Chapter 12
Fate and Translocation of (Nano)Particulate Matter in the Gastrointestinal Tract

Andreas Frey, Katrin Ramaker, Niels Röckendorf, Barbara Wollenberg, Ingmar Lautenschläger, Gabriella Gébel, Artur Giemsa, Markus Heine, Denise Bargheer and Peter Nielsen

Abstract Nanoscience has flourished with increasing use of nanoparticles in many products. The particles enter the environment and affect both biotic and abiotic components of the ecosystem. Via the water supply and the food chain, humans could be affected by ingesting those particles. In this chapter, we will discuss mechanisms by which nanoparticles or their constituents can be translocated from the gastrointestinal tract, what their fate may be and how relevant this is for human health.

12.1 Introduction

In the usual sense, the term "nanoparticle" stands for manufactured or engineered nanoparticulate matter. Several thousand tons of engineered nanoparticles per year are produced worldwide [1]. Many different classes of nanoparticles are designed that offer tuneable properties to cover many applications in materials science, electronics, dyes, pigments and paint technology, catalysis, antibiotics, as well as in nanomedicine and many others [2] (Fig. 12.1).

What is less well known is the fact that nature itself is a skilled nanotechnologist and naturally formed nanoparticles occur in volcanic ash clouds, wood soot, spring

A. Frey (✉) · K. Ramaker · N. Röckendorf
Division of Mucosal Immunology and Diagnostics, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany
e-mail: afrey@fz-borstel.de

B. Wollenberg
Department of Ear, Nose and Throat, University Hospital Schleswig-Holstein, Ratzeburger Allee 160, 23538 Lübeck, Germany

I. Lautenschläger
Clinic for Anesthesiology and Operative Intensive Care Medicine, University Hospital Schleswig-Holstein, Arnold-Heller-Strasse 3, 24105 Kiel, Germany

G. Gébel · A. Giemsa · M. Heine · D. Bargheer · P. Nielsen
Institute of Biochemisty and Molecular Cell Biology, University Medical Center Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany
e-mail: nielsen@uke.de

P. Gehr and R. Zellner (eds.), *Biological Responses to Nanoscale Particles*, NanoScience and Technology, https://doi.org/10.1007/978-3-030-12461-8_12

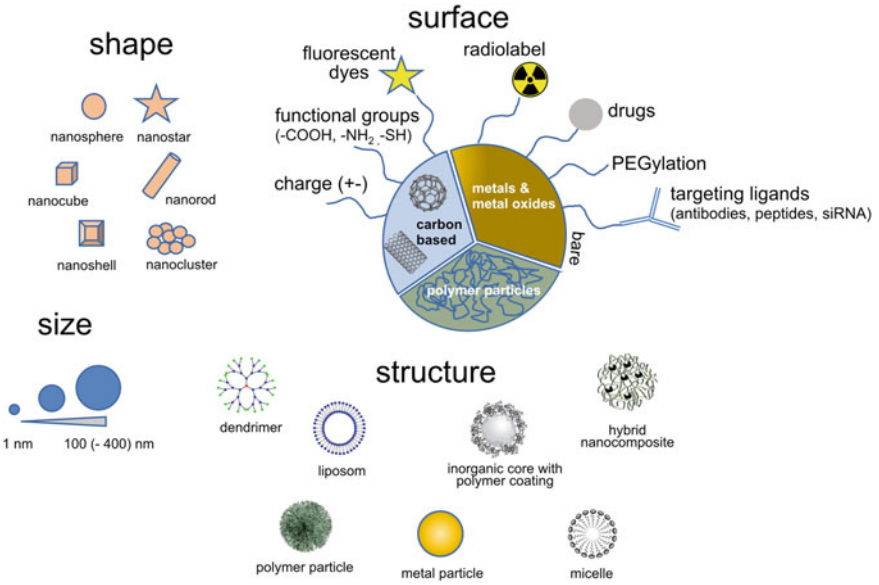

Fig. 12.1 Different classes of engineered nanoparticles. Many properties such as size, shape, surface charge, surface coating can be designed in synthesis what determines their specific physiochemical properties and may strongly influence their translocation and fate in the gastrointestinal tract when ingested

water, fine sand, and in biological materials as well [3]. These natural nanomaterials exist since millions of years on Earth.

Nanoparticles can be swallowed directly via food, beverages and drugs. Ingestion can also result from hand to mouth contact in the workplace [4], as well as from airway secretions which are contaminated with particles that have been cleared from the respiratory tract by the mucociliary escalator [5]. A new approach in nanotechnology is the field of nano-food [6, 7]. This includes the use of nanotechnology in packaging materials, farming practices, food processing, and also in the foods themselves.

Thus, nanoparticles are already in the food chain and may ever have been. The questions are: what happens to them in the gastrointestinal tract, and is there a significant health risk from ingested nanoparticles?

The uptake of particles in the gastrointestinal tract was first described by the German physiologist Gustav Herbst in 1844 when he found ingested starch bodies in the sub-millimeter range showing up in the circulation [8]. The finding was confirmed by others, forgotten, rediscovered in 1906 [9] and confirmed again [10] but always remained subject of critical debate [11].

Decades before the onset of nanotechnology, in the second half of the twentieth century, another round of studies begun, this time with better defined particulate chemical matter (asbestos, resin particles, latex particles) [12–15] but the controversy persisted. Again incorporation was detected, but the rates of uptake that were

reported varied significantly. Main reason for these conflicting results seems to be the methodology with which particle uptake was detected [16]. Bulk tissue analyses were performed often without discriminating between taken up and adherent particulate matter [17] which was later found to have a tremendous impact on detection rates [18]. Histological analyses may overcome this problem to some extent but are not quantitative [16] and one runs the risk of smearing luminal particles across the sectioned tissue during workup (own observation). Although the initial finding concerning the sub-millimeter starch particles was rejected, there was consent at that point in time that particles in the micrometer range can readily be taken up by the gastrointestinal mucosa and carried onwards within the body.

However, again it must be stated that this picture becomes less clear today, even when we apply present-day highly advanced methodology for detecting and quantifying translocation of even smaller particles into the body [19]. The reasons for this are (i) the diverse character and the limited colloidal stability of the ingested nanoparticles, (ii) the problem that the gastrointestinal tract is a site of very complex processes where even symbiotic interactions between host cells and the resident microbiome must be taken into account, (iii) the fact that the majority of literature addressing biological effects of nanoparticles in human-relevant systems concerns isolated cell systems or lung biology and less in vivo studies of processes in the gastrointestinal tract.

In the following sections we will look into detail into the anatomy and physiology of the gut, into reactions that modify or degrade nanoparticles after ingestion, into the types and features of nanoparticles that should predispose for interaction, into model systems to investigate those interactions, into the medical exploitation of nanoparticles in the alimentary tract and into the ultimate fate and the risk ingested nanoparticles may pose.

