginning to the end of desired intestinal segments with the help of two syringes and two three-way stopcock valves. After being flushed with saline, the intestinal segment is carefully placed back into the peritoneal cavity and the abdomen is covered with a cotton wool pad to avoid peritoneal liquid evaporation and heat losses. An aliquot of test solution (e.g., 5 mL) is perfused inside the intestinal segment, and a small portion of the sample (e.g., 100 μL) is periodically collected at predetermined time intervals (Lozoya-Agullo et al. 2015).

In a comprehensive study comparing the colonic permeability of 14 different compounds using in vitro, in situ, and ex vivo models, researchers found that the data from the in situ model exhibited a good correlation with Caco-2 monolayer studies and the data from human fraction of dose absorbed (F_{abs}) studies in the literature (Lozoya-Agullo et al. 2017b). Nevertheless, the results from the Ussing chamber model showed a weak correlation to the in situ results, suggesting that more in-depth mechanistic studies should be done to better understand the intestinal permeability and absorption of the Ussing chamber approach.

7. IN VIVO EVALUATION MODELS

Regardless of the complexity of in vitro or ex vivo models, in vivo evaluation is ultimately required to validate the biological performance of bioactive delivery systems and nanoparticles, particularly the release kinetics and biodistribution of the nanoparticles under in vivo physiological conditions.

7.1. Pharmacokinetic Studies

In pharmacokinetic (PK) studies, blood samples are withdrawn at predetermined time intervals after oral administration of the test bioactive substances or nanoparticles. Whole blood and serum can be used to perform analytical assays (such as chromatography) to determine the concentration of the test substances after extraction. Peak concentration (C_{max}) and time (t_{max}) are obtained directly from the plasma profile. The area under curve (AUC) of the concentration–time profile can be determined. The relative bioavailability (RB) is then calculated using the following equation (Guan et al. 2019):

$$\mathrm{RB}(\%) = \frac{(\mathrm{AUC}_{\mathrm{sample}})/(\mathrm{Dosage}_{\mathrm{sample}})}{(\mathrm{AUC}_{\mathrm{control}})/(\mathrm{Dosage}_{\mathrm{control}})} \times 100\%.$$

As an example, free astaxanthin (AST) and AST-loaded chitosan nanoparticles were fed to mice, and the PK results showed that the plasma AST concentration of the AST-loaded nanoparticles group rapidly reached a peak after 4.12 h (t_{max}), which was significantly faster than that of the free AST group (6.24 h) (Guan et al. 2019). Moreover, compared to the free AST group, the bioavailability of the AST-loaded nanoparticles group was 207%, which demonstrated that the bioavailability of a single dose of 2.4 mg/kg AST could be increased around twofold by encapsulating it within the nanoparticles.

7.2. Biodistribution Analysis

The determination of the biodistribution of bioactive agents and nanoparticles after oral administration is important to assess their efficacy and safety. As summarized in **Table 5**, numerous technologies have been developed to track the fate of nanoparticles in vivo (Arms et al. 2018). For some inorganic nanoparticles such as AgNPs or AuNPs, the biodistribution after oral administration can be visualized directly using electron microscopy. For instance, electron microscopy was used to observe the accumulation of AgNPs in duodenal and liver sections of male mice exposed to 300 mg/kg of AgNPs per day for 3 days (Narciso et al. 2020). The results showed that the AgNPs were aggregated inside membrane-bound structures or dispersed in the cytosol along microvilli (**Figure 5*a–d***). For some organic nanoparticles, in vivo bioluminescence imaging can be used with different labeling systems to visualize the movement and location of nanoparticles inside the body of an animal (Chen et al. 2010). The in vivo imaging system (IVIS) is an optical imaging technique that uses fluorescence to obtain a high signal-to-noise ratio due to the low bioluminescence of mammalian tissues. For applications of oral delivery systems, the nanoparticles are labeled with fluorescent markers such as sulforhodamine B or Cy5.5. By imaging the animal at different time points, the particle motion can be tracked. To investigate the biodistribution of nanoparticles after absorption, organs can be removed from the animal and individual organs can be further imaged ex vivo. Researchers have synthesized bioactive compound–lipid conjugates with different lipid chain lengths and formulated them into solid lipid nanoparticles (SLNs) (Ma et al. 2017). After being labeled with a hydrophobic quenching dye, the ingested SLNs were tracked by IVIS and the results showed that fluorescence signals were found throughout the entire GI tract and liver after oral administration of the SLNs. The SLNs prepared with shorter lipid chain lengths (6 carbons) were more quickly lipolyzed and absorbed along the intestine-to-circulation path,

Table 5 Comparison of various techniques for biodistribution analysis

Technique	Equipment	Advantages	Disadvantages
Histology	Light and fluorescence microscopy	A relatively low-cost qualitative method for large tissue sections	Time-consuming, low-resolution, laborious technique; susceptible to human error; not suitable for most organic NPs
Electron microscopy	TEM, SEM, STEM	A semiquantitative method that could visualize NPs in cells and cellular organelles at high resolution	Time-consuming, relatively high cost; not suitable for large tissue sections or NPs prepared with soft materials
Liquid scintillation counting	Photomultiplier tube Isotopic markers: [^{3}H]-CHE and ^{99m}Tc	A sensitive, specific, and quantitative method that can determine NPs at the organ level	Time-consuming, laborious technique; cannot provide information at cellular level; susceptible to sample composition and preparation
Measurement of drug concentration	HPLC and/or MS	A secondary quantitative measurement for whole or partial tissue samples without the need of radiation or imaging molecules	Susceptible to sample preparation; provides unreliable results; cannot provide real-time biodistribution across time points
IVIS	IVIS spectrum Fluorophores: fluorescent proteins and dyes	A highly sensitive and qualitative measurement that is simple to conduct without ionizing radiation and can perform images in a real-time pattern at the tissue level	Low spatial resolution compared to CT and MRI; susceptible to sample preparation, composition, and tissue autofluorescence; cannot provide information at cellular level or images for individual NPs
CT	CT scanner Contrast agents: iodine- or barium-based compounds	A low time-consuming qualitative method that can perform high-resolution images in a real-time pattern at the tissue level	Involves ionizing radiation and contrast imaging agents that may influence the properties and in vivo behavior of nanoparticles; cannot provide information at cellular level
MRI	MRI spectrometer Contrast agents: superparamagnetic iron oxide crystals and lanthanide metals	A direct, noninvasive technique that can perform high-resolution images in a real-time pattern at the tissue level without ionizing radiation; not limited by tissue depth	Relatively high cost and time-consuming; needs higher amount of contrast agents; cannot be used in subjects with metallic device
Nuclear medicine imaging	Single photo emission CT scanner or positron emission tomography scanner	A quantitative measurement with no restriction of depth of tissue; requires small amounts of radiolabels and able to image biochemical processes	Relatively high cost, time-consuming, and requires ionizing radiation; cannot be used for longitudinal studies because of radiolabel decay; often combined with MRI or CT

Abbreviations: CT, computed tomography; HPLC, high-performance liquid chromatography; IVIS, in vivo imaging system; MRI, magnetic resonance imaging; MS, mass spectrometry; NPs, nanoparticles; SEM, scanning electron microscopy; STEM, scanning transmission electron microscopy; TEM, transmission electron microscopy.

