Expert Opinion

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Particle uptake by Peyer's patches: a pathway for drug and vaccine delivery

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Particle uptake by Peyer's patches offers the possibility of tailoring vaccines that can be delivered orally. However, particle uptake by the follicle-associated epithelium in the gastrointestinal tract depends on several different factors that are the physicochemical properties of the particles, the physiopathological state of the animal, the analytical method used to evaluate the uptake and finally the experimental model. These parameters do not allow a clear idea about the optimal conditions to target the Peyer's patches. The goal of this review is to clarify the role of each factor in this uptake.

Keywords: microparticles, nanoparticles, oral vaccine, Peyer's patches, protein delivery

Expert Opin. Drug Deliv. (2004) 1(1):141-163

1. Introduction

In recent years, advances in biotechnology and biochemistry have led to the discovery of a large number of therapeutically active molecules, such as proteins, peptides and nucleic acids. In most cases, such compounds are indicated for chronic therapy and will need to be administered by an appropriate formulation.

The oral route of administration represents a great challenge for the development of effective and safe formulations. However, the bioavailability of proteins and nucleic acids after oral administration is limited by their degradation by digestive enzymes present in the gastrointestinal (GI) tract. The design of a carrier able to transport these fragile molecules across the mucosal barrier without degradation remains a major goal. Many delivery systems have been described as carriers to improve the transport of macromolecules across the GI mucosa [1]. Among these systems, the polymeric nano- and microparticles have been shown to allow the encapsulation of macromolecules inside the polymeric matrix and, when needed, the protection against hydrolytic and enzymatic degradation [2-6].

The uptake, after oral administration, of nano- and microparticles by cells located along the GI tract has recently received much attention because of the possibility of using these pathways to deliver macromolecules. However, in order to achieve an efficient absorption, nano- and microparticles need to be taken up from the GI tract with a sufficient rate and extent.

Since the 1980s, the gut-associated lymphoid tissue (i.e., Peyer's patches [PPs]) has been considered the primary site of particle uptake due to the presence of a follicle-associated epithelium (FAE). The FAE is characterised by the presence of M cells, which have been shown to facilitate the trancytosis of particulate material [7-10]. Despite the large amount of literature concerning particle uptake by PPs, the best physicochemical parameters (size, zeta potential, hydrophobicity, coating with adhesion factors) of particles allowing an optimal uptake by PPs are not clearly defined. Moreover, numerous discrepancies in the literature have led to an incoherent and contradicting set of data concerning the extent of particle uptake by PPs. In

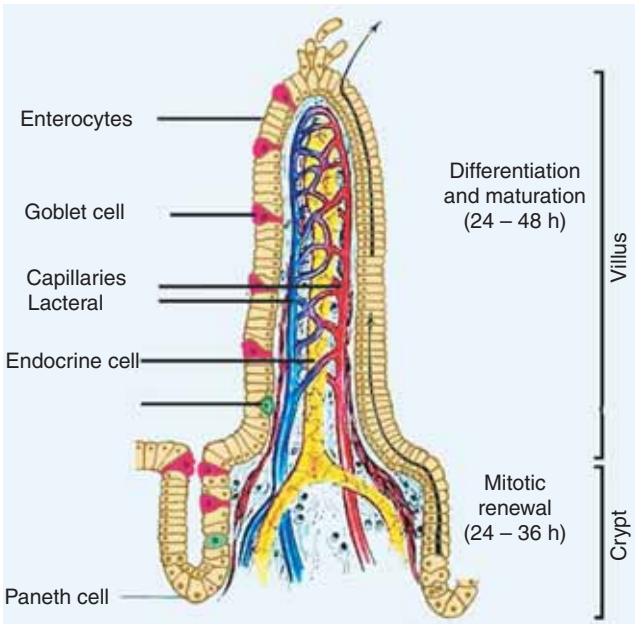

Figure 1. Structure of the intestinal villus.

addition, due to the use of a wide range of polymer types and different sizes of particles, and the large variety of analytical methods and models that have been employed to investigate particle uptake, it is difficult to draw a precise conclusion [11].

The aim of this paper is to review the physicochemical properties of particles, which have been described to affect their uptake by PPs, and their interdependence with physiological factors. The different approaches available to study particle uptake by PPs will also be discussed.

2. Structure of the mucosa of the small intestine

Although consisting of only a single layer of cells, the intestinal epithelium controls the passage of macromolecules and pathogens, and, at the same time, allows the digestive absorption of dietary nutrients. The mucosa is organised into three fundamental structures: the villi, the crypts (Figure 1) and the gut-associated lymphoid tissues (Figure 3).

2.1 The villi

Villi project into the lumen and are covered predominantly with mature, absorptive enterocytes, along with occasional mucus-secreting goblet and endocrine cells. The function of enterocytes is to take up and transport nutrients into the blood, fulfilling the basic function of the digestive system. Indeed, enterocyte apical surfaces are covered by rigid, closely packed microvilli, the tips of which contain integral membrane mucin-like glycoproteins that form a continuous, filamentous brush-border glycocalyx [12]. This thick (400 – 500 nm) layer contains adsorbed pancreatic enzymes and stalked intramembrane glycoprotein enzymes responsible for terminal digestion. It also serves as a diffusion barrier that prevents direct contact of many macromolecular aggregates, particles, viruses and bacteria with the microvillus membrane [13]. Thus, the glycocalyx has a protective function, preventing the uptake of antigens and pathogens, although also providing a highly degradative microenvironment that promotes the digestion and absorption of nutrients. The goblet cells secrete the mucus gel layer, a viscous fluid composed primarily of highly glycosylated proteins (mucins) suspended in a solution of electrolytes. The mucus gel layer has many functions, including protection against shear stress and chemical damage, and the entapment and elimination of particulate materials and microorganisms. Finally, the endocrine cells regulate the digestive function by secreting enteric hormones, such as cholecystokinin and secretin.

2.2 The crypts

Intestinal crypts, or crypts of Lieberkühn, are moat-like invaginations of the epithelium around the villi, and are lined largely with younger epithelial cells which are involved primarily in secretion (Figure 1). Towards the base of the crypts, located along the length of intestinal crypts, are undifferentiated stem cells, which continually divide to replenish the epithelial cells that die (enterocytes and goblet cells). Small intestinal crypt cells called Paneth cells, which are usually found clustered in groups of three to six, are located in the deep part of an intestinal crypt (Figure 1) [14,15]. The main defence molecules secreted by Paneth cells are α-defensins, known as cryptdins [16]. These peptides have hydrophobic and positively-charged domains that allow their insertion into biological membranes, where they interact with one another to form pores that disrupt membrane function, leading to cell death [17]. Due to the higher concentration of negatively-charged phospholipids in bacterial membranes compared with vertebrate membranes, defensins preferentially bind to, and disrupt, bacterial cells. In addition to defensins, Paneth cells secrete lysozyme and phospholipase A2 [14,15,18-20], both of which have clear antimicrobial activity [21]. These secretary molecules provide Paneth cells with a 'potent arsenal' against a broad spectrum of agents, including bacteria, fungi and some enveloped viruses.

2.3 The mucosal immune system

The mucosal immune system is divided into two sites: the inductive site, where an immune response is initiated after antigen uptake; and the effectors sites, where the immune response is expressed. The effectors sites are loosely organised and divided into the lamina propria lymphocytes (LPLs) and the intraepithelial lymphocytes (IELs). LPLs are lymphocytes scattered in the lamina propria of the mucosa. A majority of these cells are IgA-secreting B cells and memory T effector cells [22]. IELs are lymphocytes that are positioned in the basolateral spaces between luminal epithelial cells, beneath the tight junctions. IELs consist mainly of cytotoxic T cells, and have been divided into two subsets: type A and B, based on their mode of antigen recognition. Responses of type A IELs are major histocompatibility complex (MHC) restricted and

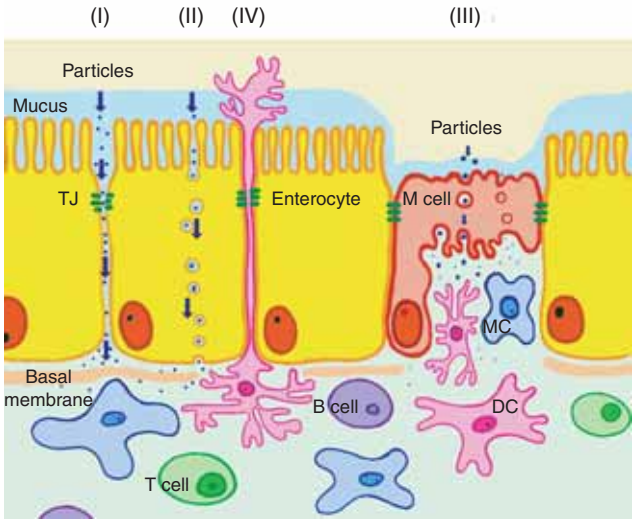

Figure 2. Schematic presentation of the FAE overlying PPs and the routes of macromolecule, microorganism and particle transport across the FAE. The FAE is characterised by the presence of highly specialised M cells. M cells differ from their neighbouring enterocytes by the absence of the brush border, a thinner mucous gel layer on the apical side and by the presence of a hollow cavity space in the basolateral side. M cells transport macromolecules, particles and microorganisms from the gut by endocytosis in the apical surface into basolateral cavity hollow space where particles or macromolecules are released by exocytosis. M cell hollow space contains lymphocytes and a few macrophage, which can interact with transported antigen or microorganisms. The transport of soluble and particulate antigen by M cells is the first step in producing a mucosal immune response. There are three possible transport routes for macromolecules, microorganisms and particles: **(I)** paracellular transport; **(II)** transcellular uptake across enterocytes by endocytic process; **(III)** endocytosis by M cells. An alternative pathway was suggested **(IV)** to uptake bacteria directly from the gut by dendritic cells [68].
DC: Dendritic cells; FAE: Follicle-associated epithelium; PP: Peyer's patch; TJ: Tight junction.

directed against peptides derived from invading pathogens. Type B IELs displaying limited T cell receptor diversity are not restricted by classical MHC molecules [23].

The inductive sites are marked by the presence of organised lymphoid tissues [24]. They generally consist of lymphoid follicles composed of assemblies of immature B cells, and adjacent T cells areas [24]. These lymphoid follicles are grouped in large PPs visible to the naked eye [24]. The FAE overlying a single follicle has unique biochemical features which make it a key portal of intestinal uptake of intact macromolecules and microorganisms.

2.3.1 Structure and function of the follicle-associated epithelium

The FAE in the intestine differs from the villus by its cellular phenotypes and biochemical properties. The FAE contains few or no goblet and enteroendocrine cells [24]. The FAE is characterised by little or no mucus gel and a decrease in defensin- and lysosyme-producing Paneth cells in the follicle-associated crypts [24,25]. Moreover, the brush border of the FAE contains low levels of membrane-associated hydrolases involved in the digestive functions [24,26,27]. The essential characteristic of the FAE is the presence of highly specialised M cells (Figure 2). The specialised epithelial M cells deliver samples of foreign material through an active transepithelial-vesicular transport from the lumen directly to intraepithelial-lymphoid cells and to organised mucosal lymphoid tissues that are designed to process antigens and initiate mucosal immune responses. The M cell transport system appears to be a key to the pathogenesis of certain bacterial and viral diseases [28] and for the effectiveness of mucosal vaccines [29].

2.3.1.1 Structure of intestinal M cells

M cells, as with enterocytes, are highly polarised with two major plasma membrane domains (apical and basolateral), and are in contact with adjacent epithelial cells through tight junctions and interdigitations (Figure 2) [30]. However, M cells and absorptive enterocytes differ from each other both morphologically and biochemically [31]. Morphologically, the microvillus of the apical surfaces of M cells are irregular, shorter in length and fewer in number than those in enterocytes (Figure 2). M cells also lack the thick filamentous brush border glycocalix and have a thinner mucus gel layer [13]. The basolateral membrane of M cells is usually deeply invaginated, forming an intraepithelial hollow space occupied by B and T lymphocytes, dentritic cells and macrophages. Biochemically, M cells also lack the secretary IgA antibodies and have very low levels of brush border enzymes and lysosomes [28]. The properties of fully differentiated M cells, particularly their poor degradative enzyme microenvironment, suggest that endocytosed macromolecules can cross the barrier intact without being significantly degraded. Hence, M cells appear to regulate the immune response by serving as gatekeepers for proteins and microorganisms in the mucosa-associated lymphoid tissue.

2.3.1.2 Origin of intestinal M cells

It has been reported that the entire epithelial substitution is accomplished every 3 – 4 days [32,33], and that the intestinal epithelium, as well as M cells, are derived from undifferentiated stem cells present in crypts. These specialised crypts supply cells to the FAE on one side and enterocytes of adjacent villi on the other side [34-37]. These observations have been supported by experimental studies using M-cell-specific lectins [38] and monoclonal anti-M antibodies [39], which demonstrate the presence of M cells within the proliferative zone of follicle crypts prior to the formation of the intraepithelial hollow space. However, these data do not exclude the possibility that fully differentiated enterocytes can be transformed into M cells under appropriate conditions such as antigenic stimulation. For example, the transfer of pathogen-free mice to a normal animal house environment [40], or *in vivo* antigenic stimulation using