12.2 Architecture of the Gastrointestinal Tract

As part of the terrestrial ecosystem humans are in constant physical and chemical interaction with their environment. Per day an adult human being breathes more than 10 m^3 air thereby resorbing about 500 l or 700 g of oxygen, ingests 1–3 l of water and consumes pounds of food of which about 300–500 g of nutrients actually are incorporated. In order to manage assimilation of such an extent in a timely manner the cellular interface where the actual transport events occur must cover a substantial surface area. In the airways, the site of gas exchange, the interface encompasses about 75 m^2, in the alimentary tract where water and solutes are taken up this area is approximately 35 m^2 [20]. The large mucosal surface area of the alimentary tract is not tailored evenly along the oro-rectal axis of the digestive system. At the site of food and water ingestion—oral cavity, oropharynx and oesophagus—the mucosal lining is plain and consists of multi-layered, partially keratinized squamous epithelia which provide a robust barrier against mechanical stress as it may be caused by movement of solid, undigested food constituents. The architecture of these epithelia

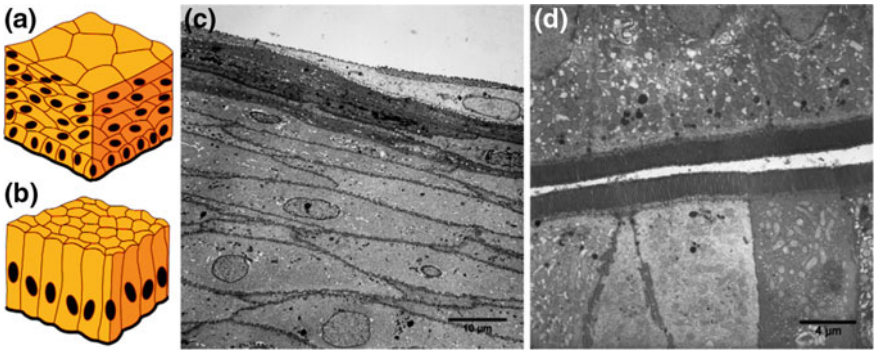

Fig. 12.2 Predominant cell types in the mucosal lining of the alimentary tract. Left: Schematic drawing of **a** stratified squamous epithelia lining the oral cavity, the oropharynx and the esophagus; **b** columnar epithelium lining the assimilation-active mucosae from the stomach downwards. Right: Electron micrographs of human tissue sections: **c** squamous epithelium of human tonsil, displaying the typical cell multilayer with masonry bond-type organization; **d** columnar epithelium of human small intestine (ileum) displaying tight cell-to-cell contacts. The images were acquired in the context of an extensive electron microscopic cell surface analysis study performed by some of the authors [24]

resembles that of a brick wall where the bricks are staggered such that each brick covers the gap beneath the rows above and below (Fig. 12.2a, c) [21]. In addition to this masonry bond some kind of "molecular grout" or "mortar" between cells exists which is hydrophobic in nature and seems to derive from membrane granules of the epithelia and which seals the gaps [21–23]. Although such an architecture is perfectly suited to withstand mechanical stress, it is inappropriate for translocation of nutrients and water. For that reason the mucosae follow a different concept to fit the needs of rapid substance exchange further down the alimentary tract. These are the sites where large amounts of digestive fluids have to be secreted and where nutrients and water have to be taken up. Thus, from the stomach downwards the mucosal lining no longer consists of stratified squamous epithelia but of columnar epithelia of various types whose hallmark is a monolayer of column-like cells that are sealed together by tight junctions or *zonulae occludens* (Fig. 12.2b, d).

Tight junctions are gasket-like ribbons of membrane proteins that are linked internally to the cellular cytoskeleton and externally to their protein counterparts in neighbouring cells, so tightly that even ions and water cannot pass through.

Although an elaborate tight stratum, the epithelium of the alimentary tract is not designed to endure over the entire life span of an individual. On the contrary, it renews rather rapidly. While the stratified squamous epithelia of the upper alimentary tract replenish themselves within about 3 weeks, the columnar epithelium of the lower gastrointestinal tract renews even faster, within less than a week [21]. In order to prevent the building of an unguarded portal of entry at the site of a dying cell, neighbouring vital columnar epithelial cells contact each other underneath a

moribund cell and form tight junctions before the cell cadaver is sloughed off into the lumen [25–28].

Tight junctions not only seal neighbouring cells together. Due to the fact that they surround the entire cell like a waistband they separate the upper membrane hemisphere from the lower one and do not allow membrane molecule diffusion from one hemisphere to the other. As a consequence of this so-called cell polarisation a structural specialization of the cell membrane in the "upper" and "lower" half and a vectorial organization of transport systems exists. With its lower/inner hemisphere the cell is anchored onto a fabric mesh of elastic extracellullar matrix proteins, the basal membrane. With its upper/outer hemisphere the cell communicates with luminal content. In order to provide more surface area for substance exchange, the luminal outer membrane of each cell is stretched over a dense array of cytoskeletal spikes thereby forming a brush-like structure, the so-called brush border or microvilli. In analogy to the cellular level, the surface area is further increased macroscopically by organizing the whole intestinal surface tissue in extensions—or villi—reaching into the luminal pipe.

Anchored in the cell membrane of the epithelial lining cells, a fluffy layer of interwoven glycoproteins, the glycocalyx, resides on top of the microvilli [24, 29]. The role of this glycocalyx is to protect the fragile membrane lipid bilayer against luminal content and to provide an unstirred fluid layer into which nutrients can diffuse and be taken up perpendicular to the bowel movement by the enterocytes, the major assimilating cells. The glycocalyx produced by each cell is able to interlace with that of neighbouring cells thereby creating a gap-less molecular fleece. Main constituents of the glycocalyx are the membrane-anchored glycoproteins MUC3, MUC12 and MUC17 [30], members of the mucin family of proteins which is named after mucus, the slippery surface coat present on mucosal surfaces. Mucus represents the outermost layer of defense on the epithelial lining. In contrast to the glycocalyx it is neither produced by regular epithelial lining cells nor firmly attached to them [31, 32]. Mucus is generated by goblet cells which mainly reside in the valleys ("crypts") between neighbouring villi. From these crypts the mucus is expelled like from a nozzle and squeezed onto the glycocalyx by the moving luminal constituents. Depending on the segment of the lower alimentary tract, the mucus blanket either consists of a single layer of low density or of a double layer with dense inner and light outer stratum [30]. The double layer is present on sites of little assimilation and aggressive luminal matter, i.e. the stomach and the large intestine, whereas the monolayer is found in the small intestine where nutrient uptake takes place, the pH is near neutral and microbial colonization is low. The double layer design may thus represent a barrier in the barrier where the outer film is offered for breakdown by stomach fluid and colorectal flora [33] and the inner one acts as protective coating, lubricant and trap for hazardous particulate luminal contents.

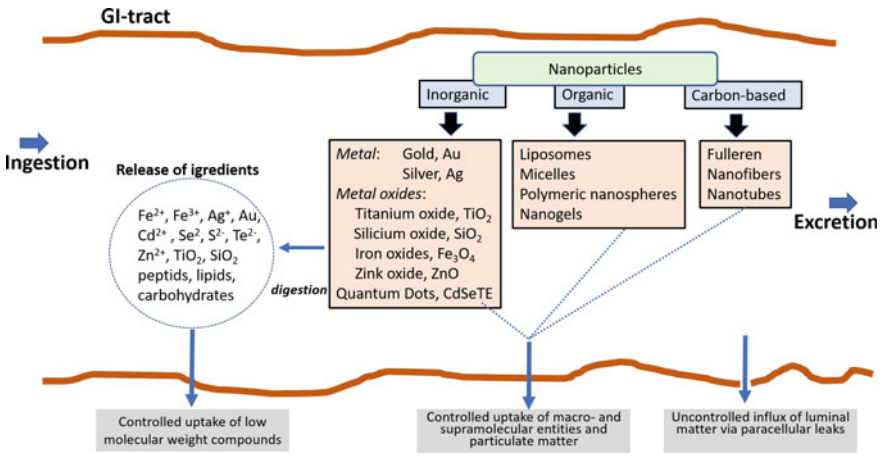

Fig. 12.3 Potential fates of nanoparticles in the gastrointestinal tract. Ingested nanoparticles are confronted first with the acidic milieu of the stomach and then with the highly active hydrolytic enzymes in the intestinal tract. It can be expected that acid- or chelator-susceptible inorganic nanoparticles will partly dissolve and small molecules, atoms or ions contained therein will be released. Organic nanoparticles consisting of carbohydrate polymers or lipids could in part be enzymatically degraded. Finally, absorption of low molecular weight components by physiological uptake mechanisms may occur, and unchanged particles or shrunken remnants may also pass across the barrier