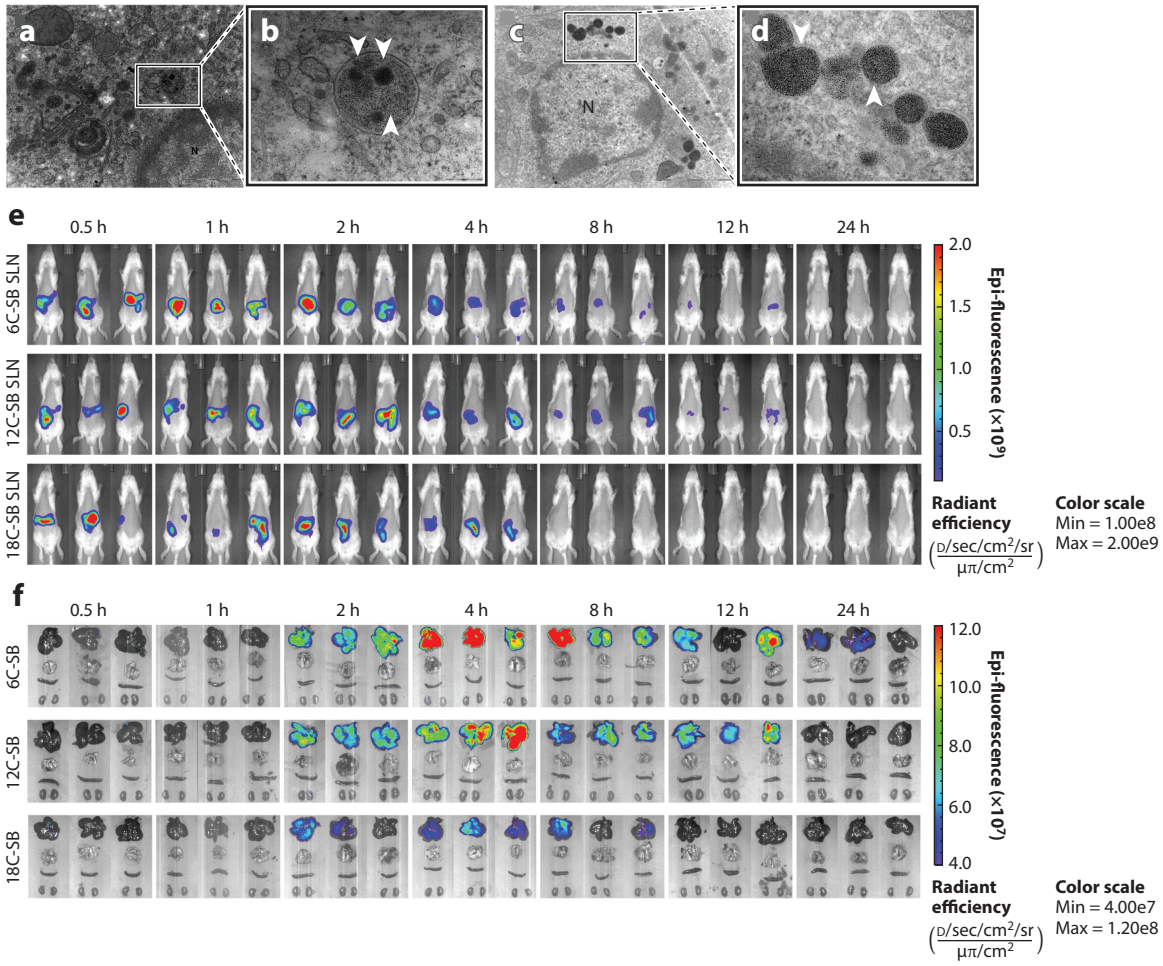

Figure 5

(*a*) Transmission electron microscope (TEM) image of liver of female mice displaying nanoparticles within a well-defined membrane-bound structure (*white arrows* in panels *b* and *d*). (*b*) Enlargement of squared area in panel *a*. (*c*) TEM images of duodenum of female mice displaying nanoparticles accumulating around the nucleus. (*d*) Enlargement of squared area in panel *c* in which tight accumulations of nanoparticles are delimited by membranes. Panels *a–d* have been adapted with permission from Narciso et al. (2020), copyright 2020 Elsevier. (*e*) In vivo live images of the digestion of solid lipid nanoparticles (SLNs) in rat after oral gavage. (*f*) Ex vivo images of biodistribution in organs and tissues. Panels *e* and *f* have been adapted with permission from Ma et al. (2017), copyright 2017 Elsevier.

compared to the other SLNs (**Figure 5*e*,*f***). In conclusion, by choosing appropriate techniques, the biodistribution of different kinds of nanoparticles can be determined and the effects and interactions between nanoparticles and the animal body can be investigated. This information can then be used to optimize product formulations.

7.3. Toxicity Analysis

The toxicity of nanoparticles is mainly affected by the exposure conditions, durations, and dosages. The in vivo toxicity assessment for nanoparticles is normally performed in animal models such as mice, rats, and zebrafish. Both acute and chronic oral toxicity studies should be done to obtain a

comprehensive toxicity profile of nanoparticles before clinical trials. In acute studies, a single dose of a high amount of nanoparticles is administered to the test animals, followed by monitoring of their mortality, general well-being, behavior patterns (such as food consumption and water intake), and sensory function tests (such as tail pinch, approach, and touch response, pupillary reflex, and acoustic startle response) at predetermined time periods (Narciso et al. 2020). In a chronic oral toxicity study, a low dose of nanoparticles is applied to the testing animals repeatedly for a predetermined period while monitoring mortality, body weight, and general clinical signs (Almansour et al. 2018). On the last day of the experiment, the animals are sacrificed and their organs are removed, fixed, and stained for histology analysis, which is used to evaluate physiological changes by examining changes in the structure and composition of the cells and organs (Delie 1998). Furthermore, hematological analysis (such as leukocytes, lymphocytes, monocytes, and granulocytes) and serum biochemical analysis (such as albumin, alkaline phosphatase, glucose, De Ritis coefficient, and total protein) can be conducted on blood samples collected at predetermined intervals (Hendrickson et al. 2016). For instance, rats were intragastrically administered with 250 and 2,000 mg/kg of body weight of AgNPs (~12 nm size) over 30 days and once, respectively (Hendrickson et al. 2016). The highest accumulation of AgNPs was observed in the liver and kidneys in acute and subacute toxicity experiments, respectively. There were no visible pathomorphological abnormalities of other internal organs or notable deviations in behavior or locomotor activity in either group. The concentration of silver detected in tissues was far smaller than the administered dosage, suggesting that most of the AgNPs were excreted from the organism through the feces and urine, resulting in no distinct toxicity under the experimental conditions used. However, more analyses such as urinalysis and fecal analysis should be conducted to confirm this.

8. MICROBIOTA-BASED EVALUATION MODELS

8.1. Nanoparticles and the Gut Microbiota

Both organic and inorganic nanoparticles may pass through the upper GI tract and reach the colon, where they can interact with the gut microbiota. Some of the most common food ingredients used to fabricate organic nanoparticles include casein, whey protein, zein, soy protein, albumin, chitosan, alginate, pectin, phytoglycogen, triacylglycerols, diacylglycerols, and phospholipids (Luo et al. 2020, McClements & Xiao 2017). The physiological effects of organic nanoparticles on the gut microbiota have been shown to exhibit health benefits for both the microbiota and the host by shifting the nutrient balance (Portune et al. 2016). Generally, food-grade organic nanoparticles can be fully digested within the upper GI tract by host-secreted enzymes, such as lipases, amylases, and proteases, and there are few reports in the literature of safety concerns with these kinds of nanomaterials on the diversity or richness of intestinal microbiota (Liu et al. 2019). Notably, the use of ingredients that are nondigestible in the GI tract but are metabolizable by the gut microbiota provides a new aspect of site-specific delivery systems. Ribeiro and colleagues (2014) prepared pectin-coated chitosan nanobeads for the controlled release of 5-aminosalicylic acid, a nonsteroidal anti-inflammatory drug, as a treatment for colon disease. Controlled release was achieved because pectin is not digested by enzymes in the upper GI tract but can be digested by pectinases secreted by the gut microbiota in the lower GI tract. The results showed that approximately 35% of the encapsulated bioactive compound was released after a 4-h incubation in simulated intestinal fluids. After the nanobeads were transferred into simulated colonic fluid, another 45% of the encapsulated ingredient was released within 2 h. These findings may lead to the development of innovative formulations for the treatment of intestinal diseases.