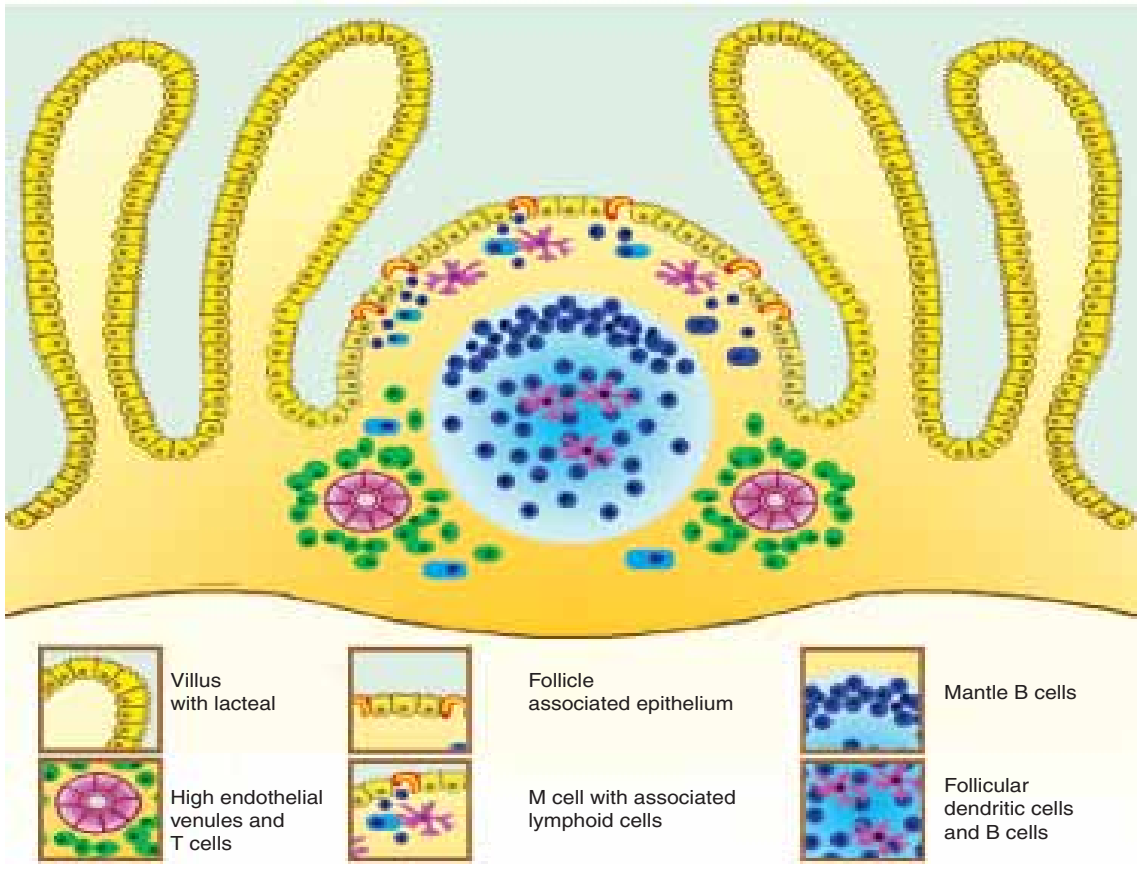

Figure 3. **Structure of Peyer's patches**. Histologically Peyer's patches can be divided into four regions: **(I)** germinal centre, which is packed with reticular cells and large lymphocytes; **(II)** follicular area surrounding the germinal centre and containing dense, small lymphocytes, among which small numbers of large lymphocytes and reticular cells are distributed; **(III)** the parafollicular area, which surrounds the follicular area. This area is T cell-dependent – predominant cell types include $CD8^+$ cytotoxic or suppressor T cell and $CD4^+$ T helper cells. In this area, lymphocytes are packed less densely than in the follicular area. This area also contains post-capillary veins and lymphatic vessels. **(IV)** The dome area is situated just above the follicular area and projects into the intestinal lumen. This area is the centre of antigen processing and presentation – predominant cell types include lymphocytes, macrophages and dendritic cells.

Salmonella typhimurium [41] or *Streptococcus pneumaniae* [42], induces an increase in M cell numbers. The authors suggest that under these conditions, enterocytes could be transformed to M cells. Others suggest that antigenic stimulation may induce an increase surface area occupied by M cells, due to the migration of lymphocytes and mononuclear cells to the M cell cavity [35,43-45]. Furthermore, enterocytes being the origin of M cells could be supported by studies demonstrating that co-culture of an intestinal epithelial cell line (Caco-2) with PP lymphocytes induces the epithelial cells to be transformed into M-like cells [46-50]. Moreover, injection *in vivo* of a similar lymphocyte preparation into the duodenal mucosa of recipient mice at sites lacking organised lymphoid tissues, also induces the formation of PP-like structures, including an M cell-containing epithelium analogous to the normal FAE [46]. These observations suggest that fully differentiated enterocytes can be transformed into M cells [48,51]. However, the transformation of enterocytes into M cell *in vitro* does not necessarily imply that similar transformations occur *in vivo* because Caco-2 cells display crypt-like cell properties (i.e., the ability to divide) [52]. However, in either case, local factors in the lymphoid tissue are likely to influence the development of PPs. Further research should be carried out to establish the factors which seem to increase M cell number, or their surface area in FAE. Results of such studies may offer a potential application in mucosal vaccine delivery.

2.3.2 Peyer's patches

PPs are collections of subepithelial lymphoid follicles burgeoning amongst villi and distributed throughout the small intestine, mainly on the antimesenteric side, from pylorus

to the ileocaecal valves, in a wide variety of animals including birds and mammals. PPs can be divided into four regions (Figure 3):

- The germinal centre, which is packed with reticular cells and large lymphocytes.
- The follicular area surrounding the germinal centre and containing dense small lymphocytes, among which a small number of large lymphocytes and reticular cells are distributed.
- The parafollicular area, which surrounds the follicular area. This area is T cell-dependent and the predominant cell types found include $CD8^+$ cytotoxic or suppressor T cell and $CD4^+$ T helper cells. In this area, lymphocytes are packed less densely than in the follicular area. This area also contains postcapillary veins and lymphatic vessels.
- The dome area situated just above the follicular area, projecting into the intestinal lumen. This area is the centre of antigen processing and presentation. Predominant cell types include lymphocytes, macrophages and dendritic cells. PP follicles are separated from the lumen by the FAE.

2.3.3 *Isolated lymphoid follicles*

Isolated lymphoid follicles (ILFs) are forms of lymphoid aggregations which have been identified in the small intestine in human [53], rabbit [54], mice [55] and guinea-pig [56]. ILFs are invisible from the serosal or mucosal surface of the small intestine. They are structurally and functionally similar to the follicular units that compose PPs and are believed to be an equivalent or complementary system to PPs for the induction of intestinal IgA antibody response [55,57,58].

3. Transport pathways of synthetic particles across the intestinal barrier

Conceptually, there are two routes by which a macromolecule or a particle can pass across the intestinal epithelia: between (paracellular uptake or transport) adjacent cells or through (transcellular uptake or transport) adjacent cells (Figure 2).

3.1 Paracelluar transport

Paracellular uptake was first described to explain the rapid apparition of orally administered particles in the blood [59-61]. It was also reported that paracellular passage between enterocytes was the major mechanism of intestinal uptake of large particles (> 1 µm). Biopsy observations by electron-scanning microscopy revealed a loosening of the tight junctions of the intestinal mucosa that occurred in the immediate neighbourhood of the goblet cells, enabling the passage of the particles. Moreover, it has been reported that polyalkylcyanoacrylate nanocapsules (< 300 nm), containing lipiodol, and administered in the lumen of an isolated ileal loop, were also able to pass through the ileal mucosa of the rat via a paracellular pathway in the non-follicular epithelium [62,63]. However, the paracellular spaces represent < 1% of the intestinal mucosa surface. They are sealed by tight junctions and the pore diameter of these junctions is < 10Å, which does not explain any significant paracellular uptake of colloidal particles. Nevertheless, paracellular transport can be improved by enhancers, such as chitosan [64], polyacrylate [65], starch [66] and thiolated polymers [67]. These polymers can enhance the paracellular transport of macromolecules by interactions between the negatively-charged cell membrane and the positive charges on the polymer, or by a complex of Ca^{2+} involved in the structure of the tight junctions. However, these observations of increasing paracellular permeability were mostly observed in cell culture models and were validated for macromolecules. Their relevance for the *in vivo* situation and particle uptake is not clear.

A recent mechanism, mediated by dendritic cells and independent of M cells has been reported for bacterial uptake [68]. Dendritic cells can express tight junction proteins and penetrate the gut epithelial monolayers to sample bacteria. There are no data, at present, about particle uptake using this pathway.

3.2 Transcellular uptake

Transcytosis is a particular process by which macromolecules or particles, are taken up by enterocytes lining the intestinal epithelia. This occurs through an endocytic process which takes place at the apical plasma membrane of the epithelial cells. It is followed by transport to the contralateral plasma membrane and release into the basolateral compartment. The transcellular uptake of particles, suggesting an endocytic process at the apical cell membrane followed by exocytic release at basolateral compartment, was first proposed by Sanders and Ashworth [69]. They observed the fate of polystyrene nanoparticles of a mean diameter of 220 nm within jejunal epithelial cells, 1 h after intragastric administration to rats. The nanoparticles were numerous in the cytoplasm of the epithelial cells, where they were enclosed in vesicles. Particles were also observed in narrow intercellular spaces. After 2 – 4 h following oral administration, nanoparticles were observed in the interstices of the lamina propria and in the lymphatic of the mucosa. The transport of nanoparticles by the transcellular pathway was also supported by Mathiowitz and collaborators [70]. They showed that nanoparticles made of poly(fumaric-co-sebacic anhydride), which display strong adhesive interactions with GI mucus and cellular linings, can pass across both the mucosal absorptive epithelium and the FAE covering the lymphoid tissue of PPs.

3.3 Lymphatic uptake via the M cells

Although there is some controversy in the literature on the extent of particle uptake by PPs, there is evidence that particle translocation can occur in PPs. It has been thought that the highly specialised M cells, which are present in the FAE overlying PPs, play a major role in the endocytosis of nano- and microparticles. However, the complete mechanism of uptake and endocytic transport by M cells is unclear, although it is generally accepted that the entry into M cells occurs via this

Table 1. Extent of particle uptake by gastrointestinal tract.

Polymer	Animal model	Administered dose (particles)	Particle size (μm)	Analytical method	Uptake (%)	Ref.
Polystyrene	Rat	Single dose 2.33×10^3 particles 2.33×10^6 particles 2.33×10^9 particles	2.139	Tissue digestion, particle extraction, direct counting by fluorescence microscopy	< 1	[149]
Polystyrene	Rat	Single dose 7.6×10^9 particles 2.8×10^8 particles	1 3	Tissue digestion, particle extraction, flow cytometry	2.1×10^{-6} 2.7×10^{-3}	[152]
Polystyrene	Rat	Single dose 2.15×10^9	2.25	Tissue digestion, particle extraction, direct counting by fluorescence microscopy	< 0.5	[132]
Polystyrene	Rat	Chronic administration (10 days) 1.25 mg/kg/day	0.05 0.1 0.3 0.5 1	Tissue digestion, polymer extraction, GPC (sum of stomach, small intestine, colon, liver, lungs, spleen, heart, kidney)	33.72 25.95 9.5 13.7 4.6	[80]
Polystyrene	Rat	Single dose: 12.5 mg/kg	0.05 0.5 1 3	GPC (blood)	9 1 0 0	[163]
Polystyrene	Rat	Chronic administration (5 days) 14 mg/kg/day	0.06	Tissue digestion, polymer extraction, GPC (sum of stomach, intestine, liver, spleen)	10	[154]
Polystyrene	Mouse	Single dose 10^6 particles 10^8 particles	2 – 9	Tissue digestion particle extraction, flow cytometry	0.01	[75]
Poly(lactide-co-glycolide)	Rat	Single dose 1.44×10^9 particles 1.83×10^8 particles	1 – 5 5 – 10	Fluorochrome extraction, fluorescence quantification	12.7 0.11	[144]

GPC: Gel permeation chromatography.

type of transport. Inert particulate can adhere to M cell surface by a nonspecific (adsorptive endocytosis) [71] or specific (active endocytosis) mechanism [72]. The particles are endocytosed and transported to the basolateral side, where they will be exocytosed into the M cell hollow space in contact with antigen-presenting cells. These particles may then be taken up by intestinal macrophages [73] or gut-associated dendritic cells [74].

4. Factors affecting particle uptake by M cells and Peyer's patches

Although a large number of studies have demonstrated that intestinal M cells have the capacity to take up particles from the gut lumen to subepithelial levels, the extent of this uptake is still a subject of discrepancy. Some authors have reported that the extent of *in vivo* uptake of polystyrene microparticles [75] as well as poly(lactide-co-glycolide) (PLGA) nano- and microspheres by PPs is < 0.01% of the total number of particles orally administered [76]. However, many other studies have shown that 2 – 3% of the ingested dose of submicron particles can be taken up (Table 1) [77]. It is evident that most

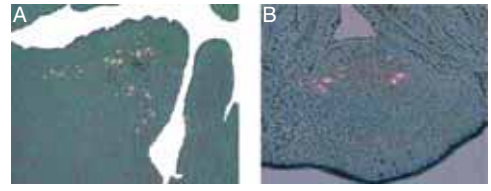

Figure 4. Histological analysis of the uptake by murine Peyer's patches 4 h after oral administration of single 20 mg of fluorescent poly(lactide-co-glycolide) nano- and microparticles. (A) Particle of mean diameter 1.1 μm. **(B)** Microparticles of mean diameter 3.9 μm. Reprinted from SHAKWEH M, CALVO P, GOURITIN B, ALPHANDARY H, FATTAL E: Uptake of biodegradable nano- and microparticles by Peyer's patches after oral administration to mice. *Proceeding of 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*, Florence, Italy (2002), with permission [84].

of the studies were conducted with non-biodegradable polymer polystyrene. However, the differences observed are largely due to the wide range of polymers used for preparing particles, their different sizes, different dose, and the large variety of analytical methods carried out to evaluate particle uptake

Table 2. The uptake of poly(lactide-co-glycolide) nano- and microparticles by murine PPs at different time intervals.

Particle size (µm)	15 min	1 h	4 h	24 h	48 h
0.3 ± 0.04	-	+++	+++	++	+
1.1 ± 0.09	-	+	+++	++	+
3.9 ± 0.62	-	+	++	+	+

(-) No fluorescence observed in PP sections;
(+++) Particle number/PP section > 100;
(++) Particle number/PP section < 100;
(+) Particle number/PP section < 10.
PP: Peyer's patch.
Reprinted from SHAKWEH M, CALVO P, GOURITIN B, ALPHANDARY H, FATTAL E: Uptake of biodegradable nano- and microparticles by Peyer's patches after oral administration to mice. *Proceeding of 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology*. Florence, Italy (2002), with permission [84].

by PPs [11]. Therefore, it is very difficult to conclude which of these studies is the more relevant. Indeed, many factors may influence the uptake of nano- and microparticles by PPs. These factors are relatively well known. They can be divided into three categories: the factors associated with the physicochemical properties of particles, such as the size, zeta potential, hydrophobicity/hydrophilicity balance and the presence of a ligand at the particle surface [78]; the factors associated with the physiology of the GI tract (gel mucus layer, glycocalyx); and the factors associated with the animal models used to evaluate the uptake by PPs, such as the animal species, age and the immuno- and physiopathological state.

4.1 Physicochemical properties of particles

4.1.1 Particle size

Particle size is a critical parameter for their uptake by PPs. It is now generally accepted that particles in the nanorange (< 1 µm) can be efficiently taken up by PPs [7,79-81]. However, it is more difficult to draw any conclusions for particles in the microrange (> 1 µm). For example, it has been shown that following oral administration, microparticles composed of PLGA of a mean diameter of < 10 µm were specifically taken up into the PPs. Particles ≥ 5 µm remained in the PPs for an extended period (35 days). Microparticles < 5 µm were disseminated in the mesenteric lymph nodes (MLNs), blood circulation and spleen [82]. However, McClean *et al.* [76] reported that microparticles of poly(lactide) (PLA) of 4 µm in size were excluded from the PPs. This observation is supported by Le Visage *et al.* [83] who reported that particles of poly(methylidene malonate 2.1.2) of 5.7 µm were not visible in the PPs. More recently, it has been shown that PLGA nano- and microparticle uptake, after oral administration in mice, was size- and time-dependent [84]. Although no particles were observed in PPs 15 min after oral administration, maximum uptake was observed at 4 h (Figure 4). Particles of 0.3, 1 and 3 µm were retained in the PPs for at least 48 h (Table 2).