12.3 Nanoparticulate Matter Confronting the Gastrointestinal Tract

Whether or not ingested particulate matter poses a risk to an individual is difficult to assess. In size, particulate matter may range from the macroscopic scale down to the nanometer range. It may vary in shape and surface texture, and its resistance to the luminal environment may differ considerably. In terms of its impact on the host, size has an enormous influence: the bigger the particle the higher the physical but the lower the physiological impact, and vice versa. A swallowed glass marble or bead may traumatize the esophagus but will remain inert towards the molecular and cellular processes in the gut. Ingested cake frosting will smoothly pass the upper alimentary tract but its nanoparticulate color pigments may interact with luminal content and the epithelial lining. For that reason ingestion of objects invisibly small such as the pigment particles in the frosting have raised considerable safety concerns in recent years. Small size, in particular in the nanometer range, enables the matter to undergo interactions on the cellular and molecular level which are difficult to predict. A lot of interactions and crossroads are possible for a nanoparticle in the GI tract. Nanoparticles can be broken down, taken up, adsorbed to luminal matter, become modified or be excreted or any of these processes may happen in combination (Fig. 12.3).

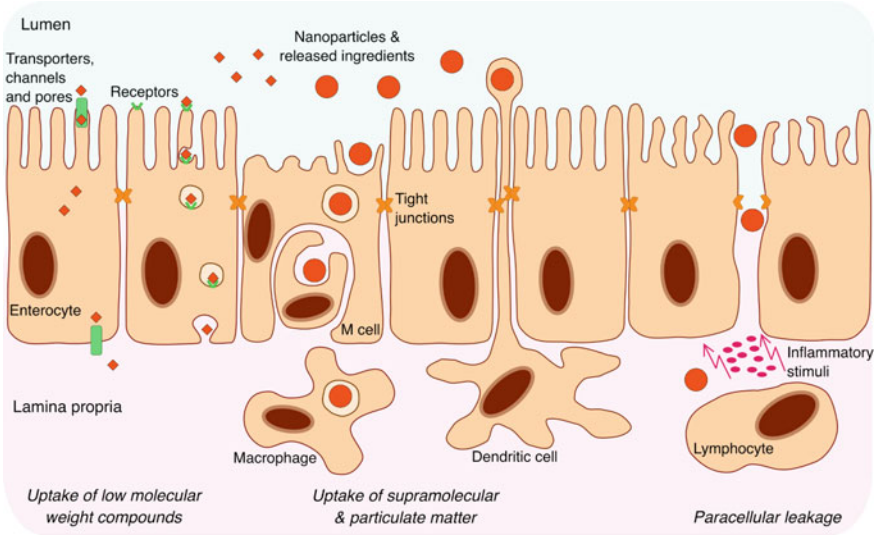

Fig. 12.4 Potential routes for nanoparticles and released ingredients across the intestinal epithelium. Enterocytes can actively or passively take up low molecular weight compounds via transporters, channels or pores, or via receptor-mediated or bulk endocytosis. Particulate matter is taken up by specialized endocytotic cells (M cells) and handed over to macrophages, or sampled from the lumen by dendritic cells. Break-up of tight junctions e.g. in a state of inflammation allows nonspecific paracellular diffusion

The mucosal lining contains gateways via which the body can sample content of the alimentary tract and can assimilate substances necessary for subsistence. These gateways in principle also constitute the entrance way for nanoparticles or their ingredients and break-down products. In a healthy individual, uptake generally occurs at designated sites which in turn results in specific uptake mechanisms. These include active or passive transport via distinct transporters or channels, receptor-mediated or bulk endocytosis/pinocytosis and uptake via specialized cells. A main determinator for uptake selectivity appears to be the size of the respective freight. In contrast, paracellular leakage is usually a consequence of some pathologic barrier disturbance and is rather non-specific regarding the transported goods (Fig. 12.4).

12.3.1 Controlled Uptake of Low Molecular Weight Compounds

For the low molecular weight level one usually discriminates between nutrients, essential salts and water, and low molecular weight xenobiotics. The former are indispensable for growth and survival of the host and thus are translocated by dedicated transport systems which reside in the apical membrane of the intestinal epithelial

cells. Specific examples for this are summarized in Table 12.1. Xenobiotics usually have no natural function inside the body, on the contrary, they even can be highly toxic for an individual. While natural/environmental origin of xenobiotics is prevalent, anthropogenic materials become increasingly relevant as a source for these substances. Uptake of such small xenobiotic molecules typically occurs by making use of existing molecular transport systems (Table 12.1).

With regard to the incorporation of (nano)particulate matter, these systems are not of relevance for the particles themselves, as even very small nanoparticles will not fit into such transporters. Yet, a particle, as any ingested matter, usually will be subject to strong physiological "attacks" in the alimentary tract. Susceptible nanoparticles may be partially or completely degraded by digestive juices and release their constituents. Of those, any metabolite of nutritional or physiological value, like e.g. carbohydrates, lipids, amino acids, or inorganic ions, will be accepted by the dedicated molecular transporters listed in Table 12.1. But nanoparticles can also harbour non-physiological and/or toxic materials or they contain an excess of an otherwise beneficial compound. Such luminally liberated substances may be viewed as a collapsed (nano)particle shrunk to fit into an otherwise irrelevant gateway. Since (nano)particulate heavy metal chalcogenids fall into this category and are abundant nanomaterials, we will examplify the "shrink-to-fit" route with two types of nanoparticles, iron oxide/sulfide nanoparticles and cadmium oxide/selenide nanoparticles. Both may stem from abrasive and melding dusts or from biomedical labelling and contrast agents.

Imaging-grade superparamagnetic iron oxide nanoparticles (SPIONs) consist of an iron oxide core (usually magnetite $Fe^{2+}(Fe^{3+})_2O_4$) and a designed shell to render them water soluble and to give them a desired function. Under the acidic pH conditions prevalent in the stomach, the core is sensitive for dissolution releasing Fe^{3+} and Fe^{2+} ions. Intestinal iron absorption in humans is specific for Fe^{2+} which is better soluble at neutral pH in the duodenum and can be absorbed by the divalent metal transporter 1 (DMT1; SLC11A2). Taken up into the assimilation-active enterocytes it is then under the control of whole body iron regulation and released via ferroportin exporter from the basolateral membrane into the blood stream, bound to apotransferrin, transported to cells in need for iron, or when in excess stored in ferritin molecules in cells of the liver, muscle, and bone marrow.