Inorganic nanoparticles may reach the colon and disturb the composition of the microbiota because of their antibacterial activities. For instance, only a very small fraction of AgNPs

remained in tissues and organs (<1%) after oral administration, suggesting that most of them were excreted in the feces, leading to potential toxicity against gut microbiota (Echegoyen & Nerín 2013). Sprague-Dawley rats (both male and female) were fed with discrete sizes of AgNPs (10, 75, and 110 nm) twice daily for 13 weeks, and it was demonstrated that the 10-nm particle size had the greatest bacterial toxicity and had a pronounced impact on host gut gene expression. 16S rRNA sequencing analyses revealed that exposure to 10-nm AgNPs caused a reduction in the Firmicutes phylum and *Lactobacillus* genus in the ileum (Williams et al. 2015). Citrate, D-glucose, sodium dodecyl sulfate, polyvinylpyrrolidone (PVP), and polysaccharides have been used to stabilize AgNPs. El Badawy et al. (2011) and Li et al. (2013) compared the antimicrobial activity of citrate-, PVP-, polyethyleneimine-, and chitosan-coated AgNPs in vitro and found that positively charged polyethyleneimine- and chitosan-coated AgNPs showed considerably stronger antimicrobial properties against *E. coli*, which were caused by an increased absorption ability of the negatively charged membrane proteins on microbiota. In contrast, other researchers found that iron nanoparticles could enhance the growth and proliferation of many microbiota, as iron is an element essential for their survival. Maghemite with sizes ranging from 2 to 540 nm in diameter was incubated with gut bacteria (*Pseudomonas aeruginosa*) in vitro. All nanoparticles increased the growth of bacteria, compared to the control group, but maghemite with the smallest particle size (2 nm) showed the greatest enhancement (Borcherding et al. 2014). The complexity of the gut microbiota means it is difficult to develop accurate models and analysis methods to study the interactions between gut bacteria and nanoparticles.

8.2. Evaluation Models

Traditionally, in vitro evaluation of nanoparticles on the gut microbiota is conducted by coincubation of nanoparticles and certain bacteria in growth media (Borcherding et al. 2014). However, because of the complexity of the GI tract, these models do not provide comprehensive information on the interactions between the host microbiota and nanoparticles. Therefore, an in vitro simulator of the human intestinal microbial ecosystem (SHIME) model has been developed for this purpose. The SHIME model consists of at least a five-step multichamber bioreactor that mimics the progressive digestive processes of the stomach, small intestine, and colon (**Figure 2*a***). This model has been widely used in the nutritional and pharmaceutical fields to determine the bioaccessibility, efficacy, and digestion behavior of food ingredients and bioactive compounds (Molly et al. 1993, Van de Wiele et al. 2015). Recently, a microbiota-on-a-chip model, which enables the gut microbiome community and host intestinal epithelial cells to be mimicked, was developed by incorporating Caco-2 cells and certain types of bacteria as described above in Section 2 (Kim et al. 2016). Until now, the ex vivo approaches have not been extensively reported in gut microbiome studies because the stability of the microbiota community structure during experimental anaerobic cultivation is uncertain and difficult to control. The in vivo models, conversely, are more controllable by using standardized diet and housing conditions (Wu et al. 2020). For instance, mice have been orally administered increasing dosages of AgNPs for 28 days, and the gut microbiota integrity and composition were investigated using 16S rRNA sequencing (van den Brûle et al. 2015). Although there were no overall toxicity or body weight changes after oral administration of AgNPs at different dosages, dose-dependent disturbances of both bacterial homogeneity and population were revealed. At the family level, Lachnospiraceae and the S24-7 family mainly accounted for the increase in Firmicutes and decrease in Bacteroidetes, respectively. A major limitation of in vivo microbiome studies is that the compositions of the murine and human microbiome communities differ considerably. Humanized mouse models have been developed by introducing a human gut microbiome community into germ-free mice (Collins et al. 2015). However, because

the human-derived microbiota species was not evolutionarily adapted to the mouse gut environment or genetic determinants, certain host responses such as immune responses in mice were not appropriately developed and the results were not representative for clinical studies (Zhou et al. 2019). Overall, the development of in vitro, ex vivo, and in vivo models that can study the interactions among nanoparticles, gut microbiota, and host is still in its early phases and more in-depth mechanistic studies are needed to address this knowledge gap.

9. CONCLUSIONS

The growing interest in nutritional applications of nanotechnologies opens new prospects in oral delivery systems. However, the oral route presents many obstacles to the delivery of encapsulated bioactive compounds, such as harsh pH conditions, various enzymatic activities, low mucus permeation, and low transportation rate. Until now, various materials have been tested to prepare nanocarriers that may overcome these physiological obstacles, thus improving the bioavailability of encapsulated cargos, extending retention times in the GI tract, and enhancing the transportation to the systemic circulation. The interactions of nanoparticles with the gut microbiota are complex and still poorly understood. Organic nanoparticles prepared with food-derived biomaterials may serve as nutrients for the gut microbiota and thus exhibit low toxicity against them. However, inorganic nanoparticles may alter the composition of the microbiota due to their antibacterial activities. Analyses of the biological fate and safety of newly developed nanomaterials should be performed prior to clinical trials. This review covers common models used for this purpose, including in vitro simulated GI fluid models, in vitro cell models, ex vivo models, in situ models, and in vivo animal models. Although the models that investigate the direct impact of nanoparticles on cells or animals are well-developed and suitable to evaluate the biological fate of most types of nanoparticles, models considering the interactions between the gut microbiota and the host system are still lacking.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

Abuznait AH, Qosa H, O'Connell ND, Akbarian-Tefaghi J, Sylvester PW, et al. 2011. Induction of expression and functional activity of P-glycoprotein efflux transporter by bioactive plant natural products. *Food Chem. Toxicol.* 49(11):2765–72

Ahadian S, Finbloom JA, Mofidfar M, Diltemiz SE, Nasrollahi F, et al. 2020. Micro and nanoscale technologies in oral drug delivery. *Adv. Drug Deliv. Rev.* 157:37–62

Ahmad N, Alam MA, Ahmad R, Umar S, Jalees Ahmad F. 2018. Improvement of oral efficacy of irinotecan through biodegradable polymeric nanoparticles through in vitro and in vivo investigations. *J. Microencapsul.* 35(4):327–43

Ahn J, Ko J, Lee S, Yu J, Kim Y, Jeon NL. 2018. Microfluidics in nanoparticle drug delivery; from synthesis to pre-clinical screening. *Adv. Drug Deliv. Rev.* 128:29–53

Al Rubeaan K, Rafiullah M, Jayavanth S. 2016. Oral insulin delivery systems using chitosan-based formulation: a review. *Expert Opin. Drug Deliv.* 13(2):223–37

Albert-Bayo M, Paracuellos I, González-Castro AM, Rodríguez-Urrutia A, Rodríguez-Lagunas MJ, et al. 2019. Intestinal mucosal mast cells: key modulators of barrier function and homeostasis. *Cells* 8(2):135

Almansour M, Alarifi S, Jarrar B. 2018. In vivo investigation on the chronic hepatotoxicity induced by intraperitoneal administration of 10-nm silicon dioxide nanoparticles. *Int. J. Nanomed.* 13:2685–96

Anby MU, Nguyen TH, Yeap YY, Feeney OM, Williams HD, et al. 2014. An in vitro digestion test that reflects rat intestinal conditions to probe the importance of formulation digestion versus first pass metabolism in danazol bioavailability from lipid based formulations. *Mol. Pharm.* 11(11):4069–83

Araujo F, Pereira C, Costa J, Barrias C, Granja PL, Sarmento B. 2016. In vitro M-like cells genesis through a tissue-engineered triple-culture intestinal model. *J. Biomed. Mater. Res. B* 104(4):782–88

Arms L, Smith DW, Flynn J, Palmer W, Martin A, et al. 2018. Advantages and limitations of current techniques for analyzing the biodistribution of nanoparticles. *Front. Pharmacol.* 9:802