4.1.2 Hydrophilicity/hydrophobicity balance

Apart from particle size, the surface hydrophobicity is also of importance for their uptake by PPs [82,85,86]. Indeed, it has been reported that hydrophobic polystyrene nanoparticles showed a strong affinity to M cells [80]. Furthermore, the uptake by PPs, following the oral administration to mice, of microparticles made of polymers with different hydrophobicity was investigated [82]. The microparticles composed of hydrophobic polymers such as polystyrene, poly(methyl methacrylate), poly(hydroxbutyrate), PLA and PLGA were taken up by PPs to a larger extent than those consisting of hydrophilic polymers, such as ethyl cellulose or cellulose acetate. In a chicken model, only microparticles made of hydrophobic PLGA (60 and 100 kDa) were taken up by PPs, whereas microparticles made of a less hydrophobic PLGA (20 kDa) were not visible in PPs [82].

4.1.3 Zeta potential

The zeta potential has been suggested to play an important role in particle uptake by PPs because M cells and the mucus gel layer (mucin and glycocalix) have a global negative charge, which should permit electrostatic interactions with particles from the opposite charge. It has been shown that carboxylated polystyrene microparticles, thus negatively charged, were taken up by PPs to a lesser extent than non-ionised particles. Non-ionised particles appear to have a greater affinity for M cells than ionised particles [7]. In order to better understand the role of particle zeta potential in PP uptake, nano- and microparticles of PLGA possessing different zeta potential were prepared using three different surface stabilising agents [87]. These agents were: poly(vinyl alcohol) (PVA), which is known to stabilise the PLGA particle surface; hydrophobically modified hydroxyethylcellulose (HMHEC) used to obtain more hydrophobic and neutral PLGA particles; and polyethyleneimine (PEI), selected to obtain positively-charged particles. The uptake by PPs, after oral administration have shown that PEI-coated positively-charged nano- and microparticles, had less affinity for PPs than PVA- and HMHEC-coated nano- and microparticles, which were negatively and neutrally charged, respectively. It is likely that strong electrostatic interactions between positive charges of PEI-coated nano- and microparticles and negative charges of glycocalix and the mucus gel layer slow down the progression and penetration of these particles towards the epithelial cell surface, thus reducing their uptake. However, PVA- and HMHEC-coated nano- and microparticles, of negative or close to neutral zeta potential, respectively, can move and penetrate across the mucus gel layer more easily because of interactions between the hydrophilic intestinal mucus and hydrophilic particle surface. This may allow particles to more efficiently reach the surface of the epithelial cell,

where they will be internalised. However, if zeta potential seems to play a role in the uptake, concerns could be raised regarding the changes that might occur in the surface charge when particles are in the GI tract medium.

4.1.4 Targeting particles to M cells

In order to enhance particle uptake by PPs, many authors have suggested the introduction of chemical modification on the surface of nano- and microparticles using specific ligands of M cell surface receptors. These ligands include M cell-specific lectins [72,88-91], secretary IgA [92,93] and anti-M cell mAbs [94].

It has been demonstrated that surface glycocalix of intestinal epithelial cells surface is composed of membrane-anchored glycoconjugates [91,95]. The composition of these glycoconjugates is dependent on their location in the intestine and the animal species [43,96,97]. Many studies have shown that the glycocalix of enterocytes of the FAE differs in the carbohydrate composition from that of enterocytes of adjacent villi [91,96,98]. Moreover, the lectin-binding properties of the M cell apical surface exhibites considerable variations, which are animal species- and intestinal site-dependent [43,98-102]. Lectin-binding sites being M cell-specific may, therefore, offer a potential means for M cell targeting using lectin-mediated delivery of nano- and microparticles. The interaction of latex nanospheres (0.5 µm) with mouse PPs was studied in a mouse loop model after the oral administration of microparticles covalently coated with lectins *Ulex europaeus* 1 (UEA1) or bovine serum albumin (BSA) [90]. UEA1-coated nanospheres bound to M cells at a level 100-fold greater than BSA-coated nanosphere. However, binding to enterocytes was unchanged, demonstrating the specificity of such targeting. This strategy presents some drawbacks. Indeed, the receptors for UAE1 are not specific to humans. Nevertheless, the presence of lectin on the particle surface could favour interactions with the mucus, even in a nonspecific fashion, and thus, increase the residence time. However, sugars from food might compete with this interaction and reduce the efficiency of such an approach.

Many studies have shown that immunoglobulins, particularly IgAs, can specifically interact with the M cell surface [103,104]. This is due to the presence of an IgA-specific receptor on the apical surface of M cells, which mediates the transepithelial transport of IgA from the intestinal lumen to the underlying gut-associated organised lymphoid tissues [105]. In order to demonstrate that this receptor may be useful for M cell targeting, the uptake of IgA-coated latex particles by M cells was evaluated in an intestinal loop model [92]. The results have shown that IgA-coated particles are efficiently taken up by M cells. However, coating particles with BSA caused a 20-fold reduction in the uptake. The use of anti-M cell mAbs may allow optimal M cell targeting. Furthermore, anti-M cell mAbs coated with polystyrene microparticles were found to accumulate in PPs of the rabbit intestinal loop at a level 3.5-fold higher than unconjugated particles [94].

Further studies in enhancing PP targeting using nano- and microparticles can thus focus on three principal axes. The first is to develop anti-M cell surface antigen mAbs [100,106-108]. The second may be the use of bacterial mechanisms of epithelial cell entry. As several microorganisms can specifically interact with intestinal M cells to invade the host [29,109,110], the use of the microbial adhesins responsible for their binding and internalisation by M cells may permit specific M cell targeting [111]. The third is to engineer biodegradable nano- and microparticles with a surface that is covalently coated with M cell-specific ligands (lectins, anti-M cell mAbs and/or bacterial adhesions). The potential of these carriers to target M cells should be tested *in vivo*. Indeed, it has not been shown in large animals or humans that this strategy is more efficient than the administration of a very high number of particles.

4.2 Gastrointestinal tract physiology

The initial step in particle uptake by PPs is the attachment to the apical surface of the intestinal epithelial cells. Particles, must, therefore, get over the mucus gel layer and bind to cell surface receptors by specific or nonspecific interactions. They can then be endocytosed. Different parameters related to the physiological conditions of the GI tract can influence the first steps before uptake occurs.

4.2.1 The intestinal mucus gel layer

Intestinal mucus, composed of a high-molecular-weight glycoprotein, covers the mucosa with a continuous adherent layer. The primary function of the mucus is to protect the GI mucosa from harmful pathogens and chemicals. The thickness of the mucus gel layer varies regionally in the range of 50 – 500 µm. The major component of the mucus is mucin, a highly heterogeneous glycoprotein that is negatively charged because of the of sulfate and sialic acid content [112]. The mucus acts as a barrier to particle uptake by entrapping them, causing their agglomeration and increasing their net size. This results in a decrease in the diffusion coefficient through the mucus, thereby restricting diffusion to the mucosa layer. *In vitro* study of the permeability of the intestinal mucin to carboxylate-modified polystyrene microparticles of five different sizes in the range of 0.1 – 1 µm were carried out [113]. A sharp decrease in mucin permeability with increasing particle diameter was observed. A gradual decrease in the permeability was then observed as the diameter was increased to 0.5 µm, after which no further significant decrease occurred. Furthermore, the extent of association of nanoparticles of different surface composition was investigated using a mucus-secreting cell culture [71]. The results showed that mucus significantly decreased the association of hydrophobic polystyrene nanoparticles, whereas the association of chitosan nanoparticles with mucus increased, but almost no binding was observed for hydrophilic stealth PLA–PEG nanoparticles. It has been suggested that electrostatic interactions between positively-charged chitosan and the

negatively-charged mucus were involved [71]. *In vivo* study of the influence of the mucus gel layer on latex nano- and microparticules crossing towards apical surface of epithelial cells has shown that the gel layer does not act as an absolute barrier for the diffusion of particles, but slows down the diffusion of these particles through the mucus [114]. Moreover, in order to investigate *in vivo* in the rat intestinal loop model the effect of the intestinal mucus on the uptake of fluorescent polystyrene microparticles (3 µm), *N*-acetylcysteine (NAC) was used as a mucolytic agent. The intestinal permeability and subsequent transport of microparticles to MLNs were observed to establish the role of the mucus gel layer in this process [115]. The number of particles within PPs and MLNs in the NAC-treated animals was significantly higher than that in the control group. These data suggest that the mucus gel layer is one of the important factors for particle uptake and thus, plays a major role in the permeability of the small intestinal and subsequent translocation to MLNs.

4.2.2 The filamentous brush border glycocalix

M cells generally lack an organised brush border and a well-defined filamentous brush border glycocalix (FBBG), although their apical membranes have a thin glycoprotein coat (20 – 30 nm) [13,116]. It has been shown that the FBBG on the apical surfaces of rabbit villi and FAE enterocytes is a size-selective barrier that can prevent particles from gaining access to membrane glycolipids [13,116]. This was demonstrated using the B subunit of cholera toxin (CTB), which binds specifically to ganglioside GM1, a glycolipid whose carbohydrate head extends 2.5 nm from the plasma membrane. The results showed that when applied in soluble form, CTB (6.8 nm) has free access to GM1 on the epithelial cell, and bound to enterocytes, FAE and M cells. However, CTB-coated colloidal gold nanoparticles (28.8 nm) failed to adhere to enterocytes, but did adhere to M cells. Furthermore, CTB-coated, latex fluorescent microparticles (1.13 µm) failed to adhere to enterocytes or M cells *in vivo*, or to differentiate Caco-2 intestinal epithelial cells significantly *in vitro*. However, these particles bound to undifferentiated Caco-2 cells that lacked brush border and glycocalix. Thus, 20 – 30 nm of glycocalix was sufficient to prevent access of 1 µm microparticles to the glycolipid receptor. Moreover, it has been reported that this glycoprotein layer is sufficient to prevent CTB-coated polystyrene nanoparticles (120 nm) from gaining access to the M cell surface [116]. This observation implies that some bacterial adhesion molecules, such as CTB, may not be useful for M cell targeting of particulate vaccine carriers [116].

4.3 Factors associated with the animal model

According to the animal species, there are important variations in the numbers and distribution of M cells and in the expression of surface antigens [24]. These variations may influence the efficiency of particle uptake by PPs.

4.3.1 Animal species

The extent of particle uptake by PPs depends on the number and frequency of M cells present in the FAE. In humans, PPs develop well before birth and a considerable number can be identified at ~ 24 weeks of gestation. When adolescence is reached, > 200 patches are found in the small intestine, and at an age of > 90 years ~ 50 patches are observed. PPs are not only present in the ileum, but also in the duodenum and jejunum [117]. Moreover, in several species such as sheep, calves, and pigs, there are two types of PP in the small intestine, which differ in location, structure, cellular composition, and function. First, several discrete patches can be found in the jejunum and the upper ileum, which are comparable to human PPs. Second, a long and continuous patch is present in the terminal ileum [118]. The continuous ileal patch influences to a great extent the development of B lymphocytes. In mice, the mean number of PPs in the small intestine is nine. They are generally more numerous and larger in the distal small intestine than in the proximal small intestine [119]. In the New Zealand White rabbit, PPs appear 15 days after birth [120]. Their number increases from the duodenum to the ileum and varies with the age of the animal reaching 40 – 50 follicles.

It has been showed that the number of M cells in humans is < 10% of all the epithelial cells in the dome region of the PPs [121]. Similar numbers have been reported for rats and mice [99,122,123]. Higher numbers of M cells (30 – 50%) are present in rabbit [124-126] and pig [127] PPs. Therefore, it is not surprising that the comparison of the uptake of polystyrene microparticles by murine FAE is lower than in rabbit FAE [128]. Moreover, three rodent species were ranked according to the extent of uptake of latex microparticles (3 µm). Particle uptake was in the order: rats > hamsters > mice [129].

From these observations, it should be stressed that results of particle uptake by PPs obtained for one type of animal must be considered with caution when correlating these data to other models, especially to humans.

4.3.2 Animal age

In several species, the number and size of PPs in older animals are larger than in younger animals. Therefore, it is likely that the extent of particle uptake by PPs in older animals may be greater. Indeed, it has been reported that, after repeated oral administration of latex microparticles (1.8 µm) to young (24 days) and aged (18 months) mice, the extent of particle uptake by PPs was higher in aged animals [130]. Moreover, it has been found that in young animals, only 87 particles were counted in the lymph thoracic duct, whereas a marked increase in the uptake was observed in 5-month-old animals (up to 775), but there was a decrease to 518 particles in older animals (9 months) [131]. These findings were unexpected considering the generally rapid absorption of nutriments and small molecules by the intestine of young animals. This may result from differences in the phagocytic activities of the cells involved in particle internalisation in animals of different ages. Moreover, Lefevre *et al.* [130] suggested that the better

uptake in older animals may be due to a slower transit time, increasing the contact of particles with lymphoid tissue surfaces; therefore, augmenting the uptake. In another study, the uptake of latex microparticles (2 µm) by PPs of weanlings (25 days), adults (5 months) and aged rats (15 months) was compared after oral administration. The number of particles detected in PP excised from the small intestine did not significantly differ between animals of different age groups [132]. The authors suggested that these apparent controversial observations may result from differences in the method of particle quantification. For example, Simon *et al.* [132] expressed the number of particles/g of tissue, whereas Lefevre *et al.* [130] expressed it per six PP/mouse, and as PPs in older animals are larger than in younger animals, they are likely to contain higher numbers of particles.

4.3.3 Physiopathological state of animal

Immuno- and physiopathological factors, such as the gut microbial content [35,40,41], acute and chronic stress [133] and diabetes [134], may modulate the barrier function of the FAE and thus modify its ability to transport macromolecules and particles. Indeed, it has been shown that tacrolimus and cyclosporin, which produce immunosuppressive effects, in part by decreasing the number of immunoreactive cells in the FAE of PPs, also reduce the uptake and transport of particles into PPs [135]. The presence of bacteria can modulate particle transport. For example, the transport of latex microparticles was significantly increased in the *Streptococcus peumoniae* R36a-infected animals compared with buffer-treated controls [35]. Furthermore, chronic stress enhances the uptake of luminal antigens and bacteria via the FAE [133]. This mechanism might also occur for synthetic particles. Finally, after oral administration of latex microparticles (2 µm) to diabetic rats, it was shown that particle translocation and peripheral distribution were reduced by ~ 30%. This could be a consequence of gastric retention and altered intestinal motility and permeability, which are associated with diabetes [134].

