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Nanotechnology systems for oral drug delivery: challenges and opportunities

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Outline:

Introduction

It is nowadays a widespread and ascertained knowledge that materials in the nanometer range of size have different physical, chemical and biological properties than materials in the large scale. This unique feature of nanoparticulate structures has been widely investigated for the potential application of nanotechnology systems in the medical field. Among others, one of the most exciting and promising applications is the use of nanotechnology for drug delivery: nanotechnology systems can improve drug potency and efficacy of therapeutics administered through different routes of administration [1]. This Chapter will focus on the use of nanotechnology systems for the delivery of therapeutics through the oral route.

The oral route is the most commonly used and most-preferred route of drug administration: oral dosage forms are easy to take, enable improved patient compliance and can be cheaper than other types of dosage forms. More than 70% of all medicines are administered orally and are either solid dosage forms (i.e. tablets and capsules) or liquid dosage forms (i.e. solutions and suspensions). However, orally administered drugs, compared to those given via the parenteral route are not directly available in the systemic circulation to exert their therapeutic effect. Instead, they must first transit through the gastro-intestinal (GI) tract and be absorbed before they reach the blood. Hence, the oral route for the delivery of drugs provides at best a delayed onset of action compared to the parenteral route or at worst it can be completely precluded for those drugs that cannot reach the blood [2,3]. The chemical, physical and biological interactions of the drug with the physiological components of the GI tract determine whether or not and to which extent the therapeutic can reach intact the blood circulation and elicit a systemic effect.

In this Chapter, the extremely heterogeneous features of the GI tract and their influence on the bioavailability of orally administered drugs will be discussed. The first part of the Chapter will elucidate the potential opportunities and limitations of the oral route for the delivery of different types of therapeutics. The second and core part of this Chapter will discuss the potential of nanotechnology to overcome the limitations of the oral route and improve the delivery of many drugs orally. Here, the unique physiological interactions of nanoscale materials with the different components of the gastrointestinal (GI) tract will be considered, emphasizing how this could be of aid for the effective delivery of drugs orally. The overall aim of the Chapter is to describe how different nanotechnologies systems can be employed to improve and expand the possibilities for effective oral drug delivery.

Physiological barriers to the oral delivery of therapeutics

When it comes to therapeutics, a big distinction must be done between small molecule therapeutics and biopharmaceuticals. Small molecule drugs are those with low molecular weight (e.g. < 800 Da) produced by chemical synthesis or present as naturally occurring compounds [4]. On the other hand, biopharmaceuticals, also called biologics or biologicals, are therapeutics where the active is derived form a biological (non-plant) source. Biopharmaceuticals are also often referred to as large molecule drugs, as most of them -with the exception of small peptides- have a large molecular weight (> 5000 Da). Since the 1990s, biopharmaceuticals have seen a tremendous growth [4,5]: today this category of products constitutes circa 15% of the medicine market and this percentage could double by 2025 [6]. Biopharmaceuticals include proteins (e.g. monoclonal antibodies), peptides (e.g. insulin), nucleic acids and vaccine therapeutics [5].

Small molecules and biological therapeutics are remarkably dissimilar: the difference between a typical small molecule drug, such as aspirin (21 atoms), and a biopharmaceutical, such as an antibody (~25,000 atoms) can be compared to the difference in weight between a bicycle (~20 lbs) and a business jet (~30,000 lbs) [7]. Small molecule therapeutics and biopharmaceuticals differ also in their physicochemical characteristics. As a consequence, it is easy to imagine that their chemical, physical and biological interactions with the physiological components of the body would be also different [8]. For instance, the oral administration of small molecule therapeutics is often possible, whereas the oral delivery of biopharmaceuticals is almost completely precluded and they are therefore almost exclusively administered by injection [5]. Due to these differences, the possibilities and challenges of the oral delivery of small molecule therapeutics and of biopharmaceuticals will be discussed separately in the following two sections.

Challenges in the oral delivery of small molecule therapeutics

Oral bioavailability of drugs mirrors the rate and extent of its absorption to the systemic circulation. Factors influencing drug bioavailability can be divided into physiological factors associated with gastro-intestinal tract, physicochemical characteristics of the drug and factors related to the dosage form.

Physicochemical characteristics of drugs affecting bioavailability

Drug absorption, can be simplified by the Fick's First law of diffusion (Eq. 1):

$$J = PC \quad (1)$$

where the flux (J) of a given drug through the gastrointestinal wall is related to the permeability coefficient (P) of the gastrointestinal wall for that drug and the drug concentration (C) in the GI fluid under sink conditions[9]. The drug concentration in the GI lumen is related not only to its solubility, but also to its dissolution rate and stability in the GI environment [10]. Assuming that a drug is stable in the GI fluids, information on drug solubility and intestinal membrane permeability can provide a better understanding of its oral bioavailability. Based on solubility and permeability parameters, drugs have been subdivided into four categories in the Biopharmaceutics Classification System (BCS) classification (Figure 3.1) [11].

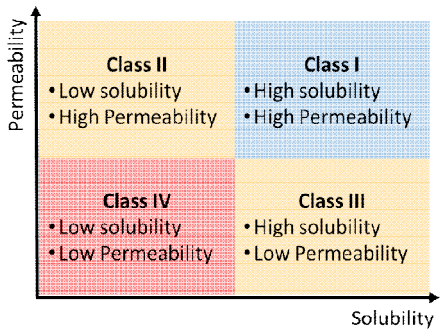


FIGURE 3.1
BCS classification system

The BCS classification has demonstrated to be an extremely effective prognostic tool to ease the development of oral drug products. Based on the BSC classification, poor oral bioavailability of drugs can be either attributed to low solubility/dissolution rate (class II), low permeability (class III), or both low solubility and permeability (class IV) [11,12]. Interestingly, the new drug compounds under development seem to present more formulation challenges than already marketed old drugs: the new pipeline seem to bear lower solubility drugs, showing a corresponding increase in BCS II compounds from ~30% to 50–60% and a decrease in BCS I compounds from ~40% to 10–20%, compared to drugs already in the market. It is therefore evident that there is an increased pressure on medicinal chemists now more than in the past to find approaches necessary to improve solubility and hence bioavailability of many new compounds [13]. This is particularly challenging for certain classes of drugs, such as HIV protease inhibitors, several anti-infective drugs and anticancer drugs, for which potency depends on the interaction with very lipophilic targets and hence maintaining potency and aqueous solubility becomes challenging. Alongside medicinal chemistry approaches to improve drug solubility, pharmaceutical scientists have also few strings to their bow. Formulation technologies designed to improve solubility and/or dissolution rate of poorly soluble drugs include preparation of solid solutions or dispersions, use of cyclodextrins and stabilization of drug in the amorphous form. In the last few years, several nanotechnology approaches, including drug nanocrystals and self-emulsifying drug delivery systems have also proved useful for this aim [14]. The use of nanotechnology systems to improve the oral bioavailability of poorly soluble drugs will be discussed in the second part of this Chapter.

Physiological factors affecting bioavailability

During its transit through the GI tract, a drug faces several potential physiological barriers to absorption. Firstly, the drug must withstand the harsh pH of the stomach. Moreover, the drug might be metabolized by the enzymes present in the stomach and in the intestine, including those produced by bacterial flora in the colon. Such type of pre-systemic metabolism of drug is defined as luminal metabolism [15,16]. If the drug remained stable, it would have to cross the mucus layer covering the epithelial cells of the GI tract, before it could be absorbed from the intestinal lumen. The risk at this point would be for the drug not to be able to diffuse through this layer or to remain bound and trapped to the mucus. In the case of small molecule drugs, generally the more lipophilic is the compound the slower is the diffusion through the GI mucus [17,18].
The passage of drugs through the GI membrane could be also severely limited by a mechanism called transmembrane efflux of drugs. This is defined as the removal of drug from the cell, i.e. GI epithelial cells in this case, via a transportation system present on the cell membrane, such as P-glycoprotein (P-gp). P-gp is highly expressed on the apical surface of a wide variety of cells, including those present in the epithelium of the jejunum [10,19,20]. Moreover, the drug could be subjected to first-pass intestinal metabolism which is a pre-systemic drug metabolism by enzymes present either in the brush border or inside enterocyte cells in the intestine. Aside from luminal and first-pass intestinal metabolism, drugs could also be subjected to first-pass hepatic metabolism: all drugs after being absorbed are firstly transported to the liver through the portal circulation. Here, several drugs could be metabolized by the many hepatic enzymes [15,21]. As the liver exerts its useful metabolic function to clear the body from many endogenous compounds and xenobiotics, many drug compounds gets also metabolized. As a matter of fact, the first pass metabolism is regarded as main cause for poor oral bioavailability of many drugs [10]. The vast majority of enzymes involved in first-pass intestinal and hepatic metabolisms belongs to the superfamily of cytochrome P450 [22].