Arranz E, Corredig M, Guri A. 2016. Designing food delivery systems: challenges related to the in vitro methods employed to determine the fate of bioactives in the gut. *Food Funct.* 7(8):3319–36

Arshad R, Gulshad L, Haq IU, Farooq MA, Al-Farga A, Siddique R, et al. 2021. Nanotechnology: a novel tool to enhance the bioavailability of micronutrients. *Food Sci. Nutr.* 9(6):3354–61

Artursson P, Palm K, Luthman K. 2001. Caco-2 monolayers in experimental and theoretical predictions of drug transport. *Adv. Drug Deliv. Rev.* 46(1–3):27–43

Bannunah AM, Vllasaliu D, Lord J, Stolnik S. 2014. Mechanisms of nanoparticle internalization and transport across an intestinal epithelial cell model: effect of size and surface charge. *Mol. Pharm.* 11(12):4363–73

Bao C, Jiang P, Chai J, Jiang Y, Li D, et al. 2019. The delivery of sensitive food bioactive ingredients: absorption mechanisms, influencing factors, encapsulation techniques and evaluation models. *Food Res. Int.* 120:130–40

Béduneau A, Tempesta C, Fimbel S, Pellequer Y, Jannin V, et al. 2014. A tunable Caco-2/HT29-MTX co-culture model mimicking variable permeabilities of the human intestine obtained by an original seeding procedure. *Eur. J. Pharm. Biopharm.* 87(2):290–98

Beguin P, Errachid A, Larondelle Y, Schneider YJ. 2013. Effect of polyunsaturated fatty acids on tight junctions in a model of the human intestinal epithelium under normal and inflammatory conditions. *Food Funct.* 4(6):923–31

Behrens I, Stenberg P, Artursson P, Kissel T. 2001. Transport of lipophilic drug molecules in a new mucus-secreting cell culture model based on HT29-MTX cells. *Pharm. Res.* 18(8):1138–45

Beig A, Miller JM, Dahan A. 2012. Accounting for the solubility–permeability interplay in oral formulation development for poor water solubility drugs: the effect of PEG-400 on carbamazepine absorption. *Eur. J. Pharm. Biopharm.* 81(2):386–91

Beloqui A, des Rieux A, Préat V. 2016. Mechanisms of transport of polymeric and lipidic nanoparticles across the intestinal barrier. *Adv. Drug Deliv. Rev.* 106:242–55

Bergin IL, Witzmann FA. 2013. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. *Int. J. Biomed. Nanosci. Nanotechnol.* 3(1–2):163–210

Bermudez-Brito M, Muñoz-Quezada S, Gómez-Llorente C, Matencio E, Romero F, Gil A. 2015. *Lactobacillus paracasei* CNCM I-4034 and its culture supernatant modulate *Salmonella*-induced inflammation in a novel transwell co-culture of human intestinal-like dendritic and Caco-2 cells. *BMC Microbiol.* 15(1):79

Berthelsen R, Klitgaard M, Rades T, Müllertz A. 2019. In vitro digestion models to evaluate lipid based drug delivery systems; present status and current trends. *Adv. Drug Deliv. Rev.* 142:35–49

Blanco E, Shen H, Ferrari M. 2015. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat. Biotechnol.* 33(9):941–51

Boegh M, Baldursdóttir SG, Müllertz A, Nielsen HM. 2014. Property profiling of biosimilar mucus in a novel mucus-containing in vitro model for assessment of intestinal drug absorption. *Eur. J. Pharm. Biopharm.* 87(2):227–35

Böhmert L, Girod M, Hansen U, Maul R, Knappe P, et al. 2014. Analytically monitored digestion of silver nanoparticles and their toxicity on human intestinal cells. *Nanotoxicology* 8(6):631–42

Borcherding J, Baltrusaitis J, Chen H, Stebounova L, Wu CM, et al. 2014. Iron oxide nanoparticles induce *Pseudomonas aeruginosa* growth, induce biofilm formation, and inhibit antimicrobial peptide function. *Environ. Sci. Nano* 1(2):123–32

Borel T, Sabliov C. 2014. Nanodelivery of bioactive components for food applications: types of delivery systems, properties, and their effect on ADME profiles and toxicity of nanoparticles. *Annu. Rev. Food Sci. Technol.* 5:197–213

Bothiraja C, Pawar A, Deshpande G. 2016. Ex-vivo absorption study of a nanoparticle based novel drug delivery system of vitamin D_3 (Arachitol Nano^{TM}) using everted intestinal sac technique. *J. Pharm. Investig.* 46(5):425–32

Burton PS, Conradi RA, Hilgers AR. 1991. (B) Mechanisms of peptide and protein absorption: (2) transcellular mechanism of peptide and protein absorption: passive aspects. *Adv. Drug Deliv. Rev.* 7(3):365–85

Butler M, Ng CY, van Heel DA, Lombardi G, Lechler R, et al. 2006. Modulation of dendritic cell phenotype and function in an in vitro model of the intestinal epithelium. *Eur. J. Immunol.* 36(4):864–74

Cao P, Xu ZP, Li L. 2022. Tailoring functional nanoparticles for oral vaccine delivery: recent advances and future perspectives. *Compos. B Eng.* 236:109826

Carrière F. 2016. Impact of gastrointestinal lipolysis on oral lipid-based formulations and bioavailability of lipophilic drugs. *Biochimie* 125:297–305

Cattani VB, Fiel LA, Jäger A, Jäger E, Colomé LM, et al. 2010. Lipid-core nanocapsules restrained the indomethacin ethyl ester hydrolysis in the gastrointestinal lumen and wall acting as mucoadhesive reservoirs. *Eur. J. Pharm. Sci.* 39(1–3):116–24

Chang C, Wang T, Hu Q, Zhou M, Xue J, Luo Y. 2017. Pectin coating improves physicochemical properties of caseinate/zein nanoparticles as oral delivery vehicles for curcumin. *Food Hydrocoll.* 70:143–51

Chen CC, Tsai TH, Huang ZR, Fang JY. 2010. Effects of lipophilic emulsifiers on the oral administration of lovastatin from nanostructured lipid carriers: physicochemical characterization and pharmacokinetics. *Eur. J. Pharm. Biopharm.* 74(3):474–82

Chen H, Yao Y. 2017. Phytoglycogen to increase lutein solubility and its permeation through Caco-2 monolayer. *Food Res. Int.* 97:258–64

Chen Y, Xue J, Wang T, Hu Q, Luo Y. 2020. Carboxymethylation of phytoglycogen and its interactions with caseinate for the preparation of nanocomplex. *Food Hydrocoll.* 100:105390

Chivere VT, Kondiah PP, Choonara YE, Pillay V. 2020. Nanotechnology-based biopolymeric oral delivery platforms for advanced cancer treatment. *Cancers* 12(2):522

Chou DB, Frismantas V, Milton Y, David R, Pop-Damkov P, et al. 2020. On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology. *Nat. Biomed. Eng.* 4(4):394–406

Christophersen PC, Christiansen ML, Holm R, Kristensen J, Jacobsen J, et al. 2014. Fed and fasted state gastro-intestinal in vitro lipolysis: in vitro in vivo relations of a conventional tablet, a SNEDDS and a solidified SNEDDS. *Eur. J. Pharm. Sci.* 57:232–39

Ciappellano SG, Tedesco E, Venturini M, Benetti F. 2016. In vitro toxicity assessment of oral nanocarriers. *Adv. Drug Deliv. Rev.* 106:381–401

Collins J, Auchtung JM, Schaefer L, Eaton KA, Britton RA. 2015. Humanized microbiota mice as a model of recurrent *Clostridium difficile* disease. *Microbiome* 3(1):35

Cone RA. 2009. Barrier properties of mucus. *Adv. Drug Deliv. Rev.* 61(2):75–85

Dahan A, Hoffman A. 2007. The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats. *Eur. J. Pharm. Biopharm.* 67(1):96–105