4.4 Experimental conditions

Many experimental protocols have been carried out to study the uptake of particles by PPs. These have shown that differences in particle dose and numbers [9,75,130,131], diet composition [136] and the volume of the particle suspension medium [137] can significantly influence the GI uptake of particles.

It has been demonstrated that the uptake of latex microparticles by PPs is dose dependent. The highest single dose of latex microparticles was correlated with the greatest number of particles retained in PPs [75,131]. Moreover, the uptake of latex microparticles (5.7 µm) by PPs was studied in mice after single (2×10^8 particles) or chronic (2×10^8 particles/day for 30 days or 4.5×10^8 particles/days for 60 days) oral administration. The 5.7 µm particles were observed in PPs and MLNs only for the higher dose given for 60 days. Moreover, at 77 days, these particles were still present in the tissues. Differences in particle uptake by PPs between fed and fasted mice have been observed. A greater number of particles were detected in PPs of fed mice. This may be due to an increase in GI transit time and/or an increase in M cell activity because of the presence of food [75]. Moreover, it has been shown that diet composition can influence particle uptake. A larger number of particles was transported from the gut lumen to the internal organs, including the MLN, spleen and liver of animals fed a solid pelleted diet, compared with those maintained on a fluid diet 4 h after oral administration [136]. Finally, it has been reported that the rapid appearance in the bloodstream and the number of latex microparticles (0.87 µm) increased with the volume of water used as the suspending vehicle. However, increasing the tonicity of the fluid was not as effective as using water as suspending medium for the transferring particles [137].

5. Evaluation methods of particle uptake by intestinal mucosa

In order to determine the extent and efficiency of nano- and microparticle uptake by PPs, and their binding to villi after oral administration, a wide variety of analytical methods are used. The large number of evaluation method may partially explain the difficulty in defining the best physicochemical parameters of particles which allow them to be efficiently and rapidly taken up by the GI tract, and particularly by PPs [11].

5.1 Qualitative determination of particle uptake

Histological methods have been mainly used to investigate qualitatively the fate of particles after oral administration. This allows to observe the presence and location of particles within the intestinal mucosa to be observed. After oral administration of particles, intestinal samples are collected and washed to reduce the contamination coming from particles that remain in the lumen. Tissue samples are then prepared by an adapted procedure for observation by light, fluorescence and/or electron microscopy.

5.1.1 Light and fluorescence microscopy

To observe non-labelled particles in intestinal mucosa by light microscopy, the whole PPs are fixed in 70% alcohol and cleared by a potassium hydroxide–glycerol mixture [9,78,138-140]. Particles taken up by PPs are distinguished by their round shape and refractivity. However, the analysis of tissue sections by fluorescence or confocal microscopy is preferable because of the high sensibility of these methods. These last techniques have been widely applied to the analysis of the tissular presence of fluorescent nano- and microparticles [82,86,141-144]. Confocal microscopy allows a direct observation of the specimen and determination of the exact particle location within the tissue, and not merely adherent to the tissue surface as an artefact of the sectioning process [92,145,146]. Moreover, simultaneous labelling of the cell membrane, nucleus and particles with different fluorochrome allowes evidence of the intracellular presence of fluorescent particles, as shown in leukocytes

of PPs [71]. Light [9,138-140] and fluorescence microscopy [13,116,134,145,146] have also been used to quantify the efficacy of particle uptake by PPs. In this case, the quantification of uptake by PPs allows to demonstate the influence of the physicochemical properties of particles on their uptake by PPs and interactions with villi to be determined. However, one should consider particle quantification by direct visual counting in histological section as a semiquantitative method, because of the difficulty of particle enumeration in the whole tissue sample.

5.1.2 *Electron microscopy*

Scanning and transmission electron microscopy have not only been used to study the morphological aspects of the FAE, PPs and M cells [25,147], but also to determine the cellular location and transport pathway of particles taken up from the intestinal lumen [63,148]. Observations by scanning electron microscopy of the non-follicular ileal mucosa and PP biopsies after intraluminal administration of lipiodol-loaded nanocapsules of polyalkylcyanoacrylate in an isolated ileal segment of the rat has revealed that nanocapsules are able to pass through the ileal mucosa, via a paracellular pathway in the non-follicular epithelium, and most predominantly, via M cells and adjacent enterocytes in PPs [63]. Furthermore, the use of transmission electron microscopy was applied to study the uptake of particulate material by PPs of pigs [148]. Uptake from the gut lumen was shown to be restricted to the dome epithelium and was almost exclusively performed by the M cells.

In summary, although histology is a fastidious method for following particle uptake, it gives precious information about the intestinal distribution of particles and the different parameters that may influence their uptake by PPs and binding to villi.

5.2 Quantitative determination of particle uptake

5.2.1 *Particle counting*

Particle quantification in the whole intestinal tissue can be assessed after tissue digestion, either by acidification [9] or alkalinisation [134,145]. This method has been applied to the quantification of non-biodegradable particles, whether fluorescent [130,143,149-151] or not [9]. Using this method, it has been possible to detect and quantify by flow cytometry a small number of particles, to measure the course of uptake and clearance, and to determine the tissue distribution of absorbed particles [75,93]. Flow cytometry has also been used to quantify the latex particles directly in the mesenteric lymph collected via a cannula connected to the major lymphatic vessels of the intestinal regions containing PPs [152]. For particles made of biodegradable polymers, such as PLGA or PLA, particle number can be determined by the quantification of the total fluorescence emitted by the sample. In this case, the fluorochrome encapsulated in biodegradable particles is extracted from the tissue sample before being quantified [81,153] or measured directly after tissue digestion [76]. To avoid fluorescence quenching due to the conditions of preparation and the interactions between the fluorescent marker and intestinal proteins, it is important to prepare series of standard from control tissue to which known amounts of fluorescent particles are added. Moreover, this approach does not allow distinguishing between fluorochrome released from particles before their uptake and fluorochrome remained associated to particles after tissue penetration. It is, therefore, suitable to minimise the release of fluorochrome from particles by multiple washing steps and particle dialysis and to determine the kinetics of release of fluorochrome from particles in artificial gastric and intestinal media [87]. Furthermore, as particles will cross cellular membranes and will be in contact with lipidic compounds, it is suggested that probe release should be checked in both aqueous and lipidic phases in the biphasic mode (e.g., liposomes) [11].

5.2.2 *Gel permeation chromatography*

Gel permeation chromatography (GPC) is based on the separation of polymeric materials as a function of their molecular weight. After tissue dissolution, particles are solubilised and the polymer is then extracted before GPC analysis. This technique necessitates the determination of the efficiency of the extraction procedure, and quantification is subsequently obtained using a calibration curve. This technique has been adopted by Jani *et al.* [80,154,155] to quantify the uptake and translocation of latex nano- and microparticles orally administered to animals. This method is particle size-independent, but it can give evidence on the tissular presence of particles and seems to be more reliable than the use of radiolabelled polymer.

5.2.3 *Radioactivity assay*

Radiolabelled nano- and microparticles are presented as an interesting approach to obtain quantitative information concerning the fate of orally administered particles and their subsequent tissular distribution, because of its high sensitivity which allows the detection of a small amount of radiolabelled polymer in different organs [156]. Radiolabelling of particles may be realised using a radioactive polymer [83,157,158] or by the encapsulation of a radiolabelled molecule as a tracer [159-161]. After oral administration of radiolabelled particles, samples of different organs are harvested and the radioactivity is quantified. In this method, it may be difficult to distinguish between the radioactivity due to particles themselves and that given by possible degradation products of the polymer [157]. Indeed, the presence of radioactivity in the bladder after oral administration of radiolabelled particles has been suggested as evidence of the polymers' degradation products of particles excreted in urine [80,157]. In an *in vitro* study of the transport of poly(butylcyanoacrylate) nanoparticles through PPs in an intestinal segment freshly isolated from pigs, it has been found that the amount of radioactivity transported across the intestine during 4 h was 1.1%. Part of this radioactivity that was not determined may result from degradation products and not from intact particles [158]. In order to know whether radiolabelled particles cross the intestinal barrier as particles,

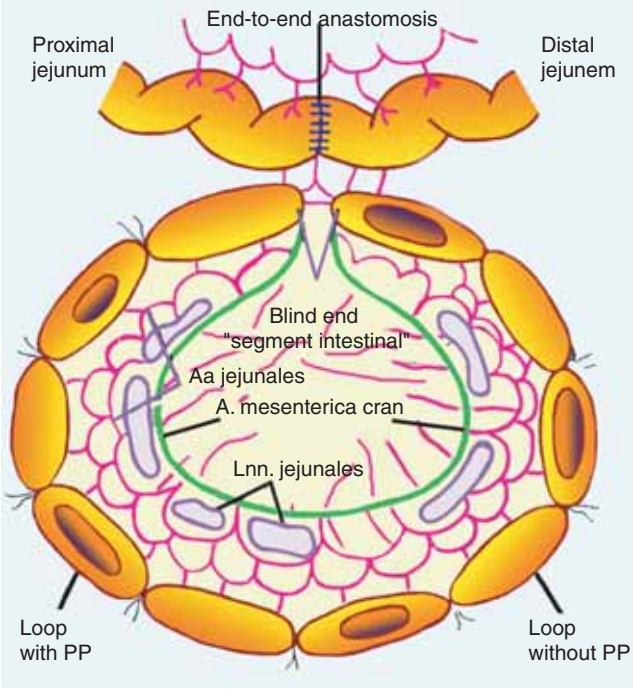

Figure 5. Presentation of the multiple intestinal loops model used as an *in vivo* model to analyse multiple mucosal immune responses [171,172]. Reprinted from *Journal of Immunological Methods*, 256, GERDTS V, UWIERA RR, MUTWIRI GK *et al.*, Multiple intestinal 'loops' provide an *in vivo* model to analyse multiple mucosal immune responses, 19-33, Copyright (2001), with permission from Elsevier.
PP: Peyer's patch.

or as degradation products, the degradation of the of particles or the kinetic release of the encapsulated radioactive molecule in simulated gastric and pancreatic fluids, need to be determined [83,157,160].

6. Experimental models

Various experimental models have been used to investigate the interaction between particles and the GI epithelium, and their eventual uptake by PPs. It has been suggested that differences between these models might be a source of contradictory reports regarding the extent and efficacy of particle uptake by the GI tract [11]. Experimental parameters, such as dosing modes, experimental settings, animal species, and the nature of harvested organs, might be the cause of the wide diversity of data obtained for particle uptake (Table 1).

6.1 *In vivo* models

6.1.1 Means of administration

In *in vivo* models, particles are either directly introduced into bottles containing drinking water, which will be taken by the animal for several days or even months [138-140,162], or given by direct repeated daily gavages of known doses [7,9,80,86,130,163] or a single known dose [83,84,87,149,150]. When introduced in to the drinking water, the amount of particles is not well defined, as the exact volume drunk by the animal is not known precisely [11]. The uncertainty regarding the dose and coincident events of absorption and elimination make these experiments difficult to interpret. Therefore, investigators have preferred to use a known dose fed by repeated daily gavages, or a single dose, which make the interpretation easier.

6.1.2 In situ isolated gut loop model

This model has been widely used to investigate particle transport across the GI tract and uptake by PPs [115,164]. This technique consists, in the administration, of particle suspension in an isolated intestinal segment from the gut, and the ligation of its two extremities without cutting the connection with blood and lymphatic supplies. It is then possible to compare the uptake of particles and their binding to intestinal epithelia in different zones of the intestine, with or without PPs, in the same animal. Moreover, as particle suspension is administered *in situ*, this model minimises variations due to gastric emptying or transit time. However, this technique can only be achieved in an unconscious animal and anaesthesia has been reported to reduce the lymphatic flow [165-168].

6.1.3 In vivo chronically isolated ileal loop model

In this model, the animal is conscious throughout the experiment. The surgical technique consists of a long-term isolation of an intestinal segment inside the animal. This isolated loop is used in perfusion experiments. The effects of anaesthesia and surgical trauma on the intestinal mobility and the lymph flow rate are absent. In addition, absorption kinetics can be analysed on the basis of luminal concentrations or blood levels of the perfused molecules [169]. This model was first used to study the absorption kinetics of soluble molecules [169]. It has since been applied to the determination of the intestinal transit of the bioadhesive microparticles after *in situ* injection into the perfused gut segment [170]. The results revealed that the model is particularly useful to evaluate and differentiate the bioadhesive properties of each delivery system.

More recently, a multiple sterile intestinal loop model has been described in sheep to analyse the potential of antigen-loaded microparticles as an oral vaccine delivery system (Figure 5) [171,172]. After animal anaesthesia, a midline abdominal incision was made and the jejunum exteriorised. A total of eight consecutive jejunal PPs were identified and the intestinal segment containing the selective PP was transected at each end. The continuity of the jejunum was re-established with an end-to-end anastomosis. To subdivide the intestinal segment into multiple loops, silk ligatures were tied 20 cm proximal and distal to each PP to create a space containing an individual PP. Antigen-loaded microparticle were injected into an individual loop. This model permitted induction of multiple mucosal immune responses in a single animal. It also provides the opportunity to deliver antigen into interspaces

devoid of PPs and to investigate the role of diffuse lymphoid nodules in the induction of mucosal immunity.

6.2 *Ex vivo* models

The successful application of *ex vivo* models of intestinal drug absorption depends on the extent to which the model comprises the relevant characteristics of the *in vivo* biological barrier. Despite the difficulties associated with trying to reproduce all the characteristics of the intestinal mucosa *ex vivo*, various systems have been developed which mimic, to varying degrees, the relevant barrier properties of the intestinal mucosa.

6.2.1 Excised intestinal tissue

Excised intestinal tissues have been used to study intestinal drug, nutrient absorption and particle uptake, and their binding to intestinal mucosa. The solution containing the test compound is applied to one side of the mucosa and the rate of drug absorption is determined by measuring either the disappearance of the drug or particle from the dosing solution, or the appearance of the drug or particle in the serosal side. Although they vary in complexity and versatility, excised tissue preparations share two important advantages: the preservation of the architectural integrity; and the ability to determine absorption across different GI segments. A common disadvantage is the limited viability of this type of preparation.

6.2.1.1 Perfused intestinal segments

In this model, part of the continuous ileum, with or without PPs, is excised and subdivided into small segments. Segments are then filled with the suspension of particles or drug solution, ligaturated and placed in an adequate medium. After intervals, the particle suspension is removed from the segment, which is longitudinally cut and washed to remove residual adhering particles. Torche *et al.* [164] used this model to investigate the ability of pig ileal segments to transport PLGA microparticles from the intestinal lumen across the mucosa. They have demonstrated that PLGA microparticles were translocated by M cells and transported through the FAE in the dome area. This model was also used to study the influence of chitosan microparticles on the transport of prednisolone sodium phosphate across the GI tract [173]. The permeability of prednisolone entrapped in chitosan microparticles was enhanced drastically compared with the drug solution [173].