Dosage form related factors affecting bioavailability

Both physiological factors and physiochemical characteristics of the drugs influence oral bioavailability of drugs. However, the type and characteristics of the dosage form in which the drug is included can also have a crucial impact on the bioavailability of the drug. For example, the amount of drug reaching the blood can be finely modulated by the use of modified release systems. As already mentioned, delivery systems have been developed to improve the bioavailability of poorly soluble drugs [14]. The study of the different types of dosage forms and approaches used to improve the oral bioavailability of drugs is outside the purpose of this Chapter. Instead, this Chapter focuses on the exploitation of nanotechnologies for the development of delivery systems that can improve oral bioavailability of therapeutics.

Challenges in the oral delivery of biopharmaceuticals

If the oral bioavailability of small drugs can be in several cases an issue, the delivery of larger biopharmaceuticals, including proteins, peptides and nucleic acids is almost completely precluded. This can be simply explained by the fact that the digestive apparatus is naturally designed to digest macromolecules, such as proteins from diet, into smaller subunits that can be absorbed. In a similar fashion, macromolecular therapeutics (e.g. insulin) would undergo the same faith, as they themselves get metabolized and thus do not get absorbed intact upon oral administration [23]. For this reason, the achievement of successful oral delivery of biopharmaceutics is extremely problematic and frequently impossible [5,24]. The barriers to the oral delivery of biopharmaceuticals can be divided into physiochemical, biochemical and physical barriers.

Physiochemical barriers to the oral delivery of biopharmaceutical

In the case of oral delivery of peptide or protein based therapeutics, the first threat to their physical stability is the harsh pH of the gastric fluid, which generally ranges from pH 1 to 2.5 under normal fasting state conditions [25]. Such low pH can compromise the physical structure of a protein by denaturation, as well as by possibly inducing oxidation, deamidation or hydrolysis[26]. Moreover, these conformational changes generally determine protein deactivation and increase the susceptibility to enzymatic degradation [27]. Similarly, the acidic pH of the gastric fluids can induce denaturation and depurination of the nucleic acid when gene therapeutics are given orally, thereby affecting their stability and effectiveness [28].

Biochemical barriers to the oral delivery of biopharmaceutical

Oral delivery of peptides and proteins therapeutics is also vastly hindered by the presence of digestive enzymes present throughout the different regions of the GI tact. These include pepsin in the stomach, intestinal endopeptidases (trypsin, chymotrypsin and elastase) and exopeptidases (aminopeptidases and carboxypeptidases) and peptidases present in the brush-borders and in the cytosol of the epithelium of the small intestine. In the colon, the presence of proteolytic enzymes is relatively low [27]. Hence, it has been proposed that site-specific drug targeting to the colon could minimize protein-based therapeutics degradation and thus improve bioavailability [29]. In the case of gene therapeutics, the main threat to their gastro-intestinal stability is the presence of pancreatic nucleases enzymes, which are secreted in the intestine and are able to digest nucleic acids into nucleosides [28,30].

Physical barriers to oral delivery of biopharmaceuticals

One major physical obstacle to proteins and peptides delivery through the oral route is the extremely limited capability of such macromolecules to cross the intestinal epithelium and hence be absorbed [5]. The large size is a main cause of poor intestinal absorption for macromolecules: it has been shown that absorption of polyethylene glycol (PEG) of only 2 kDa molecular weight is less than 2% when administered to mice via gavage, while shorter PEG molecules showed good absorption [31]. This also applies to proteins and peptides, which, if remained intact and not digested, cannot generally be absorbed due to their large molecular weight and hydrophilicity that generally limit both paracellular and transcellular transport [27,32,33]. Another potential physical barrier could be constituted by the presence of mucus covering the epithelium of the GI tract, in which large proteins could diffuse slowly or be trapped. However, it has been demonstrated that diffusion of many different large proteins through this thick mucus layer is almost as fast as in water [34]: the mucus barrier, considered for long a diffusion barrier, does not actually hinder the diffusion of many proteins.

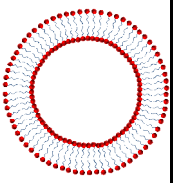
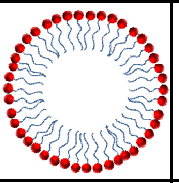
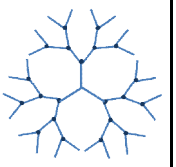
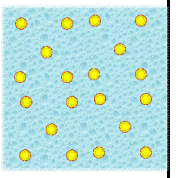
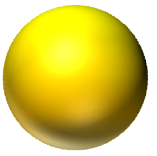
P-gp efflux and first-pass intestinal and hepatic metabolism barriers

At last, it should be mentioned that peptides in a similar manner to small molecules have shown to be susceptible to P-gp efflux and to intestinal and hepatic metabolism by enzymes (cytochrome P450 family) present inside enterocytes cells and in the liver [35–37].
Finally, the reader must be reminded that the importance of each of these aforementioned barriers to the overall bioavailability varies considerably. For instance, enzymatic digestion is a major obstacle for most protein, peptide or nucleic acid based therapeutics, as these gastro-intestinal enzymes (e.g. pepsin) often have specificity over a broad range of substrates [38]. Similarly, absorption through the gut is precluded to virtually all biopharmaceuticals, being large in size and often hydrophilic. In short, the stability of small molecules in the GI fluids and the intestinal permeability can limit the oral bioavailability of specific groups of drugs, whereas GI instability and poor absorption render oral bioavailability of almost all biopharmaceuticals virtually null. Several approaches have been investigated in order to improve the oral bioavailability of biopharmaceuticals, including chemical modification [20], use of enzyme inhibitors [39], use of absorption enhancers [40], use of mucoadhesive polymer systems [41] and use of membrane transporter and receptor targeting [42,43]. Moreover, particulate carrier systems have been also largely investigated for this purpose.

Nanotechnology systems as tools to maximize oral drug delivery

Among other approaches to improve the oral bioavailability of small molecule drugs and biopharmaceuticals, nanotechnologies have shown to be very promising. Different nanotechnology systems have shown the potential to boost oral delivery by overcoming to some extent one or more of the aforementioned challenges presented by the oral route of administration. This Section presents the recent advances in nanotechnologies based on their potential uses to overcome the different oral delivery barriers. A brief description of different nanotechnology systems used in drug delivery is shown in Table 3.1.

TABLE 3.1
Description of different nanotechnology systems used in drug delivery

	Definition	Size	Schematic representation	Ref.
Solid polymeric and lipid nanoparticles	Are solid constructs in the nanometre range, made of natural or synthetic polymers, high melting point lipids or proteins. Drugs can be either incorporated within the matrix or attached to the surface.	1-10 nm or larger		[1,44]
Liposomes	Are closed spherical vesicles that consist of one or more amphiphilic lipid bilayer surrounding aqueous compartments. Both water-soluble and lipophilic moieties can be incorporated within the liposome construct, whereas some can be adsorbed to the surface.	From 30 nm up to several µm		[1,45]
Micelles	Are aggregates of surfactant molecules or amphiphilic macromolecules that self-assemble in aqueous solutions into a core-shell structure. The micellar structure has a hydrophobic core and a hydrophilic surface and can thus work as solubilizing agents.	< 100 nm		[44,46]
Dendrimers	Are highly branched three-dimensional polymeric macromolecules, and have defined sizes and molecular weights. They consist of a central core, layers of polymer branching and an exterior surface onto which drug molecules, targeting groups or hydrophilic polymers can be added. Drug molecules can be also carried within the construct.	10-20 nm		[1]
Nanoemulsions and Self-emulsifying drug delivery systems (SEDDS)	Nanoemulsions are made of two immiscible liquids in which one liquid is dispersed as droplets through the other phase. O/W emulsion is made of a blend of oil and surfactants dispersed in aqueous phase. SEDDS consist of drug dissolved in oils and stabilized by surfactants that form o/w micro- or nano-emulsions in situ upon exposure to aqueous environment.	> 500 nm		[10,44,47]
Microspheres	Are spherical particles in the micrometre size range that can be made from polymers, lipids or proteins.	> 1 µm		[1]

Approaches to impart protection of drugs against the harsh GI environment

Upon ingestion of the dosage form, the first barrier encountered by therapeutics, in particular large molecules, is the harsh environment of the GI fluids. Several nanotechnology approaches have been developed in order to protect drugs from physical and enzymatic degradation. Most of these approaches are based on the incorporation of the drug into particulate carriers that can provide protection against the destructive action of acid and enzymes. The extent of protection however varies based on the physicochemical characteristics of the nanocarriers: factors including size of the carrier and properties of the materials forming these carriers are of primary importance [48–51]. Besides this direct shielding action, some materials used to fabricate nanocarriers can also have enzyme inhibitory function. For example, polymethacrylic acid–chitosan–polyethylene glycol (PCP) nanoparticles have shown up to 50% inhibitory effect on the intestinal enzyme trypsin, as measured using casein as enzyme substrate [52].