Dahiya DK, Renuka PM, Shandilya UK, Dhewa T, et al. 2017. Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: a review. *Front. Microbiol.* 8:563

Dawson M, Krauland E, Wirtz D, Hanes J. 2004. Transport of polymeric nanoparticle gene carriers in gastric mucus. *Biotechnol. Prog.* 20(3):851–57

Dawson M, Wirtz D, Hanes J. 2003. Enhanced viscoelasticity of human cystic fibrotic sputum correlates with increasing microheterogeneity in particle transport. *J. Biol. Chem.* 278(50):50393–401

de Sousa IP, Cattoz B, Wilcox MD, Griffiths PC, Dalgliesh R, et al. 2015. Nanoparticles decorated with proteolytic enzymes, a promising strategy to overcome the mucus barrier. *Eur. J. Pharm. Biopharm.* 97:257–64

Deat E, Blanquet-Diot S, Jarrige JF, Denis S, Beyssac E, Alric M. 2009. Combining the dynamic TNO-gastrointestinal tract system with a Caco-2 cell culture model: application to the assessment of lycopene and α-tocopherol bioavailability from a whole food. *J. Agric. Food Chem.* 57(23):11314–20

Delie F. 1998. Evaluation of nano- and microparticle uptake by the gastrointestinal tract. *Adv. Drug Deliv. Rev.* 34(2–3):221–33

des Rieux A, Fievez V, Théate I, Mast J, Préat V, Schneider YJ. 2007. An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle transport by M cells. *Eur. J. Pharm. Sci.* 30(5):380–91

des Rieux A, Ragnarsson EG, Gullberg E, Préat V, Schneider Y-J, Artursson P. 2005. Transport of nanoparticles across an in vitro model of the human intestinal follicle associated epithelium. *Eur. J. Pharm. Sci.* 25(4–5):455–65

Dickinson PA, Rmaileh RA, Ashworth L, Barker RA, Burke WM, et al. 2012. An investigation into the utility of a multi-compartmental, dynamic, system of the upper gastrointestinal tract to support formulation development and establish bioequivalence of poorly soluble drugs. *AAPS J.* 14(2):196–205

Ding X, Yu Q, Hou K, Hu X, Wang Y, et al. 2020. Indirectly stimulation of DCs by Ganoderma atrum polysaccharide in intestinal-like Caco-2/DCs co-culture model based on RNA-seq. *J. Funct. Foods* 67:103850

Dos Santos T, Varela J, Lynch I, Salvati A, Dawson KA. 2011. Effects of transport inhibitors on the cellular uptake of carboxylated polystyrene nanoparticles in different cell lines. *PLOS ONE* 6(9):e24438

Dünnhaupt S, Kammona O, Waldner C, Kiparissides C, Bernkop-Schnürch A. 2015. Nano-carrier systems: strategies to overcome the mucus gel barrier. *Eur. J. Pharm. Biopharm.* 96:447–53

Durán-Lobato M, Martín-Banderas L, Lopes R, Gonçalves L, Fernández-Arévalo M, Almeida A. 2016. Lipid nanoparticles as an emerging platform for cannabinoid delivery: physicochemical optimization and biocompatibility. *Drug Dev. Ind. Pharm.* 42(2):190–98

Echegoyen Y, Nerín C. 2013. Nanoparticle release from nano-silver antimicrobial food containers. *Food Chem. Toxicol.* 62:16–22

Ekert JE, Johnson K, Strake B, Pardinas J, Jarantow S, et al. 2014. Three-dimensional lung tumor microenvironment modulates therapeutic compound responsiveness in vitro: implication for drug development. *PLOS ONE* 9(3):e92248

El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM. 2011. Surface charge-dependent toxicity of silver nanoparticles. *Environ. Sci. Technol.* 45(1):283–87

Esch MB, Mahler GJ, Stokol T, Shuler ML. 2014. Body-on-a-chip simulation with gastrointestinal tract and liver tissues suggests that ingested nanoparticles have the potential to cause liver injury. *Lab Chip* 14(16):3081–92

Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. 2013. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine* 9(1):1–14

Fairstein M, Swissa R, Dahan A. 2013. Regional-dependent intestinal permeability and BCS classification: elucidation of pH-related complexity in rats using pseudoephedrine. *AAPS J.* 15(2):589–97

Fatouros DG, Deen GR, Arleth L, Bergenstahl B, Nielsen FS, et al. 2007. Structural development of self nano emulsifying drug delivery systems (SNEDDS) during in vitro lipid digestion monitored by small-angle X-ray scattering. *Pharm. Res.* 24(10):1844–53

Fredrikzon B, Olivecrona T. 1978. Decrease of lipase and esterase activities in intestinal contents of newborn infants during test meals. *Pediatr. Res.* 12(5):631–34

Frontela-Saseta C, López-Nicolás R, González-Bermúdez CA, Martínez-Graciá C, Ros-Berruezo G. 2013. Anti-inflammatory properties of fruit juices enriched with pine bark extract in an in vitro model of inflamed human intestinal epithelium: the effect of gastrointestinal digestion. *Food Chem. Toxicol.* 53:94–99

Gerloff K, Pereira DI, Faria N, Boots AW, Kolling J, et al. 2013. Influence of simulated gastrointestinal conditions on particle-induced cytotoxicity and interleukin-8 regulation in differentiated and undifferentiated Caco-2 cells. *Nanotoxicology* 7(4):353–66

Ghadimi D, Helwig U, Schrezenmeir J, Heller KJ, de Vrese M. 2012. Epigenetic imprinting by commensal probiotics inhibits the IL-23/IL-17 axis in an in vitro model of the intestinal mucosal immune system. *J. Leukoc. Biol.* 92(4):895–911

Giampetruzzi L, Barca A, De Pascali C, Capone S, Verri T, et al. 2018. Human organ-on-a-chip: around the intestine bends. In *Convegno Nazionale Sensori*, pp. 181–88. Cham, Switz.: Springer

Goldberg M, Gomez-Orellana I. 2003. Challenges for the oral delivery of macromolecules. *Nat. Rev. Drug Discov.* 2(4):289–95

Guan L, Liu J, Yu H, Tian H, Wu G, et al. 2019. Water-dispersible astaxanthin-rich nanopowder: preparation, oral safety and antioxidant activity in vivo. *Food Funct.* 10(3):1386–97

Guerra A, Etienne-Mesmin L, Livrelli V, Denis S, Blanquet-Diot S, Alric M. 2012. Relevance and challenges in modeling human gastric and small intestinal digestion. *Trends Biotechnol.* 30(11):591–600

Guimarães M, Statelova M, Holm R, Reppas C, Symilllides M, et al. 2019. Biopharmaceutical considerations in paediatrics with a view to the evaluation of orally administered drug products—a PEARRL review. *J. Pharm. Pharmacol.* 71(4):603–42

Gullberg E, Keita ÅV, Sa'ad YS, Andersson M, Caldwell KD, et al. 2006. Identification of cell adhesion molecules in the human follicle-associated epithelium that improve nanoparticle uptake into the Peyer's patches. *J. Pharmacol. Exp. Ther.* 319(2):632–39

Gullberg E, Leonard M, Karlsson J, Hopkins AM, Brayden D, et al. 2000. Expression of specific markers and particle transport in a new human intestinal M-cell model. *Biochem. Biophys. Res. Commun.* 279(3):808–13

Hendrickson OD, Klochkov SG, Novikova OV, Bravova IM, Shevtsova EF, et al. 2016. Toxicity of nanosilver in intragastric studies: biodistribution and metabolic effects. *Toxicol. Lett.* 241:184–92

Hu Q, Bae M, Fleming E, Lee JY, Luo Y. 2019. Biocompatible polymeric nanoparticles with exceptional gastrointestinal stability as oral delivery vehicles for lipophilic bioactives. *Food Hydrocoll.* 89:386–95