6.2.1.2 Everted intestinal sac

In the everted intestinal sac method, a segment of the intestine is harvested from an animal, everted, ligated at the ends and filled with an oxygenated buffer. The sac is then introduced into an adequate medium containing a known amount of microparticles or compound solution. The sac is then removed. The microparticles are then isolated, washed and lyophilised. The percentage of binding to the sac is determined by subtraction of the weight of residual spheres from the original tare weight. This technique was first used to study the intestinal absorption of solutes [174,175] and to evaluate the GI bioadhesive ability of nano- and microparticles [176-178].

6.2.1.3 Ussing chamber

To prepare intestinal mucosal sheets suitable for mounting in Ussing chambers, a longitudinal cut of an intestinal segment is made to produce a long mucosal sheet. This sheet is cut as needed to produce mucosal strips of adequate size to fit in the opening of the diffusion (Ussing) chamber. Although mucosal sheets are used with or without the underlying muscle layer, *in vivo*, the muscle layer is not a barrier to absorption. Thus, the removal of this muscle layer, a process known as stripping, is advantageous for two reasons. First, it removes an artificial permeability barrier, and second, stripped tissues can be oxygenated more efficiently. An Ussing chamber consists of two half-cells joined together, with the mucosal specimen mounted as a sheet between them. After mounting the tissue, the chamber is immediately placed in a heating block. The compound to be analysed is added in the donor chamber and samples are taken out of the receiving chamber for analysis. Ussing chambers are usually connected to voltage clamps, which are used to make electrical measurements during the course of experiments. These measurements can be used to monitor tissue integrity and viability. This model has been widely used to study drug and macromolecule transport across intestinal mucosa [179,180]. However, it does not give satisfying results for particle uptake by the GI tract. Although the literature provides evidence that particles adhere and cross intestinal mucosa after oral administration in rabbits, no uptake or adsorption of particles (fluorescent polystyrene, 0.05 – 3 µm) on the surface of intestinal tissue has been observed after incubation in the presence of excised rabbit intestine (with or without PPs) [11]. Moreover, when the Ussing chamber method was used to test uptake in excised intestinal tissues, of nanoparticles of polystyrene and PLGA, during oxygenation, nanoparticles were caught in oxygen bubbles and massively adhered to the plastic of the chamber [181].

In summary, these *ex vivo* models have the advantages of being rapid, thus allowing the analysis of several pieces of tissue at the same time. Therefore, it offers the possibility of running experiments on PP-containing and -free tissue in parallel. However, a common disadvantage is the limited viability of the tissue. Indeed, it has been demonstrated that the intestinal tissue from an abattoir degenerated 25 min post-mortem, resulting in progressive lysis, shedding and exfoliation of part of the epithelium, and an almost complete loss of villus architecture [181]. The authors concluded that nanoparticle uptake into the epithelium and the subepithelial tissue observed in the study could be due to autolytic artefacts. They suggested avoiding small intestine obtained from abattoirs and to use tissue from freshly sacrificed animals, which is excised within a few minutes post-mortem.

Table 3. Non-exhaustive tests of immune responses induced following oral administration of antigen-loaded particles.

Polymer	Antigen	Species	Particle size (µm)	Observations	Ref.
PLA	OVA	Mice	0.6, 1.0, 4.0, 7.0, 11.0, 15.0, 21.0, 26.0	Only microparticles of 4.0 µm enhanced serum antibody Microparticles of 7.0 µm enhanced IgA secretion to a significant extent, whereas those with 26.0 µm in were ineffective	[197]
SBPVA-g-PLGA	TT	Mice	0.1, 0.5, 1.5	Microparticles > 1 µm and TT in solution did not increase the serum IgG and IgA The highest antibody titres were found in the case of 0.1 µm	[198]
PLGA	PC–Thyr	Mice	< 10	Induction of specific IgA response in intestinal, pulmonary and vaginal secretions, as well as a strong specific systemic immune response	[3]
PLGA	ETEC-CS6	Human (healthy volunteers)	ND	Four of five volunteers had significant ASC IgA and significant serum IgG responses No protection studies were carried out	[199]
PLGA	BSA	Mice	0.20, 0.50, 1.0	Microparticles of 1 µm diameter elicited a higher serum IgG response than those of 0.50 or 0.20 µm diameters The immune response for particles of 0.50 µm diameter is similar than that obtained with particles of 0.20 µm diameter given by the s.c. and the p.o., and higher by the i.n. route	[200]
PLGA	OVA	Mice	3.0	After primary immunisation, IgA antibodies were detected in saliva, vaginal and gut washes, which were significantly greater than those detected with soluble antigen After secondary immunisation, higher antibody titres were found in three fluids The specific activity (antibody/µm IgA) of antibodies in vaginal fluid and saliva was significantly greater than in serum or gut washes	[193]
PELA	HPL	Mice	3.20 – 4.05	Increase in sIgA and IgG production in salivary and gut washes and antibody-secreting cells in PPs	[201]
Alginate	PSA	Sheep	9 –14	Induction of humoral immune responses However, PSA-specific T cell responses were not induced	[172]

ACS: Circulating antibody-secreting cells; BSA: Bovine serum albumin; ETEC-CS6: Enterotoxigenic *Escherichia coli*; HPL: *Helicobacter pylori* lysates; i.n.: Intranasal; i.p.: Intraperitoneal; OVA: Ovalbunin; PC–Thyr: Phosphorylcholine linked to thyroglobulin; PELA: Poly (D,L-lactide)-polyethylene glycol; p.o.: By mouth (*per os*); PSA: Porcine serum albumin; SBPVA-g-PLGA: Poly(sulfobutyl-vinyl-alcohol)-graft-poly(lactide-co-glycolide); s.c.: Subcutaneous; TT: Tetanus toxoid.

6.2.2 In vitro *models*

Human colon adenocarcinoma cell line, Caco-2, has been widely used to investigate particle interaction and transport across the human intestinal epithelium [71,76,153]. This cell culture model possesses many advantages, including the opportunity to study cellular mechanisms of particle transport across intestinal epithelium and rapid screening under controlled conditions. However, this cell culture model cannot be representative of PPs because, on the one hand, it is compromised by the lack of certain cell types present in normal intestinal epithelium (e.g., goblet cells and M cells), and, on the other hand, they do not produce the gel mucus layer. Therefore, a heterologous co-culture composed of freshly isolated murine PP cells and Caco-2 cells has been characterised and presented as an *in vitro* M cell model [46,47,49]. The morphology of M-like cells was studied by transmission electron microscopy, which demonstrated an irregularity in shape and fewer microvilli compared with the Caco-2 cells [49]. The phenotypic changes that occurred when enterocytes developed into M cells were then confirmed by quantification of M cell markers, such as the low expression of alkaline phosphatase, redistribution of villin, a cytoskeletal protein associated with the scaffold of microvilli, loss of brush border structure from the apical surface to cytoplasm, indicating the expression of a $5\beta_1$-integrin at the apical surface of M cells [50]. This co-culture was able to transport nano- (0.2 µm) as well as microparticles (1 – 2 µm) [49] of polystyrene. To obtain a more homologous co-culture based entirely on human cells, murine lymphocytes were replaced by a human Raji B cell line [48]. The apparition of human M-like cells was

confirmed by the identification of the human M cell marker, Sialyl Lewis A antigen. It has been demonstrated that this co-culture of Caco-2 cells and Raji B cells is not only able to transport latex nano- and microparticles [48], but also chitosan microparticles [182], which have been shown to be taken up by PPs *in vivo* [141].

It has been suggested that M cells may not be the only cell type involved in antigen sampling in PPs. Co-culture of Caco-2 cells and dendritic-like cells has been characterised. It has been showed that dendritic cells express tight junction proteins and penetrate gut epithelial monolayer to sample *Salmonella typhimurium* bacteria [68]. The presence of dendritic cells in such a co-culture model may be more representative to *in vivo* PPs and allow the study of their eventual role in the processing of particle transport across the FAE.

7. Potential of nano- and microparticles as oral drug/vaccine delivery systems

7.1 Nano- and microparticles for oral delivery of drugs

A number of studies have demonstrated that biodegradable particles can enhance the bioavailability of many drugs that are normally poorly absorbed following oral administration. The increase of bioavailability of tavincamine entrapped in poly(hexylcyanoacrylate) nanoparticles has been demonstrated [62]. These nanoparticles were also used for the oral delivery of insulin, resulting in prolonged hypoglycaemia in fasted rats [183]. In another study, nanoparticles made of polyanhydride copolymers of fumaric and sebacic acids have been shown to display strong adhesive interactions with the mucus gel layer [70]. These particles were shown to traverse the absorptive enterocytes and FAE. They increased the oral absorption of three model substances of different molecular weight (dicumarol, insulin and plasmid DNA). Both studies regarding insulin delivery led to impressive results. However, the question as to why they were not followed by other studies is still open. Recent work regarding the delivery of insulin-containing nanocapsules of poly(alkylcyanocrylate) has shown that there is a lot of variability in the concentration of insulin crossing the intestinal barrier. In addition, the authors did not observe any modification of the glycaemia, suggesting there are still questions regarding the first data and the decrease of glycaemia after oral administration of insulin-containing nanocapsules [184]. Moreover, nanoparticles have been shown to enhance the absorption of luteinising hormone-releasing hormone [185]. Microparticles of PLA were also used for the oral delivery of IFN [159].

7.2 Nano- and microparticles for oral delivery of vaccines

As most infections agents enter the body through mucosal surfaces, immunisation of these surfaces represents a potent mechanism of warding off the pathogen at the site of entry. The GI, nasopharyngeal, pulmonary and genitourinary surfaces are bathed in mucus that contain secretory IgA (sIgA) derived from the plasma cells underlying the mucosal membrane. Local sIgA is an important component of the mucosal immune system. The presence of sIgA can prevent infection of epithelial host cells. IgA can also remove antigen that crosses the epithelial barrier by transporting the antigen across the epithelium [186]. Because of their ability to induce a local secretary IgA response, oral vaccines potentially offer the advantage of increased efficacy in comparison with parenteral vaccines. Successful oral vaccination requires improvements to be made in the protection and delivery of peptide antigens in the GI tract, as well as their presentation to cells of the gut associated lymphoid tissue (GALT); such requirements might be met by using microparticles as vaccine carriers. Table 3 presents a non-exhaustive list of oral vaccination tests using biodegradable particles.

Polylactide (PLA), polyglycolide (PGA), and their copolymers (PLGA) are the principal polymers used to encapsulate vaccines. These polymers have the advantages of biocompatibility, biodegradability and a history of safe use in humans [187]. Particle degradation rate can be manipulated by changing factors, such as the molecular weight of the components, surface area, monomer stereoregularity and lactide:glycolide ratio [188-190]. Thus, considerable tailoring of the system is possible to optimise antigen presentation and, hence, the antibody response. PLG microparticles also offer the potential to entrap and deliver several antigens and adjuvants simultaneously [191]. One of the limitations of PLGA in relation to vaccine development is that these polymers are soluble in only a limited range of organic solvents and are insoluble in water. The most commonly used solvent for PLGA polymers is dichloromethane, although ethyl acetate and others have also been used. Hence, the technique most commonly used for the preparation of microencapsulated vaccines implies the emulsification of aqueous solutions of antigen into organic solvents containing the polymer. The resulting primary emulsion is then emulsified in external aqueous phase containing a surfactant. After organic solvent elimination by extraction or evaporation, the particles can be obtained by centrifugation. A significant problem with PLGA microencapsulation is the possibility of antigen denaturation as a consequence of exposure to organic solvent, elevated temperature and aqueous–organic interfaces during the procedure of particle preparation. Nevertheless, a number of proteins has been successfully encapsulated in PLGA particles with a full maintenance of structural and immunologic integrity and ability to induce mucosal immune response [2,3,5,79,192].

Following oral administration of PLG microparticles containing a toxoid vaccine for staphylococcal enterotoxin B to mice, circulating toxin-specific antibodies and a concurrent sIgA anti-toxin response in saliva, gut-wash fluid and bronchial alveolar wash fluid were detected, whereas no response was measured after oral dosing with the soluble antigen [82]. Another study demonstrated that the model antigen

ovalbumin, entrapped in 3 µm PLG particles, induced significantly enhanced sIgA and systemic IgG antibody responses following oral administration to mice, in comparison with the free protein [193]. The maximum salivary IgA response to antigen was 50-times greater than the response to the soluble antigen [193].

Alginate microparticles constitute a further type of microparticulate carrier that has demonstrated efficacy for the oral administration of vaccines [171,172,194,195]. The advantages of alginate in vaccine microencapsulation are that it is soluble in water and does not necessitate the use of organic solvents. Oral delivery of alginate microparticles elicited the production of sIgA at the mucosal surfaces of mice, rabbits and cattle, and resulted in an increased delayed-type hypersensitivity reaction in chickens [194]. Mild conditions of synthesis are required for the preparation of alginate microparticles, making them highly suitable as oral carriers for labile antigens.

8. Conclusion

In conclusion, nano- and microparticles offer considerable potential for drug and vaccine delivery via the GI tract. Perhaps greater therapeutic success can be achieved by using microparticles as carriers for vaccines, rather than for therapeutic drugs, because of the relative amount of antigen that is required to induce an immune response in comparison with the amount of drug required to produce a pharmacological response. Moreover, antigen-loaded nano- and microparticles offer the potential to be highly efficient because of their ability to induce a local and systemic immune response. Microparticles that target immune cells within PPs may be optimal for initiating IgA responses, but nanoparticles that can escape from the PP region to peripheral lymphoid organs might be chosen for generation of systemic IgG responses [196]. Nevertheless, this review has shown that optimisation of particulate delivery through targeting receptors of the M cells and/or using particles displaying the best size and the right surface properties is needed to obtain satisfactory systems that are relevant for human applications.

9. Expert opinion

Using particle uptake by PPs to deliver drugs or vaccines has raised a lot of interest in the last 20 years. It is now known that the extent of particle uptake by the PPs is very low and depends on the physicochemical properties of the particles, the intestinal environment and animal state. It is likely that because of this low uptake, only vaccine, and not drug, delivery could be achieved by this pathway. Indeed, stimulation of the mucosal immune response does not require a dose level such as for obtaining a pharmacological effect, and this allow a better prediction of suitability of this uptake for oral vaccine delivery. Moreover, controlling the time-release profile is probably less critical in the case of vaccines than for drugs, thus making the formulation of vaccinal products easier compared with drug delivery devices, which are intended to create a precisely defined pharmacokinetic profile. However, even in this case, from the promising results obtained in animal models, only a few systems have gone into clinical trials and at the moment there is no vaccine delivered by particles on the market. Therefore, in order to gain efficiency in the delivery of vaccine by particulate carriers, their uptake by adequate cells needs to be enhanced. What would be the best option?

- The identification of M cell-specific surface antigens and receptors that may permit the production of specific antibodies and ligands for M cell-selective targeting.
- The development of particles having optimal surface properties (i.e., hydrophobicity, zeta potential, coating).