Using a radiolabelled-SPION as a model system for metal oxide nanoparticles, the fate of these particles in the gastrointestinal tract was studied in mice. The uptake of ^{59}Fe into the body from two different preparations was measured by whole-body-counting after 7–14 days, when the non-absorbed part was completely excreted in the faeces (Fig. 12.5) [58]. It should be noted that this technique is the most sensitive and reliable method for detecting the iron absorption in vivo [59]. In one preparation, the oleic acid-stabilized lipophilic iron oxide cores (11 nm) were coated with a polymer containing COOH-groups at the surface to make the particles water soluble (polym.-SPION). In the other form, the lipophilic cores were embedded into lipoprotein—micelles that transport lipids and other hydrophobic substances in human blood ("nanosomes") [60]. It was found that from both forms about 5–7% of the ^{59}Fe-label were absorbed into the body and used for haemoglobin synthesis, clearly indicating

Table 12.1 Selection of molecular transporters residing in the apical membrane of intestinal epithelial cells

Nutrient type	Freight	Foreign cargo	Transport/coupling ions	Name of apical intestinal transporter (Abbreviation)	SLC-Classification	References
Amino acids	Basic, neutral and aromatic amino acids		No coupling	Neutral and Basic amino-acid transporter (NBAT, rBAT)	SLC3A1	[34, 35]
	Glycine	Nothing known	Coupled/$Na^+ + Cl^-$	Glycine transporter 1 (GLYT1)	SLC6A9	[36, 37]
	Large neutral amino acids		Coupled: Na^+	Sodium-dependent neutral amino acid transporter (B^0AT1)	SLC6A19	[37, 38]
	Imino acids, sarcosine pipecolate		Coupled: Na^+	Sodium/imino-acid transporter/imino transporter (SIT1, IMINO, NTT4/XT1)	SLC6A20	[37, 39]
	Small, unbranched, zwitterionic α-, β-, and γ-amino and imino acids, short chain fatty acids	D-amino acids, β-alanine, 5-amino-levulinic acid, N-methylated amino acids, 3-amino-1-propanesulphonic acid, 1-aminocyclopropane-carboxylic acid, etc.	Coupled: H^+	Proton-coupled amino acid transporter (hPAT1)	SLC36A1	[40]

(continued)

Table 12.1 (continued)

Nutrient type	Freight	Foreign cargo	Transport/coupling ions	Name of apical intestinal transporter (Abbreviation)	SLC-Classification	References
Carbo-hydrates	Fructose		No coupling	Fructose transporter (GLUT5)	SLC2A5	[41]
	Glucose, galactose	C-linked glycosides of polyphenols	Coupled: Na^+	Sodium-glucose cotransporter (SGLT1)	SLC5A1	[42, 43]
Non-alkali metal ions	Ca^{2+}	Ba^{2+}, Sr^{2+}	No coupling, but GSH- and vitamin D-dependent	Apical calcium channel CaT1 or ECAC2 (TRPV6)	–	[44, 45]
	Fe^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}	Cd^{2+}, Pb^{2+}, Cu^+ (not Cu^{2+})	Coupled: H^+	Divalent metal ion transporter-1 (DMT1)	SLC11A2	[45, 46]
	Zn^{2+}	(Cu^{2+})	Not known	Zip4	SLC39A4	[45, 47]
	Mg^{2+}	Ba^{2+}, Ni^{2+}	No coupling	TRPM6	–	[47, 48]
	Cu^{2+}			CTR1	SLC31A1	[47]
Cholesterol	Cholesterol, vitamins D, E and K, carotenoid			Scavenger receptor class B-I (SR-BI)		[49, 50]
	Cholesterol, vitamins D, E and K, phytosterols		Clathrin-mediated endocytosis	NPC1-like cholesterol transpoter 1 NPC1L1		[49, 50]

(continued)

Table 12.1 (continued)

Nutrient type	Freight	Foreign cargo	Transport/coupling ions	Name of apical intestinal transporter (Abbreviation)	SLC-Classification	References
Fatty acids	Fatty acids			FATP4	SLC27A4	[51]
Peptides	Di- and tripeptides	Various drugs: beta-lactam antibiotics, ACE-inhibitors, 5-amino-levulinic acid	Coupled: H^+	Oligopeptide transporter 1 PEPT1	SLC15A1	[52]
Vitamins	Vitamin C (ascorbate)	Various ascorbate-derivatives (used for drug delivery)	Coupled/Na^+	SVCT1	SLC23A1	[53]
	Vitamin B2 (riboflavin)		Coupled: H^+	Riboflavin transporter RFVT3	SLC52A3	[54]
	Folate		Coupled: H^+	Proton-coupled folate transporter PCFT	SLC46A1	[55]
	Thiamin		Coupled: H^+	Thiamin Transporter ThTr 2	SLC19A3	[55, 56]
Organic compounds	L-carnitine/organic cations		Coupled/Na^+/No coupling	OCTN2	SLC22A5	[57]
	Organic anions: cholate, bile acids, steroids, thyroxine,	Various toxins (microcys-tins), drugs (statins, quino-lines, antibiotics, metho-trexate)	No coupling	Organic anion transporting polypeptides OATP1A2	SLCO1A2	[57]

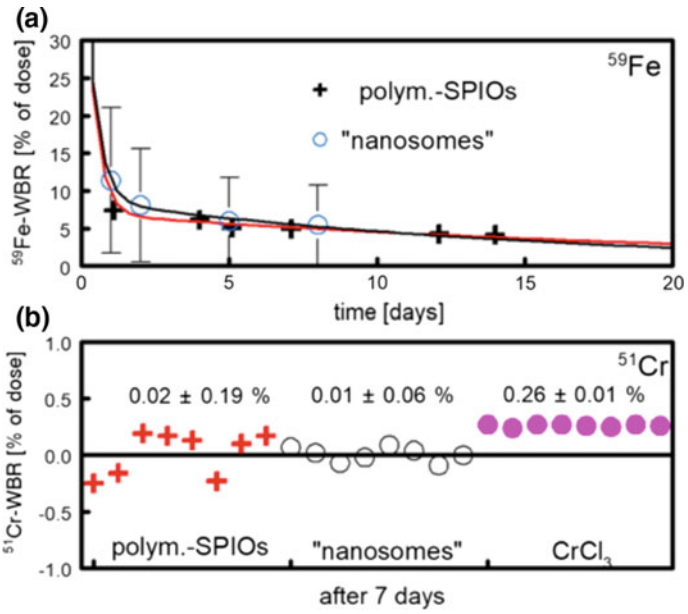

Fig. 12.5 Absorption of ^{59}Fe- or ^{51}Cr-labeled SPIONs in mice [58]. **a** ^{59}Fe-labeled polymer-coated SPIONs or so-called "nanosomes" (oleic acid stabilized, hydrophobic SPIONs embedded in chylomicron-like lipid micelles were administered by gavage to groups of mice (n = 4–5) and the ^{59}Fe-whole-body-retention (WBR) was measured after 1-14 days. **b** Same procedure with ^{51}Cr-labelled SPIONs, nanosomes, or $CrCl_3$ (n = 8). Individual values of ^{51}Cr-whole-body-retention (WBR) after 7 days (=apparent gastrointestinal absorption) are given (figure taken from [58] under Beilstein-Institut Open Access License 1.1; https://www.beilstein-journals.org/bjnano/terms#lic11)

a significant absorption from the gut (Fig. 12.5a). However, it remained questionable if this numbers truly represented intact particle uptake. To test this, the iron oxide cores were exchange-labelled with $^{51}CrCl_3$ in a stable and homogenous form [58].