Hu Q, Hu S, Fleming E, Lee J-Y, Luo Y. 2020. Chitosan-caseinate-dextran ternary complex nanoparticles for potential oral delivery of astaxanthin with significantly improved bioactivity. *Int. J. Biol. Macromol.* 151:747–56

Hu Q, Lu Y, Luo Y. 2021. Recent advances in dextran-based drug delivery systems: from fabrication strategies to applications. *Carbohydr. Polym.* 264:117999

Hutchinson M, Mallatt J, Marieb EN, Wilhelm PB. 2007. *A Brief Atlas of the Human Body*. London: Pearson

Jørgensen SDS, Al Sawaf M, Graeser K, Mu H, Müllertz A, Rades T. 2018. The ability of two in vitro lipolysis models reflecting the human and rat gastro-intestinal conditions to predict the in vivo performance of SNEDDS dosing regimens. *Eur. J. Pharm. Biopharm.* 124:116–24

Kämpfer AA, Urbán P, Gioria S, Kanase N, Stone V, Kinsner-Ovaskainen A. 2017. Development of an in vitro co-culture model to mimic the human intestine in healthy and diseased state. *Toxicol. Vitro* 45:31–43

Kämpfer AA, Urbán P, La Spina R, Jiménez IO, Kanase N, et al. 2020. Ongoing inflammation enhances the toxicity of engineered nanomaterials: application of an in vitro co-culture model of the healthy and inflamed intestine. *Toxicol. Vitro* 63:104738

Kamstrup D, Berthelsen R, Sassene PJ, Selen A, Müllertz A. 2017. In vitro model simulating gastro-intestinal digestion in the pediatric population (neonates and young infants). *AAPS PharmSciTech* 18(2):317–29

Kang YB, Sodunke TR, Lamontagne J, Cirillo J, Rajiv C, et al. 2015. Liver sinusoid on a chip: long-term layered co-culture of primary rat hepatocytes and endothelial cells in microfluidic platforms. *Biotechnol. Bioeng.* 112(12):2571–82

Kaukonen AM, Boyd BJ, Charman WN, Porter CJ. 2004. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm. Res.* 21(2):254–60

Ke Z, Guo H, Zhu X, Jin Y, Huang Y. 2015. Efficient peroral delivery of insulin via vitamin B12 modified trimethyl chitosan nanoparticles. *J. Pharm. Pharm. Sci.* 18(2):155–70

Keiper A. 2003. The nanotechnology revolution. *New Atlantis* 2:17–34

Kernéis S, Bogdanova A, Kraehenbuhl JP, Pringault E. 1997. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* 277(5328):949–52

Kim HJ, Huh D, Hamilton G, Ingber DE. 2012. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. *Lab Chip* 12(12):2165–74

Kim HJ, Ingber DE. 2013. Gut-on-a-chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integr. Biol.* 5(9):1130–40

Kim HJ, Li H, Collins JJ, Ingber DE. 2016. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. *PNAS* 113(1):E7–E15

Kimura H, Yamamoto T, Sakai H, Sakai Y, Fujii T. 2008. An integrated microfluidic system for long-term perfusion culture and on-line monitoring of intestinal tissue models. *Lab Chip* 8(5):741–46

Klitgaard M, Sassene PJ, Selen A, Müllertz A, Berthelsen R. 2017. Studying furosemide solubilization using an in vitro model simulating gastrointestinal digestion and drug solubilization in neonates and young infants. *Eur. J. Pharm. Sci.* 109:191–99

Kucharzik T, Lügering N, Rautenberg K, Lügering A, Schmidt M, et al. 2000. Role of M cells in intestinal barrier function. *Ann. N. Y. Acad. Sci.* 915(1):171–83

Lamichhane S, Yde CC, Forssten S, Ouwehand AC, Saarinen M, et al. 2014. Impact of dietary polydextrose fiber on the human gut metabolome. *J. Agric. Food Chem.* 62(40):9944–51

Langguth P, Bohner V, Heizmann J, Merkle H, Wolffram S, et al. 1997. The challenge of proteolytic enzymes in intestinal peptide delivery. *J. Control. Release* 46(1–2):39–57

Le Feunteun S, Al-Razaz A, Dekker M, George E, Laroche B, van Aken G. 2021. Physiologically based modeling of food digestion and intestinal microbiota: state of the art and future challenges. An INFOGEST review. *Annu. Rev. Food Sci. Technol.* 12:149–67

Lennernäs H, Palm K, Fagerholm U, Artursson P. 1996. Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in vivo. *Int. J. Pharm.* 127(1):103–7

Leonard F, Collnot EM, Lehr CM. 2010. A three-dimensional coculture of enterocytes, monocytes and dendritic cells to model inflamed intestinal mucosa in vitro. *Mol. Pharm.* 7(6):2103–19

Li C, Fu R, Yu C, Li Z, Guan H, et al. 2013. Silver nanoparticle/chitosan oligosaccharide/poly (vinyl alcohol) nanofibers as wound dressings: a preclinical study. *Int. J. Nanomed.* 8:4131–45

Liu J, Leng P, Liu Y. 2021. Oral drug delivery with nanoparticles into the gastrointestinal mucosa. *Fundam. Clin. Pharmacol.* 35(1):86–96

Liu X, Zhang B, Sohal IS, Bello D, Chen H. 2019. Is "nano safe to eat or not"? A review of the state-of-the art in soft engineered nanoparticle (sENP) formulation and delivery in foods. *Adv. Food Nutr. Res.* 88:299–335

Lowrence RC, Subramaniapillai SG, Ulaganathan V, Nagarajan S. 2019. Tackling drug resistance with efflux pump inhibitors: from bacteria to cancerous cells. *Crit. Rev. Microbiol.* 45(3):334–53

Lozoya-Agullo I, Araújo F, González-Álvarez I, Merino-Sanjuań M, González-Álvarez M, et al. 2017a. Usefulness of Caco-2/HT29-MTX and Caco-2/HT29-MTX/Raji B coculture models to predict intestinal and colonic permeability compared to Caco-2 monoculture. *Mol. Pharm.* 14(4):1264–70

Lozoya-Agullo I, González-Álvarez I, González-Álvarez M, Merino-Sanjuán M, Bermejo M. 2015. In situ perfusion model in rat colon for drug absorption studies: comparison with small intestine and Caco-2 cell model. *J. Pharm. Sci.* 104(9):3136–45

Lozoya-Agullo I, Zur M, Fine-Shamir N, Markovic M, Cohen Y, et al. 2017b. Investigating drug absorption from the colon: single-pass versus Doluisio approaches to in-situ rat large-intestinal perfusion. *Int. J. Pharm.* 527(1–2):135–41

Lundquist P, Artursson P. 2016. Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations and studies in human tissues. *Adv. Drug Deliv. Rev.* 106:256–76

Luo Y. 2020. Food colloids binary and ternary nanocomplexes: innovations and discoveries. *Colloids Surf. B* 196:111309

Luo Y, Wang Q, Zhang Y. 2020. Biopolymer-based nanotechnology approaches to deliver bioactive compounds for food applications: a perspective on the past, present, and future. *J. Agric. Food Chem.* 68(46):12993–3000

Ma Y, Adibnia V, Mitrache M, Halimi I, Walker GC, Kumacheva E. 2022. Stimulus-responsive nanoconjugates derived from phytoglycogen nanoparticles. *Biomacromolecules* 23(5):1928–37

Ma Y, He H, Xia F, Li Y, Lu Y, et al. 2017. In vivo fate of lipid-silybin conjugate nanoparticles: implications on enhanced oral bioavailability. *Nanomedicine* 13(8):2643–54

Madan JR, Ansari IN, Dua K, Awasthi R. 2020. Formulation and in vitro evaluation of casein nanoparticles as carrier for celecoxib. *Adv. Pharm. Bull.* 10(3):408–17