Surface engineering and modification is much more accessible than attaching ligands to particles and monitoring their recognition efficiency. However, most of the models are not close to human application, which means they do not take into account parameters such as physicochemical and/or biochemical interactions with the diet or physiological state.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. HUSSAIN N, JAITLEY V, FLORENCE AT: Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv. Drug Deliv. Rev.* (2001) 50:107-142.
2. ALLAOUI-ATTARKI K, PECQUET S, FATTAL E *et al.*: Protective immunity against *Salmonella typhimurium* elicited in mice by oral vaccination with phosphorylcholine encapsulated in poly(DL-lactide-co-glycolide) microspheres. *Infect. Immun.* (1997) 65:853-857.
3. ALLAOUI-ATTARKI K, FATTAL E, PECQUET S *et al.*: Mucosal immunogenicity elicited in mice by oral vaccination with phosphorylcholine encapsulated in poly (D,L-lactide-co-glycolide) microspheres. *Vaccine* (1998) 16:685-691.
4. ALLEMANN E, LEROUX J, GURNY R: Polymeric nano- and microparticles for the oral delivery of peptides and peptidomimetics. *Adv. Drug Deliv. Rev.* (1998) 34:171-189.
5. FATTAL E, PECQUET S, COUVREUR P, ANDREMONT A: Biodegradable microparticles for the mucosal delivery of antibacterial and dietary antigens. *Int. J. Pharm.* (2002) 242:15-24.

•• Summary of application of oral administration of particles for stimulation and suppression of the immune system.

6. FATTAL E, ROQUES B, PUISIEUX F, BLANCO-PRIETO MJ, COUVREUR P: Multiple emulsion technology for the design of microspheres containing peptides and oligopeptides. *Adv. Drug Deliv. Rev.* (1997) 28:85-96.
7. JANI P, HALBERT GW, LANGRIDGE J, FLORENCE AT: The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. *J. Pharm. Pharmacol.* (1989) 41:809-812.
8. JEPSON MA, SIMMONS NL, SAVIDGE TC, JAMES PS, HIRST BH: Selective binding and transcytosis of latex microspheres by rabbit intestinal M cells. *Cell Tissue Res.* (1993) 271:399-405.
9. LEFEVRE ME, HANCOCK DC, JOEL DD: Intestinal barrier to large particulates in mice. *J. Toxicol. Environ. Health.* (1980) 6:691-704.
10. JEPSON M, SIMMONS N, O'HAGAN DT, HIRST BH: Comparison of poly(DL-lactide-co-glycolide) and polystyrene microsphere targeting to intestinal M cells. *J. Drug Target.* (2003) 11:269-273.
11. DELIE F: Evaluation of nano- and microparticle uptake by the gastrointestinal tract. *Adv. Drug Deliv. Rev.* (1998) 34:221-233.
12. MAURY J, NICOLETTI C, GUZZO-CHAMBRAUD L, MAROUX S: The filamentous brush border glycocalyx, a mucin-like marker of enterocyte hyper-polarization. *Eur. J. Biochem.* (1995) 228:323-331.
13. FREY A, GIANNASCA KT, WELTZIN R *et al.*: Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting. *J. Exp. Med.* (1996) 184:1045-1059.
14. OUELLETTE AJ, SATCHELL DP, HSIEH MM, HAGEN SJ, SELSTED ME: Characterization of luminal paneth cell alpha-defensins in mouse small intestine. Attenuated antimicrobial activities of peptides with truncated amino termini. *J. Biol. Chem.* (2000) 275:33969-33973.
15. OUELLETTE AJ, SELSTED ME: Paneth cell defensins: endogenous peptide components of intestinal host defense. *FASEB J.* (1996) 10:1280-1289.
16. AYABE T, SATCHELL DP, PESENDORFER P *et al.*: Activation of Paneth cell alpha-defensins in mouse small intestine. *J. Biol. Chem.* (2002) 277:5219-5228.
17. AYABE T, SATCHELL DP, WILSON CL, PARKS WC, SELSTED ME, OUELLETTE AJ: Secretion of microbicidal alpha-defensins by intestinal Paneth cells in response to bacteria. *Nat. Immunol.* (2000) 1:113-118.
18. DUBOUIX A, CAMPANAC C, FAUVEL J *et al.*: Bactericidal properties of group IIa secreted phospholipase A(2) against *Pseudomonas aeruginosa* clinical isolates. *J. Med. Microbiol.* (2003) 52:1039-1045.
19. OUELLETTE AJ: Paneth cells and innate immunity in the crypt microenvironment. *Gastroenterology* (1997) 113:1779-1784.
20. OUELLETTE AJ: Defensin-mediated innate immunity in the small intestine. *Best Pract. Res. Clin. Gastroenterology.* (2004) 18:405-419.
21. PORTER EM, BEVINS CL, GHOSH D, GANZ T: The multifaceted paneth cell. *Cell Mol. Life Sci.* (2002) 59:156-170.
22. BRANDTZAEG P, FARSTAD IN, HARALDSEN G: Regional specialization in the mucosal immune system: primed cells do not always home along the same track. *Immunol. Today* (1999) 20:267-277.
23. CAMPBELL DJ, BUTCHER EC: Intestinal attraction: CCL25 functions in effector lymphocyte recruitment to the small intestine. *J. Clin. Invest.* (2002) 110:1079-1081.
24. KRAEHENBUHL JP, NEUTRA MR: Epithelial M cells: differentiation and function. *Ann. Rev. Cell Dev. Biol.* (2000) 16:301-332.
•• Complete description of M cells.
25. OWEN RL: Uptake and transport of intestinal macromolecules and microorganisms by M cells in Peyer's patches-a personal and historical perspective. *Semin. Immunol.* (1999) 11:157-163.
26. OWEN RL, BHALLA DK: Cytochemical analysis of alkaline phosphatase and esterase activities and of lectin-binding and anionic sites in rat and mouse Peyer's patch M cells. *Am. J. Anat.* (1983) 168:199-212.
27. SAVIDGE TC, SMITH MW: Evidence that membranous (M) cell genesis is immuno-regulated. *Adv. Exp. Med. Biol.* (1995) 371A:239-241.
28. SIEBERS A, FINLAY BB: M cells and the pathogenesis of mucosal and systemic infections. *Trends Microbiol.* (1996) 4:22-29.
29. NEUTRA MR, FREY A, KRAEHENBUHL JP: Epithelial M cells: gateways for mucosal infection and immunization. *Cell* (1996) 86:345-348.
30. FASANO A: Novel approaches for oral delivery of macromolecules. *J. Pharm. Sci.* (1998) 87:1351-1356.
31. NEUTRA MR, MANTIS NJ, FREY A, GIANNASCA PJ: The composition and function of M cell apical membranes: implications for microbial pathogenesis. *Semin. Immunol.* (1999) 11:171-181.
•• Description of the mechanism of penetration of microorganisms through the M cell.
32. BHALLA DK, OWEN RL: Cell renewal and migration in lymphoid follicles of Peyer's patches and cecum-an autoradiographic study in mice. *Gastroenterology* (1982) 82:232-242.
33. SMITH MW, SYME G: Functional differentiation of enterocytes in the follicle-associated epithelium of rat Peyer's patch. *J. Cell Sci.* (1982) 55:147-156.
34. BYE WA, ALLAN CH, TRIER JS: Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. *Gastroenterology* (1984) 86:789-801.
35. GEBERT A, STEINMETZ I, FASSBENDER S, WENDLANDT KH: Antigen transport into Peyer's patches: increased uptake by constant numbers of M cells. *Am. J. Pathol.* (2004) 164:65-72.
36. KITAGAWA H, HOSOKAWA M, TAKEUCHI T, YOKOYAMA T, IMAGAWA T, UEHARA M: The cellular differentiation of M cells from crypt undifferentiated epithelial cells into microvillous epithelial cells in follicle-associated epithelia of chicken cecal tonsils. *J. Vet. Med. Sci.* (2003) 65:171-178.
37. LELOUARD H, SAHUQUET A, REGGIO H, MONTCOURRIER P: Rabbit M cells and dome enterocytes are distinct cell lineages. *J. Cell Sci.* (2001) 114:2077-2083.
38. GEBERT A, HACH G: Differential binding of lectins to M cells and enterocytes in the rabbit cecum. *Gastroenterology* (1993) 105:1350-1361.
39. PAPPO J: Generation and characterization of monoclonal antibodies recognizing follicle epithelial M cells in rabbit gut-associated lymphoid tissues. *Cell. Immunol.* (1989) 120:31-41.

•• One of the first papers describing targets on M cells.

40. SMITH MW, JAMES PS, TIVEY DR: M cell numbers increase after transfer of SPF mice to a normal animal house environment. *Am. J. Pathol.* (1987) 128:385-389.

41. SAVIDGE TC, SMITH MW, JAMES PS, ALDRED P: Salmonella-induced M-cell formation in germ-free mouse Peyer's patch tissue. *Am. J. Pathol.* (1991) 139:177-184.

42. BORGHESI C, REGOLI M, BERTELLI E, NICOLETTI C: Modifications of the follicle-associated epithelium by short-term exposure to a non-intestinal bacterium. *J. Pathol.* (1996) 180:326-332.

43. GEBERT A, POSSELT W: Glycoconjugate expression defines the origin and differentiation pathway of intestinal M-cells. *J. Histochem. Cytochem.* (1997) 45:1341-1350.

44. GEBERT A, FASSBENDER S, WERNER K, WEISSFERDT A: The development of M cells in Peyer's patches is restricted to specialized dome-associated crypts. *Am. J. Pathol.* (1999) 154:1573-1582.

45. SANSONETTI PJ, ARONDEL J, CANTEY JR, PREVOST MC, HUERRE M: Infection of rabbit Peyer's patches by Shigella flexneri: effect of adhesive or invasive bacterial phenotypes on follicle-associated epithelium. *Infect. Immun.* (1996) 64:2752-2764.

46. KERNEIS S, BOGDANOVA A, KRAEHENBUHL JP, PRINGAULT E: Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. *Science* (1997) 277:949-952.

47. KERNEIS S, PRINGAULT E: Plasticity of the gastrointestinal epithelium: the M cell paradigm and opportunism of pathogenic microorganisms. *Semin. Immunol.* (1999) 11:205-215.

48. GULLBERG E, LEONARD M, KARLSSON J *et al.*: Expression of specific markers and particle transport in a new human intestinal M-cell model. *Biochem. Biophys. Res. Commun.* (2000) 279:808-813.

49. LIANG E, KABCENELL AK, COLEMAN JR, ROBSON J, RUFFLES R, YAZDANIAN M: Permeability measurement of macromolecules and assessment of mucosal antigen sampling using *in vitro* converted M cells. *J. Pharmacol. Toxicol. Methods.* (2001) 46:93-101.

•• First description of *in vitro* model of PPs.

50. TYRER P, RUTH FOXWELL A, KYD J, HARVEY M, SIZER P, CRIPPS A: Validation and quantitation of an *in vitro* M-cell model. *Biochem. Biophys. Res. Commun.* (2002) 299:377-383.

51. KUCHARZIK T, LUGERING N, RAUTENBERG K *et al.*: Role of M cells in intestinal barrier function. *Ann. NY. Acad. Sci.* (2000) 915:171-183.

52. JEPSON MA, CLARK MA: Studying M cells and their role in infection. *Trends Microbiol.* (1998) 6:359-365.

53. MOGHADDAMI M, CUMMINS A, MAYRHOFER G: Lymphocyte-filled villi: comparison with other lymphoid aggregations in the mucosa of the human small intestine. *Gastroenterology* (1998) 115:1414-1425.

54. KEREN DF, HOLT PS, COLLINS HH, GEMSKI P, FORMAL SB: The role of Peyer's patches in the local immune response of rabbit ileum to live bacteria. *J. Immunol.* (1978) 120:1892-1896.

55. HAMADA H, HIROI T, NISHIYAMA Y *et al.*: Identification of multiple isolated lymphoid follicles on the antimesenteric wall of the mouse small intestine. *J. Immunol.* (2002) 168:57-64.

56. ROSNER AJ, KEREN DF: Demonstration of M cells in the specialized follicle-associated epithelium overlying isolated lymphoid follicles in the gut. *J. Leukoc. Biol.* (1984) 35:397-404.

57. LORENZ RG, CHAPLIN DD, MCDONALD KG, MCDONOUGH JS, NEWBERRY RD: Isolated lymphoid follicle formation is inducible and dependent upon lymphotoxin-sufficient B lymphocytes, lymphotoxin beta receptor, and TNF receptor I function. *J. Immunol.* (2003) 170:5475-5482.

58. FAGARASAN S, HONJO T: Intestinal IgA synthesis: regulation of front-line body defences. *Nat Rev Immunol.* (2003) 3:63-72.

59. VOLKHEIMER G, SCHULZ FH: The phenomenon of persorption. *Digestion* (1968) 1:213-218.

60. VOLKHEIMER G, SCHULZ FH, AURICH I, STRAUCH S, BEUTHIN K, WENDLANDT H: Persorption of particles. *Digestion.* (1968) 1:78-80.

61. ALPAR HO, FIELD WN, HYDE R, LEWIS DA: The transport of microspheres from the gastro-intestinal tract to inflammatory air pouches in the rat. *J. Pharm. Pharmacol.* (1989) 41:194-196.

62. APRAHAMIAN M, MICHEL C, HUMBERT W, DEVISSAGUET JP, DAMGE C: Transmucosal passage of polyalkylcyanoacrylate nanocapsules as a new drug carrier in the small intestine. *Biol. Cell.* (1987) 61:69-76.

63. DAMGE C, APRAHAMIAN M, HUMBERT W, PINGET M: Ileal uptake of polyalkylcyanoacrylate nanocapsules in the rat. *J. Pharm. Pharmacol.* (2000) 52:1049-1056.

64. SCHIPPER NG, OLSSON S, HOOGSTRAATE JA, DEBOER AG, VARUM KM, ARTURSSON P: Chitosans as absorption enhancers for poorly absorbable drugs 2: mechanism of absorption enhancement. *Pharm. Res.* (1997) 14:923-929.

65. PREDA M, LEUCUTA SE: Oxprenolol-loaded bioadhesive microspheres: preparation and *in vitro/in vivo* characterization. *J. Microencapsul.* (2003) 20:777-789.

66. BJORK E., ISAKSSON U., EDMAN P., ARTURSSON P: Starch microspheres induce pulsatile delivery of drugs and peptides across the epithelial barrier by reversible separation of the tight junctions. *J. Drug Target.* (1995) 2:501-507.

67. BERNKOP-SCHNURCH A, KAST CE, GUGGI D: Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems. *J. Control. Release* (2003) 93:95-103.

68. RESCIGNO M, URBANO M, VALZASINA B *et al.*: Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* (2001) 2:361-367.

• Demonstration of uptake of micoorganisms by dendritic cells.