It is well known that the intestinal absorption of ionic Cr^{3+} in contrast to Fe^{2+} is extremely low in rodents [61]. The results from analogous experiments in mice using the two forms of $^{51}Cr^{3+}$-spiked SPION showed that the absorption of Cr^{3+} was extremely low as expected (0.01–0.02% absorption rate) (Fig. 12.5b). Thus, the in vivo experiments virtually excluded the uptake of the two different forms of intact nanoparticles, namely a typical polymer-coated iron oxide as well as a micelle-type nanoparticle in mice. Therefore, the ^{59}Fe-results in Fig. 12.5a must be interpreted as a partial digestion of SPIONs in the stomach followed by absorption of released ionic Fe^{2+}.

Cadmium selenide is present in certain nanoparticulate semiconductors—so called quantum dots (Qdots)—which are used as replacement for classical fluorescent dyes in biomedical imaging due to their extreme photostability. Being a heavy metal chalcogenide like iron oxide, CdSe is also susceptible to digestive fluids and

the potential release of Cd^{2+} from ingested Qdots is a major concern with respect to biosafety. Many studies have demonstrated the toxicity of various Qdots in cell culture [62–67]. And as CdSe crystals are sensitive to acidic or oxidizing environments, Qdots survive a gastrointestinal transit only when coated with special shells [68, 69]. Otherwise they rapidly lose their fluorescent properties, due to etching processes (own observation; [70, 71]). In the latter case, the Cd^{2+} is liberated and it is assumed that cadmium ions misuse molecular transporters for divalent metals such as those for Fe^{2+}, Zn^{2+}, Mn^{2+} or Ca^{2+} thereby entering the body. For example, Cd accumulation is enhanced in animals fed a diet deficient in zinc (Zn), iron (Fe), and calcium (Ca). Obviously, Cd can be absorbed through two main transporters: the divalent metal transporter (DMT) 1, a preferential Fe-transporter, and calcium transporter (CaT) 1 [72]. However, when realistic dosing was applied in mice and rats no abnormal behaviour or tissue damage was observed over the several months' time span even after systemic administration of Qdots [73–75].

Unlike with SPIONs, where the released but not absorbed iron is simply integrated into the much larger pool on nonabsorbed food iron, the Cd^{2+} release from unprotected Qdots may cause an intoxication of the host not only from ingested Qdots. Released Cd^{2+} could also affects the luminal flora. It was shown in animal studies that chronic oral Cd^{2+} intake reduced the total number of intestinal bacteria among which the growth of *Bacteroidetes* spp. was significantly suppressed whereas the growth of *Lactobacillaceae* spp. was not inhibited [76] or even increased [77]. This is in line with another observation where certain lactobacillus species not only showed a high tolerance against Cd^{2+} but also bound this heavy metal ion and thus may act as detoxifying "enterosorbent" if present [78]. Moreover, the complete lack of an intestinal flora and thus the lack of any microbial Cd^{2+} binders obviously increases cadmium uptake and induces the expression of metallothioneins—heavy metal scavenger proteins—by the host [79]. The widely used nanosilver when ingested could also work in this direction. A chronic feeding of AgNP (50 and 200 ppm) over 60 days showed nephrotoxic effects in Wistar rats [80]. These concentrations are below previously reported lowest observed adverse effect level for bulk silver. The high surface of the particle could release oxidised Ag^{+} with the know high reactivity towards bacterial proteins. All in all, heavy metal metabolism in the alimentary tract is a complex process, and the intake of such substances may have far reaching physiological consequences. This holds also true if those heavy metals are contained in nanoparticulate matter because they may become luminally released.

However, regarding the release of toxic metal ions and chalcogenides such as Cd^{2+}, Ag^{+}, Se^{2-}, Te^{2-} etc., noble metal atoms such as Au, Ag, Pt, or any other ingredient of nanoparticles in the gastrointestinal tract, it should be noted that this represents not an entirely new situation for human health because these substances are present already in the environment and in the food chain [81]. Especially the heavy metals burden (Cd, Pd, Hg, Ag) follows the industrial development in many countries and the impact to human health mainly from uncontrolled working processes in the past are quite well investigated and are the basis of non-occupational or occupational exposure limits. That does not mean that no effects are awaited. In the lack of conclusive study data on effects and mechanism in the gut, it can only be estimated that the amount

of toxic materials released from ingested natural or engineered nanoparticles in the gut should be below harmful levels of environmental, health and safety protection.

12.3.2 Controlled Uptake of Macro- and Supramolecular Entities and Particulate Matter

As the digestive system usually breaks down biopolymers into their monomeric building blocks, the advance of polymers that survived digestion ("too-fit-to-shrink") is suspicious. At best the polymer is an innocuous food constituent such as cellulose or chitin. Being digestion-resistant it would be useless for the body but difficult to excrete. At worst the polymer evolved to withstand the lumen of the gastrointestinal tract for a particular purpose. Toxins of diarrhoea-inducing bacteria, such as cholera toxin, are one example of the latter. They are secreted by pathogenic bacteria in order to manipulate the host in favour of their own survival and dissemination. Cholera toxin possesses a hydrodynamic diameter of about 6 nm [29]. If structures this small can already pose a risk to the body and are metabolically worthless, incorporation of even larger structures such as microbes is even less desirable. Nevertheless, for the healthy organism it is indispensable to sample flora from the gut lumen in order to monitor and re-adjust microbial homeostasis within the alimentary pipe. Thus, several different, but always well-guarded doors along the gastrointestinal tract wall exist in case of a vital, healthy mucosal epithelium (Fig. 12.4). At such sites, the epithelium actively or passively samples macromolecules or larger entities and forwards them to the underlying mucosal immune system for further inspection. Due to the inherent danger of those substances, their presence may even trigger de novo formation of uptake/monitoring sites as is the case e.g. for cholera toxin which induces formation of an epithelial cell type specialized in uptake and translocation of macromolecular and particulate matter—the so-called M cells [82]. M cells are a unique variant of columnar intestinal epithelial cells. They lack a prominent glycocalyx, and it could be shown that M cells are able to pick up digestion resistant matter that adheres to their luminal cell membrane, such as choleratoxin B subunit-coated gold or polystyrene nanoparticles [29, 83] (Fig. 12.6). The cargo is translocated into a pocket which each M cell forms on the basolateral side and handed over to residing phagocytic cells for immunological survey [84, 85]. Due to the local abundance of specific differentiation inducing molecules M cells are most prominent above nodes of organized mucosa-associated lymphoid tissue (O-MALT), the so-called Peyer's patches, which usually reside opposite the vascularization and abdominal suspension of the gut pipe (mesenterium) [82, 86–89], but M cells can also be found within the epithelial lining outside Peyer' patches [90] and possibly be induced by proinflammatory stimuli [82]. While M cells are mere transporters for attached matter, dendritic cells (DCs) actively gather luminal content along the alimentary tract. In order to gain access to the lumen, DCs either cooperate with M cells sending their protrusions through a pore which the M cell forms through its cytoplasm [91], or they push their