Malekjani N, Jafari SM. 2021. Modeling the release of food bioactive ingredients from carriers/nanocarriers by the empirical, semiempirical, and mechanistic models. *Compr. Rev. Food Sci. Food Saf.* 20(1):3–47

Masiiwa WL, Gadaga LL. 2018. Intestinal permeability of artesunate-loaded solid lipid nanoparticles using the everted gut method. *J. Drug Deliv.* 2018:3021738

McClements DJ. 2013. Edible lipid nanoparticles: digestion, absorption, and potential toxicity. *Prog. Lipid Res.* 52(4):409–23

McClements DJ, Xiao H. 2017. Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. *NPJ Sci. Food* 1:6

Mercuri A, Passalacqua A, Wickham MS, Faulks RM, Craig DQ, Barker SA. 2011. The effect of composition and gastric conditions on the self-emulsification process of ibuprofen-loaded self-emulsifying drug delivery systems: a microscopic and dynamic gastric model study. *Pharm. Res.* 28(7):1540–51

Minekus M, Marteau P, Havenaar R. 1995. Multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. *Altern. Lab. Anim.* 23(2):197–209

Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. 2021. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* 20(2):101–24

Molly K, Woestyne MV, Verstraete W. 1993. Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. *Appl. Microbiol. Biotechnol.* 39(2):254–58

Monda V, Villano I, Messina A, Valenzano A, Esposito T, et al. 2017. Exercise modifies the gut microbiota with positive health effects. *Oxidative Med. Cell. Longev.* 2017:3831972

Mosgaard MD, Sassene P, Mu H, Rades T, Müllertz A. 2015. Development of a high-throughput in vitro intestinal lipolysis model for rapid screening of lipid-based drug delivery systems. *Eur. J. Pharm. Biopharm.* 94:493–500

Narciso L, Coppola L, Lori G, Andreoli C, Zjino A, et al. 2020. Genotoxicity, biodistribution and toxic effects of silver nanoparticles after in vivo acute oral administration. *NanoImpact* 18:100221

Ndlovu N, Mayaya T, Muitire C, Munyengwa N. 2020. Nanotechnology applications in crop production and food systems. *Int. J. Plant Breed. Crop Sci.* 7(1):624–34

Nielsen DSG, Fredborg M, Andersen V, Purup S. 2017. Administration of protein kinase D1 induces a protective effect on lipopolysaccharide-induced intestinal inflammation in a co-culture model of intestinal epithelial Caco-2 cells and RAW264.7 macrophage cells. *Int. J. Inflamm.* 2017:9273640

Olbrich C, Müller R. 1999. Enzymatic degradation of SLN-effect of surfactant and surfactant mixtures. *Int. J. Pharm.* 180(1):31–39

Pan J, Lei S, Chang L, Wan D. 2019. Smart pH-responsive nanoparticles in a model tumor microenvironment for enhanced cellular uptake. *J. Mater. Sci.* 54(2):1692–702

Pawar VK, Meher JG, Singh Y, Chaurasia M, Reddy BS, Chourasia MK. 2014. Targeting of gastrointestinal tract for amended delivery of protein/peptide therapeutics: strategies and industrial perspectives. *J. Control. Release* 196:168–83

Porter CJ, Kaukonen AM, Taillardat-Bertschinger A, Boyd BJ, O'Connor JM, et al. 2004. Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. *J. Pharm. Sci.* 93(5):1110–21

Portune KJ, Beaumont M, Davila AM, Tomé D, Blachier F, Sanz Y. 2016. Gut microbiota role in dietary protein metabolism and health-related outcomes: the two sides of the coin. *Trends Food Sci. Technol.* 57:213–32

Pugazhendhi A, Edison TNJI, Karuppusamy I, Kathirvel B. 2018. Inorganic nanoparticles: a potential cancer therapy for human welfare. *Int. J. Pharm.* 539(1–2):104–11

Ranaldi G, Ferruzza S, Canali R, Leoni G, Zalewski PD, et al. 2013. Intracellular zinc is required for intestinal cell survival signals triggered by the inflammatory cytokine TNFα. *J. Nutr. Biochem.* 24(6):967–76

Ribeiro LN, Alcântara AC, Darder M, Aranda P, Araújo-Moreira FM, Ruiz-Hitzky E. 2014. Pectin-coated chitosan–LDH bionanocomposite beads as potential systems for colon-targeted drug delivery. *Int. J. Pharm.* 463(1):1–9

Rigat-Brugarolas L, Elizalde-Torrent A, Bernabeu M, De Niz M, Martin-Jaular L, et al. 2014. A functional microengineered model of the human splenon-on-a-chip. *Lab Chip* 14(10):1715–24

Rodriguez NJ, Hu Q, Luo Y. 2019. Oxidized dextran as a macromolecular crosslinker stabilizes the zein/caseinate nanocomplex for the potential oral delivery of curcumin. *Molecules* 24(22):4061

Rosa PM, Gopalakrishnan N, Ibrahim H, Haug M, Halaas Ø. 2016. The intercell dynamics of T cells and dendritic cells in a lymph node-on-a-chip flow device. *Lab Chip* 16(19):3728–40

Schimpel C, Teubl B, Absenger M, Meindl C, Fröhlich E, et al. 2014. Development of an advanced intestinal in vitro triple culture permeability model to study transport of nanoparticles. *Mol. Pharm.* 11(3):808–18

Sender R, Fuchs S, Milo R. 2016. Revised estimates for the number of human and bacteria cells in the body. *PLOS Biol.* 14(8):e1002533

Shahbazi M-A, Santos HA. 2013. Improving oral absorption via drug-loaded nanocarriers: absorption mechanisms, intestinal models and rational fabrication. *Curr. Drug Metab.* 14(1):28–56

Sieber S, Wirth L, Cavak N, Koenigsmark M, Marx U, et al. 2018. Bone marrow-on-a-chip: long-term culture of human haematopoietic stem cells in a three-dimensional microfluidic environment. *J. Tissue Eng. Regen. Med.* 12(2):479–89

Singh V, San Yeoh B, Chassaing B, Xiao X, Saha P, et al. 2018. Dysregulated microbial fermentation of soluble fiber induces cholestatic liver cancer. *Cell* 175(3):679–94.e622

Sjögren E, Abrahamsson B, Augustijns P, Becker D, Bolger MB, et al. 2014. In vivo methods for drug absorption–comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *Eur. J. Pharm. Sci.* 57:99–151

Sontheimer-Phelps A, Hassell BA, Ingber DE. 2019. Modelling cancer in microfluidic human organs-on-chips. *Nat. Rev. Cancer* 19(2):65–81

Speckmann B, Pinto A, Winter M, Förster I, Sies H, Steinbrenner H. 2010. Proinflammatory cytokines down-regulate intestinal selenoprotein P biosynthesis via NOS2 induction. *Free Radic. Biol. Med.* 49(5):777–85

Sriram G, Alberti M, Dancik Y, Wu B, Wu R, et al. 2018. Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. *Mater. Today* 21(4):326–40

Sung JH, Srinivasan B, Esch MB, McLamb WT, Bernabini C, et al. 2014. Using physiologically-based pharmacokinetic-guided "body-on-a-chip" systems to predict mammalian response to drug and chemical exposure. *Exp. Biol. Med.* 239(9):1225–39

Susewind J, de Souza Carvalho-Wodarz C, Repnik U, Collnot E-M, Schneider-Daum N, et al. 2016. A 3D co-culture of three human cell lines to model the inflamed intestinal mucosa for safety testing of nanomaterials. *Nanotoxicology* 10(1):53–62

Swaan PW. 1998. Recent advances in intestinal macromolecular drug delivery via receptor-mediated transport pathways. *Pharm. Res.* 15(6):826–34

Thuenemann EC, Mandalari G, Rich GT, Faulks RM. 2015. Dynamic gastric model (DGM). In *The Impact of Food Bioactives on Health*, ed. K Verhoeckx, P Cotter, I López-Expósito, C Kleiveland, T Lea, et al., pp. 47–59. Cham: Springer