Liposomal formulations

Liposomes have been investigated as carriers for improving the oral bioavailability of small molecule drugs and biopharmaceuticals by potentially overcoming some of the GI barriers, including the GI degradation. Conventional liposomes have shown to protect peptides such as insulin from digestion by intestinal enzymes, yet to some extent [53]. Despite this potential benefit, liposomes themselves can undergo degradation under the acidic conditions in the stomach and in presence of bile salts and pancreatic lipase in the intestine [54,55]. Therefore, their potential instability might render the loaded drugs unshielded and exposed to the disruptive conditions of the GI fluids. Thus, a number of systems have been investigated in order to improve the GI stability and oral delivery of liposomes. These advanced approaches comprise polymerized liposomes [56], Archaeosomes [55,57,58], bilosomes [59,60] and liposomes coated with PEG, mucin [54], silica [61,62] or thiolated polymers [63,64]. In US patent 5,762,904 [56], polymerized liposomes for the delivery of vaccines were prepared by polymerization of double-bond containing monomeric phospholipids. Polymerization has shown to improve the stability in comparison to un-polymerized liposomes both in acidic conditions and in presence of intestinal bile salts. This was associated with improved oral bioavailability in rats.

Archaeosomes are liposomes that include diether and/or tetraether lipids, typical lipids of archaeobacterial membranes, in their composition [58]. Patel et al. [58] thoroughly investigated the stability of archaeosomes under different stress conditions found in the GI tract, i.e. low pH, bile salts and pancreatic lipases and their ability to retain the encapsulated compounds. Some of the tested archaeosomes showed to be more stable than many conventional, i.e. ester phospholipids-based, liposomal formulations. Moreover, archaeosomes were investigated as potential carriers for peptides, such as insulin [57]. Insulin-loaded archaeosomes were compared to conventional liposomes in terms of *in vitro* oral stability, transport studies and *in vivo* efficacy. Insulin release from archaeosomes in simulated gastric and intestinal fluids was less than that obtained with conventional liposomes, suggesting superior stability of archaeosomes.. Although archaeosomes showed lower *in vitro* transport across Caco-2 cells, yet they resulted in lower level of blood glucose compared to conventional liposomes. The obtained superior *in vivo* efficacy of archaeosomes could be attributed to the higher stability of these constructs in the GI system, rather than to the intestinal transport [57].

Archaesomes are developed as completely new entities compared to conventional liposomes, using diether and/or tetraether lipids as liposome-forming compounds, rather than the phospholipids

generally used in conventional liposomes. Another more straight forward approach to obtain orally stable liposomal formulations is the incorporation of certain ingredients, such as lipids or bile salts into the structure of conventional liposomes. For example, a single tetraether lipid, obtained from the archaeon *Sulfurous acidocaldarius*, was used to stabilize conventional drug-loaded liposomes. The presence of the tetraether lipid in the liposomes improved their stability under physiological levels of intestinal bile salts. However, the tetraether-containing liposomes, despite remaining fairly stable in size, could not prevent major leakage of encapsulated small molecules at pH 2. These liposomes would possibly not be able to protect sensitive drugs from the high proton concentration in the gastric fluids. Thus, to overcome this stability problem incorporation of freeze-dried liposomes into enteric coated capsules could be considered [55].

Liposomes incorporating bile salts, namely bilosomes, have also been used for oral drug delivery. In a study [60], bilosomes were prepared by incorporating sodium glycocholate, a bile salt, into insulin-loaded liposomes. The presence of sodium glycocholate within the liposomal structure resulted in better protection of insulin against enzymatic degradation by pepsin, trypsin, and α-chymotrypsin, compared to conventional liposomes or liposomes containing other bile salts.

Coating the surface of liposomes with the sugar chain portion of mucin or polyethyleneglycol (PEG) was also explored as means to improve the GI stability of liposomes [54]. Such modification improved the stability of liposomes significantly in presence of physiological levels of intestinal bile salts and completely suppressed the degradation of the incorporated insulin in rat intestinal fluids, as compared to uncoated liposomes. The improved stability of insulin has been attributed to the higher resistance of the liposomes to degradation by bile salts, which in turn increased insulin retention within the liposomes. *In vivo* studies also revealed significant hypoglycemic effect of insulin-loaded surface coated liposomes, particularly those coated with PEG. Silica-coated liposomes were also investigated as a potential oral delivery system for insulin [61,62]: liposomes were used as scaffold for deposition of silica nanoparticles, creating hybrid nanocapsules. The silica coating on the liposomes protected liposomes against degradation by digestive enzymes, *in vitro*. Moreover, the release of insulin from nanocapsules in simulated gastric and intestinal media was slower compared to the release of insulin from uncoated liposomes. These results suggest that liposomal GI stability and release kinetics can be controlled by the incorporation of the silica nanoparticle layer on the liposomes [62]. Liposomes have also been coated with thiolated chitosan [63]. Among other characteristics, such as improved mucus penetration, improved absorption and effective inhibition of efflux pump, these liposomes demonstrated high stability in the gastrointestinal environment. Interestingly, chitosan-coated liposomes have also been investigated for the oral delivery of DNA-based vaccines [65].

Recently, layersomes, composed of layer by layer coating of the polyelectrolytes over liposomes were developed. Layersomes have shown higher stability than conventional liposomes upon exposure to simulated gastric and intestinal fluids. Moreover, layersomes resulted in increased oral bioavailability of several anticancer drugs, including doxurobicin and paclitaxel [10,66,67].

Emulsions

Emulsions have also been considered as possible carriers for the oral delivery of proteins and peptides. Solid-in-oil-in-water (S/O/W) emulsions of insulin have been formulated for the purpose of protecting the labile peptide from the enzymatic degradation in the GI and to enhance the intestinal permeability. It was assumed that incorporating insulin in the oil phase can prevent its degradation. This S/O/W emulsion of insulin showed significantly higher hypoglycemic activity than that of insulin solution, upon oral administration to rats [68]. In order to increase the stability of the

previous formulation during storage, an enteric coated dry emulsion formulation has been developed and tested for release in simulated gastric and intestinal fluids. Drug release was pH-dependent and also affected by the presence of intestinal lipases [69]. The use of oral insulin emulsions has also been exploited by Provalis PLC Company, with the product known as Microemulin. This insulin-loaded W/O microemulsion has reached phase II clinical trials [70,71].

Solid Lipid Nanoparticles (SLN)

Lipid-based carriers can be broadly classified into liquid and solid lipid formulations depending on the melting point of the used lipids [44]. Solid lipid nanoparticles (SLN) are made of high melting point lipids, i.e. solid at room temperature. These lipids are physiologically compatible and thus SLN are generally considered safe to be administered orally. Moreover, these solid lipid carriers are considered more robust in comparison to liposomes and emulsions and could therefore offer better protection to the encapsulated drugs [72,73]. SLN are very attractive drug delivery systems, because the can be easily manufactured in large scale and without the use of organic solvents [74]. Insulin-loaded SLN were able to partially protect insulin from pepsin degradation. Even greater protective effect was observed with insulin-loaded lectin-modified SLN. These results were confirmed by *in vivo* studies, where the relative bioavailabilities for insulin-loaded SLN and insulin-loaded lectin-modified SLN were 4.99% and 7.11%, respectively, in comparison to subcutaneous injection of insulin [75]. In another study, it was shown that the hydrophobicity of the lipids can play an important role in the release and oral bioavailability of insulin-loaded SLN [51]. SLN have also been investigated for the oral delivery of the peptide salmon calcitonin [76].