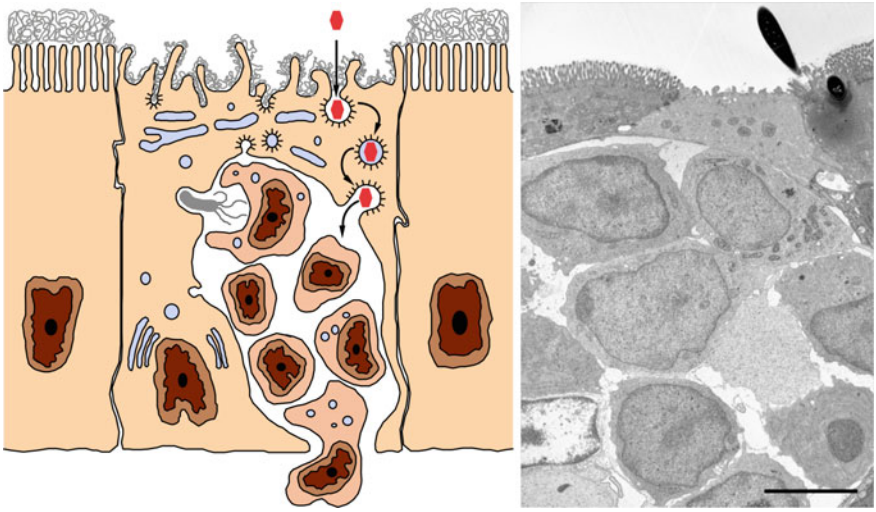

Fig. 12.6 Transcytosis via Peyer's patch M cells. Left: Schematic depiction of an M cell: the apical membrane is covered only by a rudimentary glycocalyx. Pathogens and other particulate matter are picked up from the lumen by endocytosis and transported to the basolateral side. Here they are released to be taken up by macrophages which reside in a pocket formed by the M cell. Right: Electron micrograph of an M cell from mouse ileal Peyer's patch. Macrophages in the M cell pocket await the delivery of microbes endocytosed by the M cell from the gut lumen. Bar: 5 μm

protrusions between regular columnar epithelial cells. As the respective type of DC also expresses tight junction proteins the generated hole via which the DCs pick up luminal material can be sealed on the fly [86]. More recent findings even suggest an active role of the glycocalyx for sensing of gut content. The mucin MUC17 which is a major constituent of the intestinal glycocalyx was shown to relocate from the apical surface to an intracellular vesicular pool distinct from classical endosomes upon parasympathetic stimulation (carbachol) [92]. Gut content adherent to MUC17 may be ferried into the epithelium under those conditions and offered to underlying immune cells for further examination.

A very special digestion-resistant polymer type which may be encountered in the intestine are prions. Infectious prions are extremely stable ("too-fit-to-shrink"), misfolded protein aggregates of 17–27 nm diameter [93] which—upon incorporation—catalyze misfolding of the respective counterpart in the host. Accumulation of prion aggregates will eventually result in a dementia-like disorder, the so-called spongiform encephalopathy, e.g. Creutzfeldt-Jakob disease in humans [94]. The disease is highly contagious and believed to be transmitted via the alimentary tract, simply by ingestion of prion-contaminated foodstuff. Transmission apparently occurs via M cells as it was demonstrated that mice which do not develop M cells due to a genetic defect are largely resistant to oral prion transmission [95], whereas mice being triggered to form more M cells are highly susceptible to oral prion infection [96].

As mentioned above, sampling of potential microbial dwellers or intruders from the glacis of the mucosal barrier is a controlled process exerted by M cells and dendritic cells. Hence, both cell types are capable to pick up particles of microbial size and above. M cells were reported to translocate particles of up to 10 μm in diameter [97]; dendritic cells (DCs) are able to engulf particles of up to 15 μm in vitro [98]. Yet, uptake appears to be most efficient with particles in the mid-to-high-nanometer range (500 ± 250 nm) [97–102].

This is in strong contrast to early studies which reported incorporation of orally administered particles in the size range of several hundred micrometers. In light of the more recent findings on gastrointestinal barrier function and cellular transport capacity—as described before in this chapter—it still remains an enigma how particles this big are able to traverse the mucosal lining. Passage due to paracellular leakage seems to be the most plausible explanation at this point in time.

12.3.3 Uncontrolled Influx of Luminal Matter via Paracellular Leaks

Ingress of particles considerably bigger than any of the gateways described above and the occurrence of luminal bacteria in the peritoneal cavity [103] indicates that even the staggered defences of the epithelial layer can be overcome by luminal matter. Such events seem to occur rather frequently, and the mammalian gut appears to have adjusted to such recurring epithelial leakages. For example, the polarized goblet cells of the gastrointestinal tract express toll-like receptor 5 (TLR5), an innate immune sensor for bacterial flagella, on their basolateral instead of their apical side [104] such that it can act as a "leakage detector" underneath the epithelial tight junctions. There are believed to be three main reasons for transmural flux of luminal matter: (i) bacterial overgrowth in the lower small intestines due to a defect in the gastric barrier and/or delayed intestinal clearance [103, 105]; (ii) deficiencies in the host gastrointestinal immune defense [103]; and (iii) increased permeability and damage of the intestinal barrier [103]. Barrier damage may either occur spontaneously or be caused by trauma. The latter cause is the most frequent one; physical impacts onto the abdomen, ingestion of rigid foreign objects or consumption of foods that contain bone or wood splinter may be traumatic to the gastrointestinal lining. Spontaneous perforation is less common and in most cases due to an underlying gastrointestinal affliction. Luminal bacterial pathogens may contribute by secreting specific tight junction-altering proteins or toxins [106–108]. Chronic inflammatory disorders such as Crohn's disease, colitis or celiac disease as well as intestinal infections are known triggers for spontaneous fissures, too [109–111]. The inflammatory events predispose for that by weakening tight junction integrity [112]. In an experimental set-up with intestinal biopsies from patients suffering from inflammatory bowel disease it was shown that the translocation of different nanoparticle types across the epithelium was higher in inflamed tissue than in healthy mucosa [113].

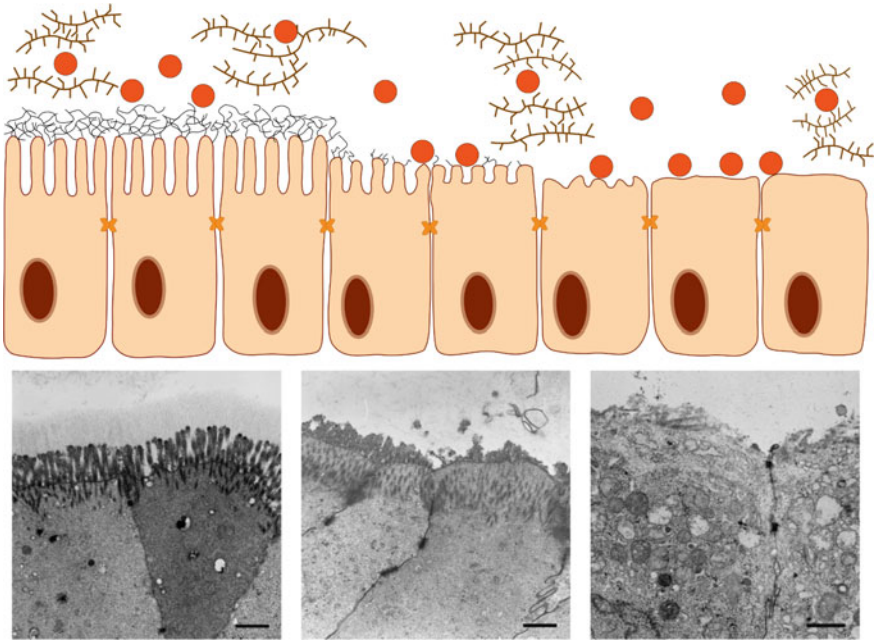

Fig. 12.7 Lack of an epithelial glycocalyx at cancerous lesions in the human intestine. The schematic drawing illustrates how the presence of a glycocalyx on fully differentiated enterocytes (left part) prevents access of nanoparticles to the epithelial surface. Lack of a glycocalyx on precancerous or adenoma regions (middle part) or on cancer cells (right part) allows attachment of particles to the unprotected apical cell membrane. Electron micrographs beneath the scheme depict typical examples of healthy epithelium (left), adenoma (middle) and cancer cells (right) in human colon. A glycocalyx is only visible on the healthy epithelial cells. Bar: 1 μm