69. SANDERS E, ASHWORTH CT: A study of particulate intestinal absorption and hepatocellular uptake. Use of polystyrene latex particles. *Exp. Cell Res.* (1961) 22:137-145.

70. MATHIOWITZ E, JACOB JS, JONG YS *et al.*: Biologically erodable microspheres as potential oral drug delivery systems. *Nature* (1997) 386:410-414.

71. BEHRENS I, PENA AI, ALONSO MJ, KISSEL T: Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm. Res.* (2002) 19:1185-1193.
• Contains an interesting technique of triple labelling for following particle uptake.

72. CLARK MA, HIRST BH, JEPSON MA: Lectin-mediated mucosal delivery of drugs and microparticles. *Adv. Drug Deliv. Rev.* (2000) 43:207-223.
• Interesting review on lectin-mediated uptake.

73. WELLS CL, MADDAUS MA, ERLANDSEN SL, SIMMONS RL: Evidence for the phagocytic transport of intestinal particles in dogs and rats. *Infect. Immun.* (1988) 56:278-282.

74. LOMOTAN EA, BROWN KA, SPEAKER TJ, OFFITA PA: Aqueous-based microcapsules are detected primarily in gut-associated dendritic cells after oral inoculation. *Vaccine* (1997) 15:1959-1962.

75. EBEL JP: A method for quantifying particle absorption from the small intestine of the mouse. *Pharm. Res.* (1990) 7:848-851.

76. MCCLEAN S, PROSSER E, MEEHAN E *et al.*: Binding and uptake of biodegradable poly(DL-lactide) micro- and nanoparticles in intestinal epithelia. *Eur. J. Pharm. Sci.* (1998) 6:153-163.

77. FLORENCE AT: The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. *Pharm. Res.* (1997) 14:259-266.

78. LEFEVRE ME, WARREN JB, JOEL DD: Particles and macrophages in murine Peyer's patches. *Exp. Cell Biol.* (1985) 53:121-129.

79. KOFLER N, RUEDL C, RIESER C, WICK G, WOLF H: Oral immunization with poly-(D,L-lactide-co-glycolide) and poly-(L-lactic acid) microspheres containing pneumotropic bacterial antigens. *Int. Arch. Allergy Immunol.* (1997) 113:424-431.

80. JANI P, HALBERT GW, LANGRIDGE J, FLORENCE AT: Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. *J. Pharm. Pharmacol.* (1990) 42:821-826.

81. DESAI MP, LABHASETWAR V, AMIDON GL, LEVY RJ: Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharm. Res.* (1996) 13:1838-1845.

82. ELDRIDGE JH, HAMMOND CJ, MEULBROEK JA, STAAS JK, GILLEY RM, TICE TR: Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the peyer's patches. *J. Control. Release* (1990) 11:205-214.
•• Comparison of uptake by Peyer's patches of several types of particles.

83. LE VISAGE C, COUVREUR P, MYSIAKINE E, BRETON P, BRU N, FATTAL E: *In vitro* and *in vivo* evaluation of poly(methylidene malonate 2.1.2) microparticles behavior for oral administration. *J. Drug Target.* (2001) 9:141-153.
•• Demonstration of particles that are not taken up by intestinal cells.

84. SHAKWEH M, CALVO P, GOURITIN B, ALPHANDARY H, FATTAL E: Uptake of biodegradable nano- and microparticles by Peyer's patches after oral administration to mice. *Proceeding of 4th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology.*, Florence, Italy (2002).

85. HILLERY AM, FLORENCE AT: The effect of adsorbed poloxamer 188 and 407 surfactants on the intestinal uptake of 60-nm polystyrene particles after oral administration in the rat. *Int. J. Pharm.* (1996) 132:123-130.

86. HOSHI S, UCHINO A, KUSANAGI K, IHARA T, UEDA S: Uptake of orally administered polystyrene latex and poly(D,L-lactic/glycolic acid) microspheres into intestinal lymphoid tissues in chickens. *Vet. Immunol. Immunopathol.* (1999) 70:33-42.

87. SHAKWEH M, BESNARD M, NICOLAS V, FATTAL E: Poly(lactide-co-glycolide) particles of different physicochemical proprties and their uptake by Peyer's patches in mice. (Submitted).

88. CLARK MA, JEPSON MA, SIMMONS NL, HIRST BH: Selective binding and transcytosis of *Ulex europaeus* 1 lectin by mouse Peyer's patch M-cells *in vivo*. *Cell Tissue Res.* (1995) 282:455-461.

89. CLARK MA, JEPSON MA, HIRST BH: Exploiting M cells for drug and vaccine delivery. *Adv. Drug Deliv. Rev.* (2001) 50:81-106.

90. FOSTER N, CLARK MA, JEPSON MA, HIRST BH: Ulex europaeus 1 lectin targets microspheres to mouse Peyer's patch M-cells *in vivo*. *Vaccine*. (1998) 16:536-541.

91. JEPSON MA, CLARK MA, HIRST BH: M cell targeting by lectins: a strategy for mucosal vaccination and drug delivery. *Adv. Drug Deliv. Rev.* (2004) 56:511-525.

92. PORTA C, JAMES PS, PHILLIPS AD, SAVIDGE TC, SMITH MW, CREMASCHI D: Confocal analysis of fluorescent bead uptake by mouse Peyer's patch follicle-associated M cells. *Exp. Physiol.* (1992) 77:929-932.

93. SMITH MW, THOMAS NW, JENKINS PG, MILLER NG, CREMASCHI D, PORTA C: Selective transport of microparticles across Peyer's patch follicle-associated M cells from mice and rats. *Exp. Physiol.* (1995) 80:735-743.

94. PAPPO J, ERMAK TH, STEGER HJ: Monoclonal antibody-directed targeting of fluorescent polystyrene microspheres to Peyer's patch M cells. *Immunology* (1991) 73:277-280.

95. GEBERT A, AL-SAMIR K, WERNER K, FASSBENDER S, GEBHARD A: The apical membrane of intestinal brush cells possesses a specialised, but species-specific, composition of glycoconjugates-on-section and *in vivo* lectin labelling in rats, guinea-pigs and mice. *Histochem. Cell Biol.* (2000) 113:389-399.

96. WOODLEY JF: Lectins for gastrointestinal targeting – 15 years on. *J. Drug Target.* (2000) 7:325-333.

97. SHARMA R, SCHUMACHER U: Carbohydrate expression in the intestinal mucosa. *Adv. Anat. Embryo.l Cell Biol.* (2001) 160:1-91.

98. GEBHARD A, GEBERT A: Brush cells of the mouse intestine possess a specialized glycocalyx as revealed by quantitative lectin histochemistry. Further evidence for a sensory function. *J. Histochem. Cytochem.* (1999) 47:799-808.

99. CLARK MA, JEPSON MA, SIMMONS NL, BOOTH TA, HIRST BH: Differential expression of lectin-binding sites defines mouse intestinal M-cells. *J. Histochem. Cytochem.* (1993) 41:1679-1687.

100. GIANNASCA PJ, GIANNASCA KT, FALK P, GORDON JI, NEUTRA MR: Regional differences in glycoconjugates of intestinal M cells in mice: potential targets for mucosal vaccines. *Am. J. Physiol.* (1994) 267:G1108-1121.

101. GIANNASCA PJ, GIANNASCA KT, LEICHTNER AM, NEUTRA MR: Human intestinal M cells display the sialyl Lewis A antigen. *Infect. Immun.* (1999) 67:946-953.

102. JEPSON MA, MASON CM, SIMMONS NL, HIRST BH: Enterocytes in the follicle-associated epithelia of rabbit small intestine display distinctive lectin-binding properties. *Histochem. Cell Biol.* (1995) 103:131-134.

103. ROY MJ, VARVAYANIS M: Development of dome epithelium in gut-associated lymphoid tissues: association of IgA with M cells. *Cell Tissue Res.* (1987) 248:645-651.

104. WELTZIN R, LUCIA-JANDRIS P, MICHETTI P, FIELDS BN, KRAEHENBUHL JP, NEUTRA MR: Binding and transepithelial transport of immunoglobulins by intestinal M cells: demonstration using monoclonal IgA antibodies against enteric viral proteins. *J. Cell Biol.* (1989) 108:1673-1685.

105. MANTIS NJ, CHEUNG MC, CHINTALACHARUVU KR, REY J, CORTHESY B, NEUTRA MR: Selective adherence of IgA to murine Peyer's patch M cells: evidence for a novel IgA receptor. *J. Immunol.* (2002) 169:1844-1851.

106. CLARK MA, HIRST BH, JEPSON MA: M-cell surface beta1 integrin expression and invasin-mediated targeting of *Yersinia pseudotuberculosis* to mouse Peyer's patch M cells. *Infect. Immun.* (1998) 66:1237-1243.

107. LELOUARD H, REGGIO H, MANGEAT P, NEUTRA M, MONTCOURRIER P: Mucin-related epitopes distinguish M cells and enterocytes in rabbit appendix and Peyer's patches. *Infect. Immun.* (1999) 67:357-367.

108. LELOUARD H, REGGIO H, ROY C, SAHUQUET A, MANGEAT P, MONTCOURRIER P: Glycocalyx on rabbit intestinal M cells displays carbohydrate epitopes from Muc2. *Infect. Immun.* (2001) 69:1061-1071.

109. CLARK MA, JEPSON MA: Intestinal M cells and their role in bacterial infection. *Int. J. Med. Microbiol.* (2003) 293:17-39.

110. SANSONETTI PJ, PHALIPON A: M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin. Immunol.* (1999) 11:193-203.

111. KEEGAN ME, WHITTUM-HUDSON JA, MARK SALTZMAN W: Biomimetic design in microparticulate vaccines. *Biomaterials.* (2003) 24:4435-4443.

112. NEUTRA MR, FORSTNER JF: *Gastrointestinal mucus: Synthesis, Secretion, and Function, Physiology of the gastrointestinal tract*. Johnson LR (Ed.), Raven Press, New York.(1987):975-1009.

113. NORRIS DA, SINKO PJ: Effect of size, surface charge, and hydrophobicity on the translocation of polystyrene microspheres through gastrointestinal mucin. *J. Appl. Poly. Sci.* (1997) 63:1481-1492.

114. SZENTKUTI L: Light microscopical observations on luminally administered dyes, dextrans, nanospheres and microspheres in the pre-epithelial mucus gel layer of the rat distal colon. *J. Control. Release.* (1997) 46:233-242.

115. KHAN J, IIBOSHI Y, CUI L, WASA M, OKADA A: Role of intestinal mucus on the uptake of latex beads by Peyer's patches and on their transport to mesenteric lymph nodes in rats. *J. Parenter. Enteral. Nutr.* (1999) 23:19-23.

116. MANTIS NJ, FREY A, NEUTRA MR: Accessibility of glycolipid and oligosaccharide epitopes on rabbit villus and follicle-associated epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.* (2000) 278:G915-923.

117. CORNES JS: Peyer's patches in the human gut. *Proc. R. Soc. Med.* (1965) 58:716.

118. GEBERT A, ROTHKOTTER HJ, PABST R: M cells in Peyer's patches of the intestine. *Int. Rev. Cytol.* (1996) 167:91H159.

119. ABE K, ITO T: Qualitative and quantitative morphologic study of Peyer's patches of the mouse after neonatal thymectomy and hydrocortisone injection. *Am. J. Anat.* (1978) 151:227-237.

120. FAULK WP, MCCORMICK JN, GOODMAN JR, YOFFEY JM, FUDENBERG HH: Peyer's patches: morphologic studies. *Cell. Immunol.* (1970) 1:500-520.

121. OWEN RL, ERMAK TH: Structural specializations for antigen uptake and processing in the digestive tract. *Springer Semin. Immunopathol.* (1990) 12:139-152.

122. CLARK MA, JEPSON MA, SIMMONS NL, HIRST BH: Differential surface characteristics of M cells from mouse intestinal Peyer's and caecal patches. *Histochem. J.* (1994) 26:271-280.

123. SMITH MW, PEACOCK MA: 'M' cell distribution in follicle-associated epithelium of mouse Peyer's patch. *Am. J. Anat.* (1980) 159:167-175.

124. PAPPO J, STEGER HJ, OWEN RL: Differential adherence of epithelium overlying gut-associated lymphoid tissue. *Lab. Invest.* (1988) 58:692-697.

125. GEBERT A, HACH G, BARTELS H: Co-localization of vimentin and cytokeratins in M-cells of rabbit gut-associated lymphoid tissue (GALT). *Cell Tissue Res.* (1992) 269:331-340.

126. JEPSON MA, SIMMONS NL, HIRST GL, HIRST BH: Identification of M cells and their distribution in rabbit intestinal Peyer's patches and appendix. *Cell Tissue Res.* (1993) 273:127-136.

127. GEBERT A, ROTHKOTTER HJ, PABST R: Cytokeratin 18 is an M-cell marker in porcine Peyer's patches. *Cell Tissue Res.* (1994) 276:213-221.

128. JEPSON MA, CLARK MA, FOSTER N *et al.*: Targeting to intestinal M cells. *J. Anat.* (1996) 189:507-516.

129. BHAGAT HR, WILLIAMS W, METELITSA D, MONATH TP: Investigation of microsphere uptake in animals. *Proceedings of the Controlled Release Society* (1994):579-580.

130. LEFEVRE ME, BOCCIO AM, JOEL DD: Intestinal uptake of fluorescent microspheres in young and aged mice. *Proc. Soc. Exp. Biol. Med.* (1989) 190:23-27.

131. SEIFERT J, HARASZTI B, SASS W: The influence of age and particle number on absorption of polystyrene particles from the rat gut. *J. Anat.* (1996) 189:483-486.

132. SIMON L, SHINE G, DAYAN AD: Effect of animal age on the uptake of large particulates across the epithelium of the rat small intestine. *Int. J. Exp. Pathol.* (1994) 75:369-373.

133. VELIN AK, ERICSON AC, BRAAF Y, WALLON C, SODERHOLM JD: Increased antigen and bacterial uptake in follicle associated epithelium induced by chronic psychological stress in rats. *Gut.* (2004) 53:494-500.

134. MCMINN LH, HODGES GM, CARR KE: Gastrointestinal uptake and translocation of microparticles in the streptozotocin-diabetic rat. *J. Anat.* (1996) 189:553-559.

135. FUJIMURA Y, OWEN RL: Tacrolimus (FK506) and cyclosporine reduce the uptake and transport of particles into rabbit Peyer's patches. *Transplantation* (2002) 73:1049-1054.

136. SIMON L, WARREN I, DAYAN AD: Effect of solid and liquid diet on uptake of large particulates across intestinal epithelium in rats. *Dig. Dis. Sci.* (1997) 42:1519-1523.

137. EYLES J, ALPAR O, FIELD WN, LEWIS DA, KESWICK M: The transfer of polystyrene microspheres from the gastrointestinal tract to the circulation after oral administration in the rat. *J. Pharm. Pharmacol.* (1995) 47:561-565.