Polymeric nanoparticles

Extensive research on the use of polymeric nanoparticles in oral drug delivery has been carried out. Polymeric nanoparticles are solid constructs made of polymers, in which drugs can be either incorporated within the matrix or attached to the surface. Despite the fact that the physicochemical and drug release properties of these carriers are highly dependent on the method of preparation, nanoparticles generally have higher GI stability than liposomes, emulsions and micelles [20]. Polymeric nanoparticles have been prepared using both natural (e.g. chitosan, dextran, gelatin, alginate and agar) and synthetic polymers [e.g. Poly(lactide) (PLA), poly(glycolide) (PGA), poly(lactide-co- glycolide) (PLGA), poly(cyanoacrylate) (PCA), polyethylenimine (PEI) and polycaprolactone (PCL)] [77]. Many of these nanoparticles have been employed for oral and peptide drug delivery. For example, insulin-loaded chitosan nanoparticles have shown 14.9% pharmacological oral bioavailability relative to subcutaneous insulin injection. This is thought to be related to the enhanced intestinal absorption of chitosan nanoparticles. Moreover, the oral bioavailability was found to be dependent on the size of the carrier: small nanoparticles were soluble in acidic media and thus resulted in lower hypoglycemic effect compared to larger nanoparticles, which were difficult to dissolve in acid. This suggests that the size of chitosan nanoparticles has a role in the protection of insulin from the harsh gut condition and hence its absorption [48].

In the last few years, several multi-component nanosized formulations have been developed with the aim of improving oral bioavailabilty of drugs that are unstable in the GI. For example, Zhang et al. [78] investigated the use of cationic β-cyclodextrin-insulin complex to control the release of insulin from alginate/chitosan nanoparticles. The complexation of the peptide with β-cyclodextrin resulted in improved protection against degradation in simulated gastric fluid and higher

cumulative insulin release in simulated intestinal fluid compared to cylclodextrin free nanoparticles. Moreover, insulin remained physically stable upon exposure to both simulated gastric and intestinal fluids. In another study, multicomponent nanoparticles were designed to maximize insulin encapsulation, stability and absorption upon oral administration [79]. These multilayered insulin-loaded nanoparticles were made of - from the outermost to innermost - a Poloxamer coating to prevent aggregation of nanoparticles, albumin to protect insulin from degradation by serving as a sacrificial protein substrate to GI proteases, chitosan to improve mucoadhesion and intestinal permeation and a calcium cross-linked network of alginate and dextran to prevent premature insulin release and enzymatic degradation in the gastric fluids. These nanoparticles retained insulin during 2 hours exposure to simulated gastric fluid, whereas more than 95% of the insulin was release during the next 3 hours exposure to simulated intestinal fluids. Moreover, *in vivo* results showed 13% oral insulin bioavailability in rats. Similarly, Morçöl et al. [80] developed an elegant formulation for the oral delivery of insulin that consists of calcium phosphate-PEG-insulin nanoparticles coated with casein, forming a calcium phosphate-PEG-insulin-casein delivery system. Casein protein is known to aggregate under acidic conditions and it is thus expected that the casein coat would protect insulin in the stomach. As expected, *in vitro* studies showed minimum leakage of insulin in simulated gastric conditions and significantly higher release of insulin at a pH similar to that of the intestinal fluid. Moreover*, in vivo* studies indicated that this delivery system has the capability of delivering biologically active insulin via the oral route [80,81]. This technology has been adopted by the company BioSante for the development of oral insulin and vaccine fomulations [70].

Stimuli-responsive nanoparticles have been developed to further improve stability of orally administered drugs. pH-sensitive nanoparticles are made of materials that are insoluble at low pH and dissolve and/or swell at higher pH, such as acrylates or anionic polymers. The pH sensitivity can enable retention and possibly protection of the cargo within the nanoparticles at low pH in the upper areas of the GI tract, while allowing drug release at the higher pH in the distal GI tract [50,82]. Most pH sensitive materials are recognized as safe and have already been used for the preparation of gastro-resistant solid dosage forms, some of which are already available in the market [83]. In a study, cyclosporine loaded pH- sensitive nanoparticles were prepared using different grades of poly (methacrylic acid-co-methyl methacrylate) copolymers (Eudragit L100-55, Eudragit L100, Eudragit S100) as pH-sensitive polymers. *In vitro* release results showed that nanoparticles made of Eudragit could provide pH-dependent release, with higher extent of drug release obtained at more basic pH-values. These formulations would potentially reduce cyclosporine degradation in the stomach and deliver it at parts of the GI tracts where absorption could take place. Moreover, *in vivo* studies demonstrated that the oral bioavailability of cyclosporine loaded in Eudragit L100-55, Eudragit L100 and Eudragit S100 based nanoparticles was higher than that of Neoral microemulsion available in the market [84]. In a similar study, it was also shown that encapsulation of insulin into pH sensitive polymethacrylic acid–chitosan–polyethylene glycol (PCP) nanoparticles could result in low insulin release at pH 1.2 and faster release at pH 7.4 [52]. More recently, Rostamizadeh et al. [85] showed that different parameters involved in the preparation of these polymethacrylic acid–chitosan–polyethylene glycol (PCP) nanoparticles can be adjusted in order to maximize their performance for oral insulin delivery. Other pH-dependent polymers have been also used for protection of peptides and proteins from gastro-intestinal degredation: the incorporation of hypromellose phthalate, another pH-dependent polymer, into insulin-loaded PLGA nanoparticles showed reduced insulin release in simulated gastric fluid and improved oral bioavailability in rats, compared to hypromellose phthalate-free PLGA nanoparticles [86]. Based on these and other similar studies [87], the use of pH-sensitive polymers in nano-

constructs can be considered a good means to confer protection of gastro-labile drugs against GI degradation. This highlights the importance of having the material of nanocarriers tailored to the desired application.

Microparticles

In a similar fashion to nanoparticles, microparticles have been extensively investigated as gastro-protective carriers. Morishita et al. [89] developed insulin-loaded poly(methacrylic acid-g-ethylene glycol) microparticles which exhibited pH-responsive behavior. Microparticles with different ratios of methacrylic acid and ethylene glycol were prepared. For those microparticles containing equimolar ratios of methacrylic acid and ethylene glycol, only 6% insulin was released upon incubation at pH 1.2 for 2 hours, while the residual insulin was released promptly when the pH was shifted to 6.8. This result suggested that such microparticles could be suitable carriers for oral delivery of peptidic drugs. This has been subsequently confirmed with *in vivo* studies, where insulin-loaded microparticles containing the same ratio of excipients as in the previous study (i.e. 1:1 methacrylic acid and ethylene glycol) provided the highest hypoglycemic effect following oral administration to healthy rats. Moreover, microparticles of the same composition showed 9.5% pharmacological availability of insulin following oral administration [90]. Other biopharmaceuticals have also been incorporated into microparticles: PLGA microparticles containing enteric coating polymers as stabilizers have been used to enhance gastro-intestinal protection of a microencapsulated antigen [91]. Moreover, inactivated microorganisms have been incorporated within gastro-resistant microparticles as possible novel oral vaccines [92,93]. Finally, microparticles have also been investigated as carriers for oral gene delivery [28,94]. Interestingly, in a recent study insulin-loaded PLGA nanoparticles were encapsulated into microcapsules of Eudragit FS 30D, a pH-dependent polymer. The resulting insulin-loaded PLGA nanoparticles composite microcapsules showed pH dependent drug release and improved insulin bioavailability in mice compared to non-encapsulated nanoparticles [95].

Approaches to overcome the mucus barrier

Another important barrier to the oral delivery of therapeutics is constituted of the mucus covering the epithelium of the whole GI tract. The physiological roles of mucus include protection of the GI epithelium itself from being degraded by the acid in stomach and digested by gastric and intestinal enzymes. The mucus is also responsible for maintaining the GI epithelium constantly lubricated. Moreover, the mucus acts as a barrier against pathogenic bacteria [96]. Mucus does not generally constitute a barrier for small molecules, as they tend to diffuse through the mucus and reach the underlining epithelium. Nevertheless, hydrophobic small molecules, such as testosterone for example diffuse slowly through the mucus gel. This is due to the fact that the more hydrophobic a compound is the longer it remains non-specifically bound and partition onto hydrophobic portions of the mucus layer [17]. In the case of larger molecules, such as proteins, it has been thought for long that diffusion through mucus would have been unlikely [97]. Surprisingly, many proteins with different molecular weights showed unhindered diffusion through mucus [34]. Although mucus does not directly set a barrier to the oral delivery of many small and large molecules therapeutics, nevertheless encapsulation of drugs within nanoparticulate carriers has been suggested in order to improve their oral bioavailability. In these cases, the capability of the carrier to interact with the mucus can determine whether the drug can reach the epithelial cells for being absorbed. Hence,

the interaction of nanoparticulate systems with the mucus gel can have a strong influence on the bioavailability of the incorporated therapeutics.