Cancerous or precancerous lesions also foster spontaneous damage of the gastrointestinal barrier [109]. Although barrier perforation was reported mostly in the context of metastatic tumors, primary tumors of the large intestines may also promote access of luminal particulate matter to the epithelial lining. It was shown that both, intestinal polyps and epithelial cancers lack a mature glycocalyx and thus allow advance of particulate matter up to the cell membranes [24] (Fig. 12.7). Since polyps and manifest cancers are not subject to epithelial renewal luminal particles may accumulate at such sites. In light of this the hypothesis that the presence of (nano)particulate luminal matter indicates tumorigenesis by these particles seems questionable. The particles may simply accumulate at this site due to the lack of epithelial renewal in combination with the lack of a glycocalyx.

Nevertheless, the possibility of luminal nanoparticles to accumulate specifically at cancerous and precancerous intestinal lesions may provide a means to detect these malignancies early on.

12.3.4 Features Qualifying (Nano)Particulate Matter for Gastrointestinal Uptake

Whether or not a nanoparticle adheres to an intestinal translocation site or is broken down on route through the alimentary tract will depend on the properties of the particle itself and on the luminal environment. Particles have a certain size, shape and special surface properties. In the initial studies presented at the beginning of this chapter particle size was the most important parameter correlated with fate. Numerous studies investigate this relationship using spherical micro- and nanoparticulate objects. However, particles are not always isotropic. Rod-, disk- or ellipsoid-like particles can also be manufactured or be generated by nature (Fig. 12.1). If their long axis exceeds the size of the cellular gateways uptake may occur only if the particle approaches in a certain orientation. This influence of orientation was impressively demonstrated for particle phagocytosis by macrophages [114] but no information exists on how intestinal dendritic cells or M cells may cope with anisotropic nanoparticles, in particular when the aspect ratio is high. Beyond size and shape the particle surface properties are an important criterion for particle handling by the host. Nanotechnologists have methodologies at hand to customize the surface of nanoparticulate matter to their needs or liking and there are also numerous analytical tools to confirm the desired surface design. Yet, most of these efforts may be futile attempts because it is naive to assume that an ingested particle will not interact with luminal matter on its way through the gut. Especially particles with hydrophobic and charged surfaces may eagerly adsorb hydrophobic or counterionic intestinal constituents (e.g. lipids, nucleic acids, proteins, solute ions) and cover themselves with a so-called corona (e.g. [115–117]). For systemically administered nanoparticulate preparations the formation, composition and consequences of a corona became subject of intense investigation in the last decade. Unfortunately, no information about a gastrointestinal nanoparticle corona exists as yet. Only a few studies with bacteria have been conducted. They show that the presence of soluble non-starch plantain-fibers suppresses intestinal infection of chicken by *Salmonella* spp. [118, 119] and blocks adhesion of intestinal pathogens and commensals to M cells [120, 121] whereas the presence of emulsifier in the diet such as polysorbate-80 increases M cell translocation of bacteria [120]. As the cells were preincubated without washing it remains unclear at this point whether the epithelial surface was passivated/activated by the soluble non-starch plantain-fibres or the detergent or whether the bacteria adopted a detergent or non-starch plantain-fibre corona that supported or prevented uptake.

In light of the latter it seems clear that luminal content can have a substantial influence on particle uptake and translocation by the intestinal epithelium. The observation in the chicken-infection-study that only broccoli- and banana- but not apple- and leek-plantain fibres excerted a protective effect again demonstrates how complex the various influences on nanoparticle-host-interactions in the gut must be.

Table 12.2 Examples of nanoparticles from natural source as well as engineered nanoparticles

Naturally occurring nanoparticles			Anthropogenic nanoparticles		
Source	Chemical structure	References	Type/Class	Constituent(s)	References
Surface water	$CaCO_3$, Al_2O_3, SiO_4^{4-}	[123–125]	Inorganic	Metals (nAu, nAg, nCu, nPt) Metal oxides (FexOy, ZnO, TiO_2) Quantum Dots (CdS, CdSe, and InAs)	[126, 127]
Volcano ashes	SiO_2				[128]
Iceberg sediments	Fe_3O_4				[129]
Soot from pyrolysis	C, nanotubes, Fullerene		Organic	Non-biogenic polymers	
Mineral wells	S				
Umbra	MnO, Fe_3O_4			Biogenic polymers, lipids	[130]
Bacteria and fungi	Se-Protein		Carbon-based	Fullerene, carbon tubes, carbon fibres	[131, 132]
Meteorites	Fulleren				
Plants and atmosphere	Adducts of oxidized terpens	[133–135]		Carbon black	[136]

12.4 Types of Nanoparticles Unintentionally Occuring in the Alimentary Tract

A basic distinction is drawn between natural and engineered nanoparticles. The mounting concern about a possible impact of nanoparticle exposure on human health is based on the tacit assumption that nanotechnology with its increasing market of engineered nanoparticles is exclusively responsible for this. However, what is disregarded in this context is the fact that nature itself is a large producer of nanoparticulate matter which presents in manifold sizes, shapes and compositions and stems from various sources (Table 12.2). It is estimated that those "natural" nanoparticles formed by biogeochemical processes amount to several billon metric tons per year which is orders of magnitude more than the current annual production of engineered nanoparticles [122, 123].

One of the main sources are eroded minerals which are carried into the atmosphere by storms over arid areas (e.g. asian dust). Estimates are that the atmosphere contains about 0.3 billion tons of submicron particles of which about a quarter precipitates in wet state [137]. Among the biggest biological sources encompassing 10–30 million metric tons per year are so-called secondary organic aerosols (SOAs) which are formed continuously in the atmosphere from terpens (e.g. α-pinene) released by plants [138]. Little is known whether and to what extent such natural nanoparticles affect the environment but it seems reasonable to assume that nature can cope with its own products. Consequently, there is a rapidly growing interest in mimicing those natural production processes by so-called phyto- and phyco-nanotechnology. With this it is already possible to generate natural-identical nanoparticles by bioreduction or abrasion in bacterial or fungal processes [139].

In the usual sense, however, the term "nanoparticle" stands for manufactured or engineered nanoparticles. Based on their composition, engineered nanoparticles (ENPs) can be classified in three main groups (Table 12.2): inorganic, organic and carbon-based nanoparticles [2].

The family of inorganic nanoparticles usually consist of elemental metal or of metal chalkogenids. Of noble metals such as Ag, Au, Pt etc. rather stable nanoparticles can be formed with physicochemical properties different to bulk material. Nanosilver (nAg) particles typically measure 25 nm. They have an extremely large relative surface area and release Ag^+ ions when placed in distilled water as solvent. These ions vastly improve the bactericidal and fungicidal effectiveness of nAg by denaturation or oxidation of respective microbiotic proteins. Based on these properties, nanosilver is widely used in cosmetics (creams, shampoos, deodorants, and body lotions) as well as in textiles (socks, shirts, trousers, and active underwear). A growing number of textiles are even fabricated with metal NPs in the structure (for example 31–2,600 ppm of nAg in T-shirts). This additive offers biocidal properties and prevents excessive sweating [127, 140].