Torisawa YS, Mammoto T, Jiang E, Jiang A, Mammoto A, et al. 2016. Modeling hematopoiesis and responses to radiation countermeasures in a bone marrow-on-a-chip. *Tissue Eng. Part C* 22(5):509–15

Tyrer P, Foxwell AR, Cripps AW, Apicella MA, Kyd JM. 2006. Microbial pattern recognition receptors mediate M-cell uptake of a gram-negative bacterium. *Infect. Immun.* 74(1):625–31

Van de Wiele T, Van den Abbeele P, Ossieur W, Possemiers S, Marzorati M. 2015. The simulator of the human intestinal microbial ecosystem (SHIME®). In *The Impact of Food Bioactives on Health*, ed. K Verhoeckx, P Cotter, I López-Expósito, C Kleiveland, T Lea, et al., pp. 305–17. Cham: Springer

van den Brûle S, Ambroise J, Lecloux H, Levard C, Soulas R, et al. 2015. Dietary silver nanoparticles can disturb the gut microbiota in mice. *Part. Fibre Toxicol.* 13(1):38

Vardakou M, Mercuri A, Barker SA, Craig DQ, Faulks RM, Wickham MS. 2011. Achieving antral grinding forces in biorelevant in vitro models: comparing the USP dissolution apparatus II and the dynamic gastric model with human in vivo data. *AAPS PharmSciTech* 12(2):620–26

Veneranda M, Hu Q, Wang T, Luo Y, Castro K, Madariaga JM. 2018. Formation and characterization of zein-caseinate-pectin complex nanoparticles for encapsulation of eugenol. *LWT* 89:596–603

Vermeer IT, Henderson LY, Moonen EJ, Engels LG, Dallinga JW, et al. 2004. Neutrophil-mediated formation of carcinogenic N-nitroso compounds in an in vitro model for intestinal inflammation. *Toxicol. Lett.* 154(3):175–82

Villenave R, Wales SQ, Hamkins-Indik T, Papafragkou E, Weaver JC, et al. 2017. Human gut-on-a-chip supports polarized infection of coxsackie B1 virus in vitro. *PLOS ONE* 12(2):e0169412

Walter E, Janich S, Roessler BJ, Hilfinger JM, Amidon GL. 1996. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: in vitro-in vivo correlation with permeability data from rats and humans. *J. Pharm. Sci.* 85(10):1070–76

Wang A, Yang T, Fan W, Yang Y, Zhu Q, et al. 2019. Protein corona liposomes achieve efficient oral insulin delivery by overcoming mucus and epithelial barriers. *Adv. Healthc. Mater.* 8(12):1801123

Wang Q, Strab R, Kardos P, Ferguson C, Li J, et al. 2008. Application and limitation of inhibitors in drug–transporter interactions studies. *Int. J. Pharm.* 356(1–2):12–18

Wang T, Bae M, Lee JY, Luo Y. 2018. Solid lipid-polymer hybrid nanoparticles prepared with natural biomaterials: a new platform for oral delivery of lipophilic bioactives. *Food Hydrocoll.* 84:581–92

Wang T, Luo Y. 2019. Biological fate of ingested lipid-based nanoparticles: current understanding and future directions. *Nanoscale* 11(23):11048–63

Wang T, Luo Y. 2021. Fabrication strategies and supramolecular interactions of polymer-lipid complex nanoparticles as oral delivery systems. *Nano Res.* 14(12):4487–501

Wang T, Xue J, Hu Q, Zhou M, Chang C, Luo Y. 2017a. Synthetic surfactant- and cross-linker-free preparation of highly stable lipid-polymer hybrid nanoparticles as potential oral delivery vehicles. *Sci. Rep.* 7(1):2750

Wang T, Xue J, Hu Q, Zhou M, Luo Y. 2017b. Preparation of lipid nanoparticles with high loading capacity and exceptional gastrointestinal stability for potential oral delivery applications. *J. Colloid Interface Sci.* 507:119–30

Wang W, Xia T, Yu X. 2015. Wogonin suppresses inflammatory response and maintains intestinal barrier function via TLR4-MyD88-TAK1-mediated NF-κB pathway in vitro. *Inflamm. Res.* 64(6):423–31

Wickham M, Faulks R, Mann J, Mandalari G. 2012. The design, operation, and application of a dynamic gastric model. *Dissolution Technol.* 19(3):15–22

Williams K, Milner J, Boudreau MD, Gokulan K, Cerniglia CE, Khare S. 2015. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats. *Nanotoxicology* 9(3):279–89

Wu Q, Chen T, El-Nezami H, Savidge TC. 2020. Food ingredients in human health: ecological and metabolic perspectives implicating gut microbiota function. *Trends Food Sci. Technol.* 100:103–17

Wusigale, Liang L, Luo Y. 2020. Casein and pectin: structures, interactions, and applications. *Trends Food Sci. Technol.* 97:391–403

Xie Y, Hu X, He H, Xia F, Ma Y, et al. 2016. Tracking translocation of glucan microparticles targeting M cells: implications for oral drug delivery. *J. Mater. Chem. B* 4(17):2864–73

Xue J, Wang T, Hu Q, Zhou M, Luo Y. 2018. Insight into natural biopolymer-emulsified solid lipid nanoparticles for encapsulation of curcumin: effect of loading methods. *Food Hydrocoll.* 79:110–16

Yamashita S, Yokoyama Y, Hashimoto T, Mizuno M. 2016. A novel in vitro co-culture model comprised of Caco-2/RBL-2H3 cells to evaluate anti-allergic effects of food factors through the intestine. *J. Immunol. Methods* 435:1–6

Yang H, Yi X, Li L, Ding B. 2017. Estimation of the iron absorption from ferrous glycinate-loaded solid lipid nanoparticles by rat everted intestinal sac model. *Food Sci. Technol. Res.* 23(4):567–73

Yang K, Zhang S, He J, Nie Z. 2021. Polymers and inorganic nanoparticles: a winning combination towards assembled nanostructures for cancer imaging and therapy. *Nano Today* 36:101046

Yu M, Yang Y, Zhu C, Guo S, Gan Y. 2016. Advances in the transepithelial transport of nanoparticles. *Drug Discov. Today* 21(7):1155–61

Zare M, Samani SM, Sobhani Z. 2018. Enhanced intestinal permeation of doxorubicin using chitosan nanoparticles. *Adv. Pharm. Bull.* 8(3):411–17

Zhang Q, Yang H, Sahito B, Li X, Peng L, et al. 2020. Nanostructured lipid carriers with exceptional gastrointestinal stability and inhibition of P-gp efflux for improved oral delivery of tilmicosin. *Colloids Surfaces B* 187:110649

Zhou H, McClements DJ. 2022. Recent advances in the gastrointestinal fate of organic and inorganic nanoparticles in foods. *Nanomaterials* 12(7):1099

Zhou M, Hu Q, Wang T, Xue J, Luo Y. 2016a. Effects of different polysaccharides on the formation of egg yolk LDL complex nanogels for nutrient delivery. *Carbohydr. Polym.* 153:336–44

Zhou M, Hu Q, Wang T, Xue J, Luo Y. 2018. Alginate hydrogel beads as a carrier of low density lipoprotein/pectin nanogels for potential oral delivery applications. *Int. J. Biol. Macromol.* 120:859–64

Zhou M, Wang T, Hu Q, Luo Y. 2016b. Low density lipoprotein/pectin complex nanogels as potential oral delivery vehicles for curcumin. *Food Hydrocoll.* 57:20–29

Zhou W, Chow K-H, Fleming E, Oh J. 2019. Selective colonization ability of human fecal microbes in different mouse gut environments. *ISME J.* 13(3):805–23

Zur M, Hanson AS, Dahan A. 2014. The complexity of intestinal permeability: assigning the correct BCS classification through careful data interpretation. *Eur. J. Pharm. Sci.* 61:11–17