At this point, a brief description of the physiology of the mucus can be useful for the reader. The mucus covering the GI epithelium is a hydrogel made of proteins, carbohydrates, lipids, salts, antibodies, bacteria and cellular debris. This gel is subjected to a continuous turnover, which has been estimated to vary between 50 to 270 minutes [98]. Mucus exists as two layers: a basal firmly adherent layer of mucus also called "unstirred" mucus that covers the epithelial cells and a luminal loosely adherent, or "stirred" layer. The firmly adherent mucus is thought to have a slower clearance than the loosely adherent mucus. Nanoparticles delivered orally can undergo one of the following pathways (Figure 3.2):

1. Nanoparticles can remain bound to the chyme and hence have a fast transit through the GI and rapid elimination through faeces.
2. Nanoparticles can bind to the loosely adherent mucus and remain bound until the mucus is cleared.
3. Nanoparticles can penetrate the mucus and either remain bound to the firmly adherent mucus or possibly enter the intestinal epithelium [99].

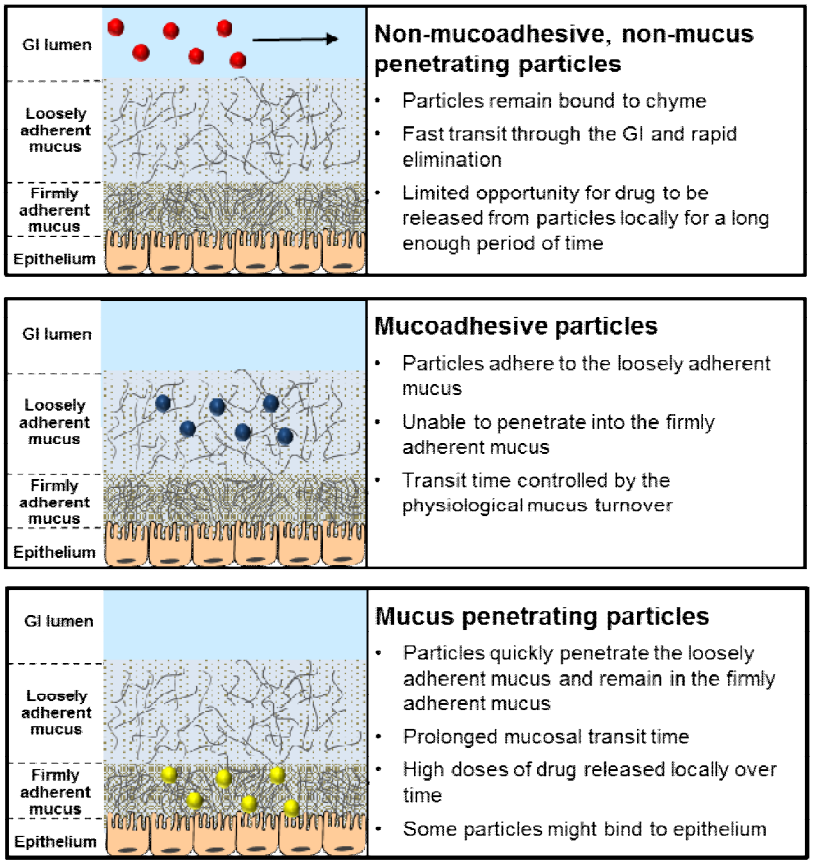


FIGURE 3.2
Interaction of particles with mucus

Many particles follow the first of the aforementioned pathways, as they do not adhere to or penetrate the mucus. Consequently, the fast transit time of these particles in the GI tract generally results in low bioavailability due to inadequate amount of drug or insufficient residence time at the site of absorption [96,99,100]. In order to overcome this problem, mucoadhesive nanoparticles have been employed: adherence of nanoparticles to the mucus slows the particle transit time in the gut, thereby increasing the time available for the entrapped drug to be released and absorbed. However, one of the major limitations of the conventional mucoadhesive particles is that they get trapped in the loosely adherent mucus due to strong interactions with the mucus, which in turn makes them unable to penetrate into the firmly adherent mucus. Thus, mucoadhesive nanoparticles remain trapped in the outermost layer of the mucus and are often incapable of reaching and crossing the intestinal epithelium. Furthermore, the transit time of these systems is basically controlled by the physiological mucus turnover: as this turnover time is 4 to 5 hours at best, mucoadhesive nanoparticles do not adhere to the gel layer for long [101].
Mucus penetrating particle systems have therefore been recently investigated, in order to overcome this problem and allow prolonged nanoparticle residence time at mucosal surfaces. The clear advantage of mucus penetrating particles is based on their ability to make their way through the mucus and get in proximity of the epithelial cells, where they can remain for longer, whereas conventional particles remain further positioned from the epithelial cells and are also cleared more quickly. Therefore, the use of mucus penetrating particles is expected to allow larger drug doses to be directly released to the epithelial cells. Additionally, some of the mucus penetrating particles may also bind to the intestinal epithelial cells, resulting in further improvement of the bioavailability of the incorporated drugs [96,99]. Both conventional mucoadhesive particles that constitute to date the most explored approach to improve mucosal delivery of drugs, as well as recent developments in mucus penetrating systems will be discussed in the following Paragraphs.

Mucoadhesive nanoparticles

Several techniques have been explored to enhance mucoadhesive properties of nanoparticles: most of these approaches are not based on targeting specific chemical structures on mucus and are therefore considered non-specific. These include hydrogen bonding, hydrophobic forces, van der Waals interactions, polymer chain interpenetration and electrostatic/ionic interactions [99].
The polymer chitosan has been widely explored for its mucoadhesive properties that are based on chitosan electrostatic interactions with the sialic groups of the mucin present in the mucus [102]. Generation of microspheres made of chitosan has shown to prolong residence time at nasal mucosal surfaces and reduce the rate of clearance from the nasal cavity [103]. Similarly, chitosan coated poly(isobutyl cyanoacrylates) nanoparticles significantly improved mucoadhesion to rat intestinal mucosal surfaces [104]. Several chitosan-based formulations have been explored for the oral delivery of proteins and peptides [48,76,78,105]. In this regard, interesting results have been achieved by Sonaje et al. [106] who formulated gastro-resistant capsules filled with insulin-loaded chitosan/poly(γ-glutamic acid) nanoparticles. *In vivo* studies in diabetic rats resulted in approximately 20% insulin relative bioavailability. In another study, chitosan nanoparticles were effective in enhancing the absorption of salmon calcitonin and reducing calcaemia levels upon oral administration to rats. This effect could be attributed to the unique mucoadhesive properties of chitosan [107]. Additionally, as it possesses reactive amino and hydroxyl group, chitosan has been chemically modified by covalently coupling it with molecules containing sufhydryl groups, forming thiolated chitosans. The thiolated form elicits stronger mucoadhesion compared to the unmodified chitosan, due to the formation of disulfide bonds between the thiolated polymer and mucus

glycoprotein [104,105,108,109]. On this basis, insulin-loaded thiolated trimethyl chitosan nanoparticles were prepared and compared to insulin-loaded trimethyl chitosan nanoparticles: the thiolated nanoparticles showed improved mucoadhesion and improved intestinal permeation compared to the non-thiolated trimethyl chitosan nanoparticles. *In vivo* experiments also showed superior hypoglycemic effect of the insulin-loaded thiolated nanoparticles, as compared to the non-thiolated nanoparticles upon oral and ileal administration in rats [110]. Thiolated chitosan nanoparticles have been also investigated as oral carriers for gene delivery [111]. In addition to chitosan-based nanoparticles, particles made of other common biomaterials, such as poly(lactic acid) (PLA), poly(sebacic acid) (PSA), poly(lactic-co-glycolic acid) (PLGA) and poly(acrylic acid) (PAA) were found to adhere to mucus through hydrogen bonding, polymeric entanglements with mucins and/or hydrophobic interactions [99].
Besides the "passive" targeting, which was previously described, some nanoparticulate systems have also been developed to actively target specific glycoproteins of mucus. Different ligands have been investigated for this purpose, including lectins [112] and invasins [113]. Coating nanoparticles with these ligands could possibly improve the binding specificity and reduce the speed of elimination by mucus turnover.