138. LEFEVRE ME, OLIVO R, VANDERHOFF JW, JOEL DD: Accumulation of latex in Peyer's patches and its subsequent appearance in villi and mesenteric lymph nodes. *Proc. Soc. Exp. Biol. Med.* (1978) 159:298-302.

139. LEFEVRE ME, VANDERHOFF JW, LAISSUE JA, JOEL DD: Accumulation of 2-micron latex particles in mouse Peyer's patches during chronic latex feeding. *Experientia* (1978) 34:120-122.

140. LEFEVRE ME, JOEL DD, SCHIDLOVSKY G: Retention of ingested latex particles in Peyer's patches of germfree and conventional mice. *Proc. Soc. Exp. Biol. Med.* (1985) 179:522-528.

141. VAN DER LUBBEN IM, KONINGS FA, BORCHARD G, VERHOEF JC, JUNGINGER HE: *In vivo* uptake of chitosan microparticles by murine Peyer's patches: visualization studies using confocal laser scanning microscopy and immunohistochemistry. *J. Drug Target.* (2001) 9:39-47.

142. MEYNELL HM, THOMAS NW, JAMES PS, HOLLAND J, TAUSSIG MJ, NICOLETTI C: Up-regulation of microsphere transport across the follicle-associated epithelium of Peyer's patch by exposure to *Streptococcus pneumoniae* R36a. *FASEB J.* (1999) 13:611-619.

143. CARR KE, HAZZARD RA, REID S, HODGES GM: The effect of size on uptake of orally administered latex microparticles in the small intestine and transport to mesenteric lymph nodes. *Pharm. Res.* (1996) 13:1205-1209.

144. DAMGE C, APRAHAMIAN M, MARCHAIS H, BENOIT JP, PINGET M: Intestinal absorption of PLAGA microspheres in the rat. *J. Anat.* (1996) 189:491-501.

145. HODGES GM, CARR EA, HAZZARD RA, CARR KE: Uptake and translocation of microparticles in small intestine. Morphology and quantification of particle distribution. *Dig. Dis. Sci.* (1995) 40:967-975.

146. JEPSON MA, SIMMONS NL, O'HAGAN DT, HIRST BH: Comparison of poly(DL-lactide-co-glycolide) and polystyrene microsphere targeting to intestinal M cells. *J. Drug Target.* (2003) 11:269-273.

147. OWEN RL, JONES AL: Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology* (1974) 66:189-203.

148. BEIER R, GEBERT A: Kinetics of particle uptake in the domes of Peyer's patches. *Am. J. Physiol.* (1998) 275:G130-137.
- Unique demonstration of particle transport kinetics across M cells.

149. LIMPANUSSORN J, SIMON L, DAYAN AD: Transepithelial transport of large particles in rat: a new model for the quantitative study of particle uptake. *J. Pharm. Pharmacol.* (1998) 50:753-760.

150. LIMPANUSSORN J, SIMON L, DAYAN AD: Intestinal uptake of particulate material by dexamethasone-treated rats: use of a novel technique to avoid intestinal mucosal contamination. *J. Pharm. Pharmacol.* (1998) 50:745-751.

151. SIMON L, SHINE G, DAYAN AD: Translocation of particulates across the gut wall – a quantitative approach. *J. Drug Target.* (1995) 3:217-219.

152. JENKINS PG, HOWARD KA, BLACKHALL NW, THOMAS NW, DAVIS SS, O'HAGAN DT: The quantitation of the absorption of microparticles into the intestinal lymph of Wistar rats. *Int. J. Pharm.* (1994) 102:261H266.

153. DESAI MP, LABHASETWAR V, WALTER E, LEVY RJ, AMIDON GL: The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. *Pharm. Res.* (1997) 14:1568-1573.

154. HILLERY AM, JANI PU, FLORENCE AT: Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. *J. Drug Target.* (1994) 2:151-156.

155. FLORENCE AT, HILLERY AM, HUSSAIN N, JANI PU: Nanoparticles as carriers for oral peptide absorption: Studies on particle uptake and fate. *J. Control. Release.* (1995) 36:39-46.

156. LEFEVRE ME, JOEL DD: Distribution of label after intragastric administration of 7Be-labeled carbon to weanling and aged mice. *Proc. Soc. Exp. Biol. Med.* (1986) 182:112-119.

157. ROPERT C, BAZILE D, BREDENBACH J, MARLARD M, VEILLARD M, SPENLEHAUER G: Fate of 14C radiolabeled poly(DL-lactic acid) nanoparticles following oral administration to rats. *Colloids and Surfaces B: Biointerfaces.* (1993) 1:233-239.

158. SCHERER D, MOOREN FC, KINNE RK, KREUTER J: *In vitro* permeability of PBCA nanoparticles through porcine small intestine. *J. Drug Target.* (1993) 1:21-27.

159. EYLES JE, ALPAR HO, CONWAY BR, KESWICK M: Oral delivery and fate of poly(lactic acid) microsphere-encapsulated interferon in rats. *J. Pharm. Pharmacol.* (1997) 49:669-674.

160. DEMBRI A, MONTISCI MJ, GANTIER JC, CHACUN H, PONCHEL G: Targeting of 3'-azido 3'-deoxythymidine (AZT)-loaded poly(isohexylcyanoacrylate) nanospheres to the gastrointestinal mucosa and associated lymphoid tissues. *Pharm. Res.* (2001) 18:467-473.

161. MLADENOVSKA K, JANEVIK EI, GLAVAS MD, KUMBARADZI EF, GORACINOVA K: Biodistribution of 131I-BSA loaded gelatin microspheres after peroral application to BALB/c mice-particle size study. *Acta. Pharm.* (2003) 53:187-197.

162. LEFEVRE ME, JOEL DD: Intestinal absorption of particulate matter. *Life Sci.* (1977) 21:1403-1408.

163. JANI PU, NOMURA T, YAMASHITA F, TAKAKURA Y, FLORENCE AT, HASHIDA M: Biliary excretion of polystyrene microspheres with covalently linked FITC fluorescence after oral and parenteral administration to male Wistar rats. *J. Drug Target.* (1996) 4:87-93.

164. TORCHE AM, JOUAN H, LE CORRE P *et al.*: *Ex vivo* and *in situ* PLGA microspheres uptake by pig ileal Peyer's patch segment. *Int. J. Pharm.* (2000) 201:15-27.

165. MCHALE NG, THORNBURY KD: The effect of anesthetics on lymphatic

contractility. *Microvasc. Res.* (1989) 37:70H76.

166. TAKESHITA T, MORIO M, KAWAHARA M, FUJII K: Halothane-induced changes in contractions of mesenteric lymphatics of the rat. *Lymphology* (1988) 21:128-130.

167. TURNER SG, BARROWMAN JA: Intestinal lymph flow and lymphatic transport of protein during fat absorption. *Q. J. Exp. Physiol Cogn. Med. Sci*. (1977) 62:175-180.

168. EDWARDS GA, PORTER CJ, CALIPH SM, KHOO SM, CHARMAN WN: Animal models for the study of intestinal lymphatic drug transport. *Adv. Drug Deliv. Rev.* (2001) 50:45-60.

169. POELMA FG, TUKKER JJ: Evaluation of a chronically isolated internal loop in the rat for the study of drug absorption kinetics. *J. Pharm. Sci.* (1987) 76:433-436.

170. LEHR CM, BOUWSTRA JA, TUKKER JJ, JUNGINGER HE: Intestinal transit of bioadhesive microspheres in an *in situ* loop in the rat – A comparative study with copolymers and blends based on poly(acrylic acid). *J. Control. Release*. (1990) 13:51-62.

171. GERDTS V, UWIERA RR, MUTWIRI GK *et al.*: Multiple intestinal 'loops' provide an *in vivo* model to analyse multiple mucosal immune responses. *J. Immunol. Methods.* (2001) 256:19-33.

172. MUTWIRI G, BOWERSOCK T, KIDANE A *et al.*: Induction of mucosal immune responses following enteric immunization with antigen delivered in alginate microspheres. *Vet. Immunol. Immunopathol*. (2002) 87:269-276.

173. MOOREN FC, BERTHOLD A, DOMSCHKE W, KREUTER J: Influence of chitosan microspheres on the transport of prednisolone sodium phosphate across HT-29 cell monolayers. *Pharm. Res.* (1998) 15:58-65.

174. DAY AJ, GEE JM, DUPONT MS, JOHNSON IT, WILLIAMSON G: Absorption of quercetin-3-glucoside and quercetin-4'-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochem. Pharmacol*. (2003) 65:1199-1206.

175. CHEN Y, PING Q, GUO J, LV W, GAO J: The absorption behavior of cyclosporin A lecithin vesicles in rat intestinal tissue. *Int. J. Pharm.* (2003) 261:21-26.

176. CARRENO-GOMEZ B, WOODLEY JF, FLORENCE AT: Studies on the uptake of tomato lectin nanoparticles in everted gut sacs. *Int. J. Pharm.* (1999) 183:7-11.

177. SANTOS CA, FREEDMAN BD, GHOSN S, JACOB JS, SCARPULLA M, MATHIOWITZ E: Evaluation of anhydride oligomers within polymer microsphere blends and their impact on bioadhesion and drug delivery *in vitro*. *Biomaterials.* (2003) 24:3571-3583.

178. SANTOS CA, JACOB JS, HERTZOG BA *et al.*: Correlation of two bioadhesion assays: the everted sac technique and the CAHN microbalance. *J. Control. Release.* (1999) 61:113-122.

179. DUCROC R, HEYMAN M, BEAUFRERE B, MORGAT JL, DESJEUX JF: Horseradish peroxidase transport across rabbit jejunum and Peyer's patches *in vitro*. *Am. J. Physiol.* (1983) 245:G54-G58.

180. KELJO DJ, HAMILTON JR: Quantitative determination of macromolecular transport rate across intestinal Peyer's patches. *Am. J. Physiol.* (1983) 244:G637-G644.

181. PIETZONKA P, WALTER E, DUDA-JOHNER S, LANGGUTH P, MERKLE HP: Compromised integrity of excised porcine intestinal epithelium obtained from the abattoir affects the outcome of *in vitro* particle uptake studies. *Eur. J. Pharm. Sci.* (2002) 15:39-47.

182. VAN DER LUBBEN IM, VAN OPDORP FA, HENGEVELD MR *et al.*: Transport of chitosan microparticles for mucosal vaccine delivery in a human intestinal M-cell model. *J. Drug Target*. (2002) 10:449-456.

183. DAMGE C, MICHEL C, APRAHAMIAN M, COUVREUR P: New approach for oral administration of insulin with polyalkylcyanoacrylate nanocapsules as drug carrier. *Diabetes.* (1988) 37:246-251.

184. COURNARIE F, AUCHERE D, CHEVENNE D, LACOUR B, SEILLER M, VAUTHIER C: Absorption and efficiency of insulin after oral administration of insulin-loaded nanocapsules in diabetic rats. *Int. J. Pharm.* (2002) 242:325-328.

185. HILLERY AM, TOTH I, FLORENCE AT: Co-polymerised peptide particles II: oral uptake of a novel co-polymeric nanoparticulate delivery system for peptides. *J. Control. Release.* (1996) 42:65H73.

186. SIMECKA JW: Mucosal immunity of the gastrointestinal tract and oral tolerance. *Adv. Drug Deliv. Rev.* (1998) 34:235-259.

187. YAMAGUCHI K, ANDERSON JM: *In vivo* biocompatibility studies of medisorb® 65/35 D,L-lactide/glycolide copolymer microspheres. *J. Control. Release.* (1993) 24:81-93.

188. KIM HK, PARK TG: Comparative study on sustained release of human growth hormone from semi-crystalline poly(-lactic acid) and amorphous poly(-lactic-co-glycolic acid) microspheres: morphological effect on protein release. *J. Control. Release* (2004) 98:115-125.

189. PARK TG: Degradation of poly(-lactic acid) microspheres: effect of molecular weight. *J. Control. Release.* (1994) 30:161-173.

190. PARK TG: Degradation of poly(lactic-co-glycolic acid) microspheres: effect of copolymer composition. *Biomaterials* (1995) 16:1123-1130.

191. LAVELLE EC, SHARIF S, THOMAS NW, HOLLAND J, DAVIS SS: The importance of gastrointestinal uptake of particles in the design of oral delivery systems. *Adv. Drug Deliv. Rev.* (1995) 18:5-22.

192. JONES DH, MCBRIDE BW, THORNTON C, O'HAGAN DT, ROBINSON A, FARRAR GH: Orally administered microencapsulated Bordetella pertussis fimbriae protect mice from B. pertussis respiratory infection. *Infect. Immun.* (1996) 64:489-494.

193. CHALLACOMBE SJ, RAHMAN D, O'HAGAN DT: Salivary, gut, vaginal and nasal antibody responses after oral immunization with biodegradable microparticles. *Vaccine* (1997) 15:169-175.

194. BOWERSOCK TL, HOGENESCH H, SUCKOW M *et al.*: Oral vaccination with alginate microsphere systems. *J. Control. Release* (1996) 39:209-220.

195. KIDANE A, GUIMOND P, JU TR, SANCHEZ M, GIBSON J, BOWERSOCK TL: The efficacy of oral vaccination of mice with alginate encapsulated outer membrane proteins of pasteurella haemolytica and one-shot. *Vaccine.* (2001) 19:2637-2646.

196. ERMAK TH, DOUGHERTY EP, BHAGAT HR, KABOK Z, PAPPO J: Uptake and transport of copolymer biodegradable microspheres by rabbit

Peyer's patch M cells. *Cell Tissue Res.* (1995) 279:433-436.

197. TABATA Y, INOUE Y, IKADA Y: Size effect on systemic and mucosal immune responses induced by oral administration of biodegradable microspheres. *Vaccine* (1996) 14:1677-1685.

198. JUNG T, KAMM W, BREITENBACH A, HUNGERER KD, HUNDT E, KISSEL T: Tetanus toxoid loaded nanoparticles from sulfobutylated poly(vinyl alcohol)-graft-poly(lactide-co-glycolide): evaluation of antibody response after oral and nasal application in mice. *Pharm. Res.* (2001) 18:352-360.

199. KATZ DE, DELORIMIER AJ, WOLF MK *et al.*: Oral immunization of adult volunteers with microencapsulated enterotoxigenic Escherichia coli (ETEC) CS6 antigen. *Vaccine.* (2003) 21:341-346.

200. GUTIERRO I, HERNANDEZ RM, IGARTUA M, GASCON AR, PEDRAZ JL: Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. *Vaccine.* (2002) 21:67-77.

201. REN JM, ZOU QM, WANG FK, HE Q, CHEN W, ZEN WK: PELA microspheres loaded *H. pylori* lysates and their mucosal immune response. *World J. Gastroenterology.* (2002) 8:1098-1102.

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