Mucus penetrating nanoparticles

It is worth remembering, that a potential drawback of mucoadhesive nanoparticles is that mucoadhesion does often result in particles being trapped in the mucus and hence unavailable to be taken up and absorbed by the underlying intestinal epithelial cells. Thus, conventional mucoadhesive nanoparticles are often not suitable for those delivery systems where not the drug alone, but the whole carrier-drug complex has to be adsorbed through the gut. Moreover, also in the case of delivery systems designed to bind to mucus, where the drug can be released to reach the underlying epithelium, the fast mucus turnover can negate the chance for high doses of drug to be released locally at the mucus surfaces. Therefore, scientists are investigating nanoparticulate systems that do not simply bind to mucus, but rather can reach the intestinal epithelium by deeply penetrating the mucus [99].
An interesting explanation on how this can be achieved has been given by Olmsted et al. [34]. The authors studied the diffusion of fluorescently labeled proteins, virus particles and polystyrene microspheres in fresh samples of human mid-cycle cervical mucus. Polystyrene nanoparticles (59 to 1000 nm), unmodified or functionalized with carboxylate, epoxy, or amino groups did not cross the mucus at all; in striking contrast some virus particles, bearing size in the order of magnitude of nanoparticles could diffuse rapidly through the mucus. This result was regarded as a "lesson from nature" and can be explained by the intrinsic properties of microorganisms, which have evolutionally adapted to infect mucosal tissues. These properties include: (1) the relatively small size, (2) the net neutral, yet hydrophilic surface (typical of many proteins) and (3) the lack of superficial hydrophobic area[34]. These properties were thought to provide the viral particles with the perfect characteristics to penetrate low viscosity pores in the mucus. However, not all viral particles could penetrate efficiently this cervical gel, probably due to their larger size and the presence of exposed hydrophobic patches that could interact with the mucus [18]. On the light of these considerations, synthetic particles could be designed with similar surface characteristics to that of viral particles: 200 and 500 nm hydrophobic polystyrene nanoparticles were densely coated with low molecular weight PEG to create a hydrophilic surface that could effectively shield the hydrophobic core of the nanoparticles. PEG coated nanoparticles showed rapid diffusion through mucus. These results demonstrate that synthetically engineered nanoparticles can rapidly

penetrate the human mucus barriers [114]. However, it must be reminded that those proofs of concept [34,114] have been obtained by penetration studies through cervicovaginal mucus, but to our knowledge they have not been demonstrated in GI mucus. Nevertheless, as cervicovaginal mucus has similar rheological and compositional characteristics to that of the mucus of the GI tract, these mucus penetrating particles are expected to have the same potential for improved oral bioavailability [96,99]. It was recently demonstrated that coating saquinavir-loaded nanoparticles with dextran–protamine complexes resulted in improved drug permeability, compared to uncoated nanoparticles, both in enterocyte-like and mucus *in vitro* models. It was suggested that the hydrophilic, yet neutral net surface charge of the dextran–protamine coating, similar to the surface of viruses, allowed efficient penetration of these coated nanoparticles across the mucus barrier [115].

Another proposed approach, that could supposedly boost penetration of particles through the mucus, is based on the development of methods that could disrupt the mucus barrier. Prior studies demonstrated that absorption of nanoparticles could be influenced by simply changing the volume of liquid through which nanoparticles were orally administered to rats. Particles administered in small volumes were found mostly bound to the intestinal mucus surface, whereas nanoparticles administered in larger volumes appeared to pass through the mucus barrier. Consequently, the larger the volume of liquid administered was, the faster and greater the appearance of particles in the systemic circulation. These results can probably be explained as a pressure driven channel formation by a low viscosity liquid (i.e. large volume of nanoparticles dispersions) through a higher viscosity liquid (i.e. mucus) [96,116]. This was the first evidence that disruption of the mucus layer can constitute a means for particle penetration through the altered gel layer. Among other attempts, N-acetyl cysteine, a mucolytic, has been explored as a mucus-disrupting agent to enhance nanoparticles mucus penetration [117]. However, serious concerns have been expressed over this general concept of disrupting the mucosal barrier, as this alteration of the normal physiology could compromise the mucosa, rendering the intestinal endothelium unprotected from the GI acidity and enzymes [118]. Moreover, the disruption of the mucus layer could increase the chances for microbial pathogens translocation through the GI mucosa [119]. Therefore, it is more probable that future research trends on the development of mucus penetrating particles will be targeted towards modification of size and surface characteristics of nanoparticles, rather the alteration of the mucus layer.

Approaches to overcome the intestinal epithelial barrier

Many oral drug candidates suffer poor intestinal permeability. These include class III and IV small molecular weights therapeutics, but also most, if not all biopharmaceuticals, i.e. large molecules. Several nanoparticulate systems can improve intestinal absorption of many encapsulated drugs to some extent [37]. Particles can be potentially transported across the intestinal epithelium via transcellular or paracellular pathways (Figure 3.3).

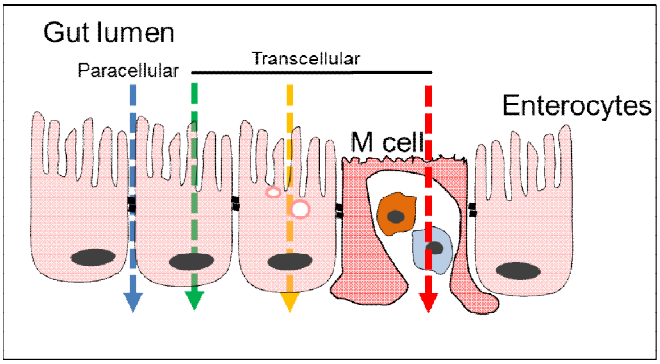


FIGURE 3.3
Transport across intestinal epithelium. Molecules and particles can cross the intestinal epithelial barrier by paracellular transport (blue line) and transcellular transport. The transcellular pathways are subdivided into passive diffusion (green line); transcytosis via normal enterocytes (orange line) or via M cells (red line)

Paracellular route

The paracellular route has not been much investigated and exploited for the delivery of nanoparticles. Under physiological conditions, the paracellular route is precluded to nanoparticles, due to the very small surface area of the intercellular spaces and to the small size (i.e. 3 to 10 Å) of the tight junctions between the epithelial cells [120]. Nevertheless, certain formulations have the ability to alter the structure of the tight junctions, allowing macromolecules and possibly small particles to permeate paracellularly. For example, chitosan is able to enhance the intestinal paracellular permeability of peptides [121]. On this basis, Sonaje et al. [122] developed insulin-loaded nanoparticles composed of chitosan (CS) and poly(γ-glutamic acid) (γPGA) that could potentially exploit the paracellular route: *in vivo* results showed a significant hypoglycemic effect in diabetic rats [123]. Subsequently, the same research group modified the previous formulation by incorporating diethylene triamine pentaacetic acid (DTPA), another tight junction opening agent, within the carrier. The latter formulation of nanoparticles was found to generate a transient and reversible increase of paracellular permeability based on the results obtained using an *in vitro* model of intestinal epithelium. Moreover, *in vivo* studies in rats revealed that these nanoparticles were effective in enhancing the bioavailability of insulin via oral administration. Similarly, particles made of poly(acrylic acids) polymers [124,125] and thiomers [126] have shown permeability enhancing properties. However, the delivery of therapeutics by the transient alteration of the permeability of the intestinal epithelium has major limitations. First, temporary opening of tight junctions is unlikely to allow intestinal uptake of particles > 50 nm [127]: in fact, paracellular transport of intact nanoparticles has not been demonstrated yet. It is instead believed that in most cases the opening of the tight junctions enables absorption of drug released from previously disintegrated particles [37]. Moreover, it has been objected that altering the physiological integrity of the intestinal epithelium to improve drug intestinal permeability might result in undesirable effects, including damaging of intestinal cell membranes and entry of unwanted xenobiotics or pathogens. This could lead to irritation and inflammation of the intestinal epithelium, as well as increasing the chances of infections [20].

Transcellular route

Particles can also be transported transcellularly via transcytosis. This process consists of the active transport of nanoparticles via initial endocytosis at the cell apical membrane, followed by transport through the cell and release at the basolateral side [128]. Two different types of epithelial cells are relevant for the oral delivery of macromolecules and particles: conventional enterocytes and M cells. Enterocytes, which constitute the majority of the epithelial cells, are thought to have very limited endocytic activities, resulting in low transcytosis of particles across the intestinal epithelium [101]. Instead, it is thought that most microorganisms, macromolecules and particles are taken up by M cells [129]. M cells are distributed in specific areas of the intestine and constitute 5% of the epithelial cells of the follicle-associated epithelium (FAE), overlying the Peyer's Patches (PPs) (Figure 3.4).

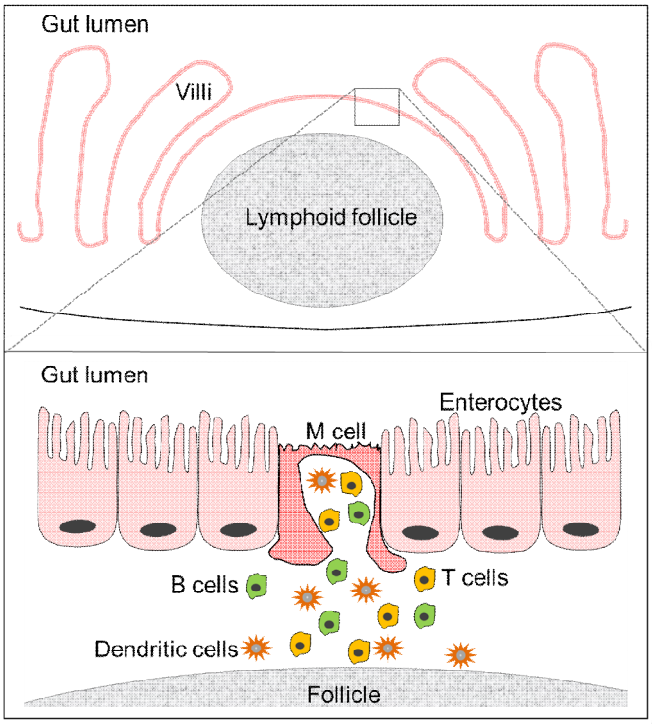


FIGURE 3.4
Overview of Peyer's Patches (PPs) with focus on the FAE. The upper illustration shows the dome-like shape of the PP lymphoid follicle, which is embedded within villi. The lower illustration is an enlarged representation of the FAE covering the PP. The FAE is constituted by conventional enterocytes and M cells. M cells show unique features: short microvilli, basolateral pocket, intimate contact with dendritic cells, T cells and B cells

PPs are major inductive sites for immune response in the gut [130]. They are mostly located in the ileum and only a limited number is present in the rest of the small intestine. PP-like structures are also found in the colon [131,132]. The main function of M cells is sampling foreign materials (i.e. antigens) from the gut lumen and transporting it to the underlying organized mucosal lymphoid tissue that induces and regulates immune responses [129] . This unique antigen-sampling activity of

M cells could be exploited in oral drug delivery: in fact, M cells have shown efficient transport of various types of particulate substances, including latex beads, carbon particles, liposomes, bacteria and viruses [133]. Conversely, the specific uptake of soluble non-particulate antigens or molecules is poor [134,135]. Based on this knowledge, it is possible to think that trans-epithelial transport of particles or pathogens is not strictly specific, as long as the substances to be transported are in particulate form. The transport mechanism, as well as the extent of transport seems to be dependent on the nature of the particles transported [129,136,137]. Uptake and transport studies with particulate materials allowed to define the influence of the physicochemical characteristics of the particles on M cell mediated transport:

- Nature of the material (i.e. particulate or non-particulate form): unlike particles, small molecules, such as dyes did not show PP-specific absorption [134]. Surprisingly, small 2.5 nm dendrimers also resulted in poor absorption in PPs, compared to larger size particles [138], but this result could be related to the low stability of those dendrimers in the gut [139].

- Size: size is one of the most important parameters that influence the transport of particles through PPs. Several studies over the years have showed that particles ranging between 50 and 500 nm in diameter are more highly transported than larger particles [101,127,138]. A recent study defined more precisely the best range for asbsorption: when 95, 130, 200, 340, 695, 1050 nm particles were given orally to mice, 95 nm particle showed the greatest transport in the PPs [134]. These results are in accordance with Jani et al. [140], who indicated that in mice 50 nm particles were better absorbed orally and distributed in the body than 100 nm particles.

- Surface charge: a positive charge on the particles could favour their uptake by M cells, considering that the cell membranes are negative. However, both the mucus and ordinary epithelial cells' membranes are also negatively charged and this could lead to entrapment of particles by enterocytes, by mucus or by the cellular membranes of M cells, due to non-specific electrostatic interaction between the positively charged particles and negatively charged physiological components [141]. Accordingly, negative and non-ionic particles have shown better M cell-mediated transport than positively charged ones [127,138].

- Hydrophobicity/hydrophilicity balance: studies with nanoparticles have shown that hydrophobic surfaces tend to have better tropism for M cells, whereas more hydrophilic particles are more suitable for enterocytes-mediated transport [101].

- Presence of targeting molecules: it is possible to increase the specific uptake and transport by M cells by decorating the surface of particles with molecules targeting M cells; increasing the specificity of M cell-mediated transport could counterbalance the unfavoured M cells/enterocytes ratio, as M cells represents only 1% of the total intestinal epithelial cells. Several particle surface modifications have been investigated. Lectins were attached to the surface of particles in order to target specific sugars on the M cells surface. Another approach involved the attachment of bacterial surface proteins on the surface of particles. This method is based on targeting particles to the same M cell receptors pathogens naturally bind to [101,142,143].

It must however be said that experimental results vary between animal species and experimental conditions and are sometimes contradictory. Albeit, there is a general agreement for what concerns the optimal particle size, with smaller particles being better absorbed than larger particles. Clear conclusions with regard to hydrophobicity and surface charge cannot be drawn yet [101].

It is worth mentioning that M cell-mediated transport is particularly suitable for oral vaccine delivery, rather than for other biopharmaceuticals. This is due to the fact that M cells do not simply sample antigen and particles: the housing of immunity cells in the M cell pocket (see Figure 3.4.) allows rapid and direct delivery of the candidate vaccine to the machinery capable of inducing immunogenic response. M cells have been defined as "a gateway of the mucosal immune system" [144]. Therefore, if M cell-mediated delivery approaches can be advantageous for other therapeutics, it could be indispensable in case of mucosal oral vaccines. Moreover, the immune system stimulation and activation by vaccines is not directly dose-dependent [135,145] and thus low intestinal absorption might not be as much of an issue with vaccines, as compared to therapeutics. Nevertheless case by case considerations must be undertaken.

Finally, several nanotechnology approaches focus on improving drug delivery of encapsulated drugs via transport through M cells. However, M cells constitute 5% of the epithelial cells of the FAE in humans [146]. This means that M cells covers only ca. 1% of the total intestinal surface. Moreover, it is known that normal enterocytes can also transport particles to a certain extent. Therefore, this recently growing interest in exploiting M cells for the oral delivery of nanoparticles could be possibly unjustified [24]. In other words, it is questionable whether the increased transcytosis capacity of M cells compared to enterocytes can counterbalance their paucity in the gut.

Approaches to enhance the solubility of poorly soluble drugs

As mentioned earlier, the oral bioavailability of drugs depends on several factors including aqueous solubility, dissolution rate and drug permeability. Upon oral administration, the drug compound must be first dissolved in the fluids of the gastrointestinal lumen before it can be absorbed. Drugs with poor solubility in aqueous environments are associated with slow dissolution as well as erratic absorption and low oral bioavailability. Thus, poor aqueous solubility represents a major obstacle in achieving adequate oral bioavailability for a large percentage of drug compounds in drug development nowadays [50]. Among other techniques, nanosytems have been investigated as a means to enhance solubility and oral bioavailability of poorly soluble drugs.

Nanocrystals

One of these approaches is nanosization or production of drug nanocrystals. It is worth to emphasize that this approach does not rely on the use of carriers, but on having the drug itself in the nanometer range of size. Nanocrystals are produced by two basic approaches: 'top down' and 'bottom up' technologies. The 'top down' approach, also referred to as nanosizing, involves size reduction of large crystalline particles into nanoparticles by means of mechanical attrition [147]. The 'bottom up' approach is a classical precipitation method, but it is less common than the former method. However, combination of approaches combining a pre-treatment step such as precipitation with a size reduction step has been also reported [148]. Nanocrystal-based formulations have been explored as a means to increase the dissolution rate of the drug compound and hence its oral bioavailability [149–151], as well as to reduce fed/fasted state variability, reduce inter-subject variability [147] and obtain fast action onset [46].

According to Noyes–Whitney equation, the rate of dissolution is affected by the effective surface area and saturation solubility of dissolved drug [152]. Reduction in particle size is one way to increase the effective surface area and thus the dissolution rate. Interestingly, moving from micronization to nanosization, i.e. < 1 μm showed to result in pronounced increase in exposed surface area and consequently in the dissolution rate of the drug. Nanosizing was also found to increase the saturation solubility of the drug compound, resulting in a further increase in the dissolution rate [153]. In addition to this direct correlation between particle size and saturation solubility described by Ostwald-Freundlich's equation, reducing particle size creates high energy surfaces rendering nanocrystals, the high energy form, more soluble as compared to the low energy form microcrystals. The saturation solubility of RMKP 22 (an antibacterial compound) powder was approximately doubled when the particle size was reduced from a mean diameter of 2.4 μm to 300 nm. This principle of action of nanoparticles has been employed in many studies. One example is the formulation of nanocrystalline dispersions which consist of submicron solid drug particles dispersed in an aqueous vehicle. Nanometer-sized particles possess high surface energy and thus stabilizers are usually added to nanosuspensions to prevent agglomeration and aggregation of drug crystals [154]. Hanafy et al. [155] determined the bioavailability of the poorly water-soluble fenofibrate following oral administration in rats of suspensions containing nanosized drug particles as compared to microsized particles. A two-fold increase in oral bioavailability was obtained upon administration of nanosuspension as compared to the reference microsuspended formulation. The increase in saturation solubility and dissolution rate upon size reduction was found to enhance the absorption and consequently increase the oral bioavailability of fenofibrate. Dispersions of nanoparticles can however be processed and used for solid oral dosage forms development. In order to achieve the goal of this drug delivery technology, these preparations are designed to redisperse into discrete, i.e. non-aggregated or non-agglomerated nanoparticulate dispersions once in contact with aqueous environment. Enhanced dissolution behavior for nanocrystal loaded tablets prepared by direct compression compared to marketed tablets was obtained by [156]. Recently, nanocrystallization and cyclodextrin complexation techniques were combined in a new attempt to improve the dissolution of poorly soluble drugs: the formulation of a nanosuspension containing oridonin/cyclodextrin inclusion complexes, drastically improved oridonin dissolution and bioavailability compared to a simple suspension of the same drug [157].
This technique is therefore ideal for oral delivery of drugs with dissolution-limited oral bioavailability, i.e. drugs of biopharmaceutical classification system (BCS) class II and IV [147], albeit it will not be of value when bioavailability is affected by metabolic- and/or permeation-related issues [154]. There are some pharmaceutical products in market and many other drug candidates in the clinical trials that utilize nanocrystals to achieve their drug delivery goals. Many of these studies concentrate on the development of nanocrystal-based oral products and in second line on i.v. injectables. However, nanocrystals could be potentially used for other routes of administration depending on the emerging needs of the market. Nanocrystals for oral administration were the first products on the pharmaceutical market. Examples on nanocrystal products on market include Rapamune® (rapamycin, immunosuppressive), Emend® (aprepitant capsule, antiemetic), Tricor® and Triglide® (fenofibrate, hypercholesterolemia).

Polymeric nanoparticles

Incorporation of drugs in nanoparticles is another approach that offers opportunities for the modulation of both solubility and permeability of the drug. Since the unique properties of these nanocarriers would be imparted to the entrapped drugs, these systems could be used to improve

the oral bioavailability of class II and IV drugs [158].The incorporation of the poorly soluble drugs into these nano-sized particles means the reduction of drug particle size down to the submicron level which could significantly increase the solubility and dissolution rate and thus improve the oral bioavailability. In addition to the reduction in particle size, having the drug dispersed in the nanoparticles is another way of enhancing the effective surface area available for dissolution. Moreover, the dissolution properties of the polymers used to construct nanoparticles could govern the specific site of drug release and thus make it more available for absorption which in turn could increase the oral bioavailability. Dai et al. [84] has reported the use of pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A, a very poorly soluble drug. Based on X-ray powder diffraction studies, the drug was thought to be either in amorphous form or molecularly dispersed within the polymer, which might enhance its oral bioavailability. Besides the physical state of the drug, the ability of these pH-sensitive polymers to release the drug at specific absorption sites reduces its degradation by gastric acid and enzymes and thus further enhances the bioavailability.

Other colloidal drug delivery systems

Emulsified systems, micelles and liposomes have been also employed to enhance oral bioavailability of poorly soluble drugs. A common feature among these systems is the ability to incorporate the poorly soluble drug in a hydrophobic reservoir [159]. Micelles are core-shell structures containing hydrophobic cores and outer hydrophilic shells [46]. Because micelles are soluble in aqueous environments, the incorporation of poorly soluble drugs into the micelle would impart solubility to the entrapped drug. Polymeric micelles were shown to increase the solubility of poorly soluble drugs in water, such as anti-cancer agents as tamoxifen [10] and paclitaxel [160,161] and could thus improve their oral bioavailability. Liposomes, which are vesicular structures composed of phospholipid bilayer surrounding an aqueous compartment, have been also investigated for enhancing the oral bioavailability of poorly soluble drugs, such as fenofibrate [162] and cyclosporine A [163]. Moreover, emulsions have been widely used for improving the oral bioavailability of class II and IV drugs [10]. More recently, microemulsions and nanoemulsions have been employed for their unique features as compared to conventional emulsions, including stability, droplet size, viscosity and energy required for manufacturing. Poorly soluble drugs are preferably incorporated into the internal phase of oil in water (o/w) emulsion to enhance their solubility and dissolution rate [45]. Enhanced oral bioavailability was reported with micro- [164] and nanoemulsion [165] based formulations. The most advanced approach of emulsion based drug delivery systems are the self-emulsifying drug delivery systems (SEDDS) [10]. These systems consist of drug dissolved in oils and stabilized by surfactants in a similar way to previously mentioned emulsions, yet they form micro- or nano-emulsions in situ upon exposure to aqueous environment. SEDDS have been reported to increase solubility and oral bioavailability of poorly soluble drugs, such as paclitaxel [166] and glipizide [167].

Conclusion

The aim of this Chapter was to elucidate the significance of nanotechnologies in overcoming some of the barriers to the oral delivery of drugs. From a physical and biological point of view, formulations in the nanometer range of size behave differently than molecules and materials in the large scale, including conventional oral formulations. Hence there is a need to understand the

unique interaction of the various nano-sized formulations with the components of the GI tract (i.e. fluids, mucus and epithelium). Certain nanosystems can favor the solubilization and bioavailability of poorly soluble drugs in the GI fluids, yet other formulations can offer protection of labile drugs against degradation in the GI fluids. Moreover, different nanoparticles can also be classified based on their interaction with the mucus. This interaction with the mucus is itself of great importance to determine the faith of the nanosystems and the overall bioavailability of the incorporated drugs. Finally, nanoparticles can be taken up and absorbed in the intestine depending on their size and surface characteristics, albeit in small amounts.

On the light of the concepts reviewed in this Chapter, a final consideration could be done. In respect to small molecules with poor solubility, the exploitation of nanotechnology systems has brought clear benefits in terms of improved oral drug bioavailability. This is testified, for example, by the fact that many dosage forms containing nanocrystals are already available in the market. Despite the potential use of nanotechnologies for the delivery of large molecule therapeutics, such as proteins and nucleic acids, these systems have not shown to provide successful oral delivery for clinical applications. An examination of the relevant literature suggests that the current approaches used for oral protein delivery, including nanotechnology "are based on a simple assumption that may not reflect the reality" [70]. Taking as most relevant example insulin: if insulin could be ever delivered orally, this would bring tremendous benefits to diabetic patients, as well as incredible profits to the manufacturers. Nevertheless, nanotechnology has not demonstrated yet that insulin can be delivered orally in sufficient amounts and even more importantly in a reproducible manner: the most promising studies have shown a maximum of 10 to 20% insulin oral bioavailability in rats [33,106]. This low bioavailability would result in high manufacturing costs to deliver standard doses of the drug, as well as variability in absorption. Such variability in absorption would be unacceptable for a drug with narrow therapeutic window, such as insulin. Therefore, there is clearly a big scientific and technological gap between showing some absorption of insulin upon oral administration in rats and producing a reliable formulation to treat diabetic patients [70]. It must however be said that although these initial proofs of concepts *in vitro* and in animals represent simple investigations without a direct correlation to humans, these studies could constitute an essential first step for a better understanding of the oral delivery of biopharmaceuticals. These initial investigations will hopefully help to pose the basis for the development of effective oral dosage forms of proteins in future.

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