Nanogold (nAu) particles are manufacturable in a wide size range between 1 and 100 nm and exhibit photothermal stability. Most valuable, however, are the special optical properties of nAu which make them interesting for all kind of medical diagnostics, e.g. liquid-phase assays electron microscopy, predominantly, transmission electron microscopy (TEM) or the so-called surface plasmon resonance spectroscopy [126].

Among the metal chalkogenids, oxides are the most versatile nanostructures because of their either metallic, semiconductor, or insulator characteristics. They offer diverse and tunable structural, physical and chemical properties and functionalities which make them widely usable as optical, optoelectronic, magnetic, electrical, mechanical, thermal, catalytic, photochemical tools. One prominent example already presented above are superparamagnetic iron-oxide nanoparticles (SPION) consisting of a γ-Fe_3O_4-core that is made water-soluble by a variety of different coating materials which easily can be functionalized by any kind of bioorganic molecules including antibodies, aptamers, and the like [128].

For nanoparticulate heavy metal sulfides, selenides and tellurides their unique optical properties are the most interesting physical feature. Upon excitation those

Quantum dots (Qdots), of which the already discussed cadmium selenide is an archetype, emit fluorescent light whose wavelength can be adjusted with particle size (2–10 nm). They are used in TV-screens and have been proposed as fluorescent labels for biomedical assays and imaging applications when fortified with a protective organic shell [129].

Organic nanoparticles make up the most diverse group of nanoparticulate materials as almost all biogenic and non-biogenic organic polymers can be formulated as nanoparticles with the right technology at hand. Family members range from spherical solid nanoparticles, dendrimers, over micelles and liposomes to drug conjugates. Organic nanoparticles are the system of choice for drug delivery as discussed below.

Carbon based nanoparticles represent the third major class of anthropogenic nanoparticles. To this class belong abundant products like amorphous carbon particles and carbon black (CB) but also more sophisticated materials such as C60 fullerenes, graphenes, carbon nanodots and single-walled carbon nanotubes (SWCNT) [131, 132]. Amorphous carbon particles, in particular carbon black, is produced in huge amounts but not all carbon black comes in the nanometer scale. While carbon black seed nanoparticles (nodules) are about 15–400 nm in diameter, also particulates in the high micrometer size can be manufactured [141, 142]. Amorphous carbon and carbon black nanoparticles (CBNP) are made use of as pigments in cosmetics [143], as catalysts [144] and possibly in future supercapacitor and flat screen designs [145, 146] as well as in bioassays [147]. Fullerenes and the other graphene-related carbon nanomaterials listed above will have an even broader field of application in the years to come ranging from sun glasses [148] over energy storage (solar cells [149], supercapacitors [150, 151], hydrogen stores [152–154]) to biomedical uses (antimicrobials [155], imaging, biosensors [156]) and possibly a lot of other applications we do not even imagine by now.

12.5 Intentional Administration: Drug Delivery and Contrast Agents

Peroral administration of drugs is the preferred route for self-medication. Unfortunately, the gastrointestinal tract is also a hostile environment in which present-day biological drugs (e.g. therapeutic antibodies, insulin, peptides, siRNA, aptamers, etc.) barely survive. For that reason pharmaceutical scientists develop micro- and nanoencapsulation systems that fortify susceptible drugs against gastrointestinal breakdown. Research in this area literally exploded within the last decade with a continuously increasing number of encapsulation techniques, matrix variants, particle surface functionalizations and drug loads. As a comprehensive presentation of this field would be beyond the scope of this chapter, we will focus here only on the basic principles of nanoparticulate gastrointestinal drug delivery systems.

12.5.1 Nanoparticulate Drug Delivery Systems

The field of nanoparticulate drug delivery emerged in the last decade of the last century, mainly driven by mucosal immunologists who realized the need for oral delivery systems in order to induce protective immunity at mucosal surfaces. Mucosal immunity mainly relies on the formation and secretion of secretory antibodies of the immunoglobulin A class which is induced if the respective antigen is taken up by the mucosal antigen gatherers, M cells and dendritic cells. As those gatherers prefer particulate matter in analogy to the bacteria they usually pick, a micro-to-nanoparticulate vaccine formulation is considered a logical solution [157–159]. Experimental mucosal immunization with particulate vaccines worked because the amount of antigen required to induce an immune reaction is low, a "catalytic" amount to use chemical terms. Nevertheless, particulate mucosal vaccines have not entered the market as yet. Excited by the pioneering work in the vaccine field pharmaceutical scientists envisioned the possibility to deliver degradation-sensitive drugs via the per-oral route by protecting them in a micro/nanocapsule. In retrospective, the delivery of encapsulated drugs across the gastrointestinal barrier "into the house" was not a success story. However, dropping the parcel "right at the doorstep" turned out to be a more favorable, yet still experimental approach. When the particles are able to reach the epithelial glycocalyx or the covering mucus layer they are as close as it gets to the recipient. In case of glycocalyx adhesion only a couple of hundred micrometers have to be passed by a drug that is released by the adhering nanoparticle. In contrast to the maze of obstacles and traps set by the luminal content the odds are considerably higher for the drug to become incorporated as long as the underlying cell has the right machinery for uptake. Using this approach, various drugs have experimentally been delivered to the desired site of uptake [158, 160–162]. To meet that goal the nanoparticulate drug formulation must be stable enough to reach their target cell environment but at the same time be so fragile that the matrix desintegrates slowly at the particle attachment site. Lipidic formulations meet that requirement [163] but also poly-lactide-coglycolide and other hydrolysis susceptible polymers [162, 164]. In order to enable nanoparticle adherence lectins are often used as particle coating because they mediate binding to the glycocalyx or the mucus [164, 165]. If the particles are to penetrate the mucus layer or have to "hide" in the mucus desintegrating enzymes or reducing compounds (thiols) are employed [166]. A disadvantage of micro- or nanoencapsulation is the slower Brownian motion of the particle as compared to the free drug and the fact that an excreted particle that did not reach its destination dumps at one push billions of drug molecules which thereby did not have a chance to enter the body. Those disadvantages are a double-edged sword. While low epithelial contact and rapid excretion of undesired nanoparticles is welcome, the same processes make life miserable for parmaceutical scientists involved in the development of nanoparticulate drug delivery systems.

12.5.2 Particulate Matter for Contrasting the Gastrointestinal Tract for Medical Imaging

The classical diagnostics of gastrointestinal diseases is based on endoscopic investigations and x-ray exams with a barium sulphate swallow. Advances in non-invasive imaging modalities including contrast enhanced ultrasound (CEUS), computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI) have in the last decades revolutionised the way in which the gastrointestinal tract can be studied. Avoiding radiation exposure, MRI is, besides CEUS, the most favourable technique combining excellent soft tissue contrast, spatial resolution, and depth of penetration [167]. A variety of contrast agents has been evaluated for MRI enterography including paramagnetic gadolinium chelates which reduce the longitudinal T1-relaxation property and result in a brighter signal, and superparamagnetic iron oxide based nanoparticles (SPION) which show size dependent T1- or T2-effects. SPION have been used in clinical MRI for more than 20 years [168], and over time, the FDA approved several SPION formulations, mainly for

Table 12.3 Characteristics of widely used clinical iron oxide nanoparticles

Generic name/product number	Trade name	Coating	Hydrodynamic diameter	Blood half-life	References
Intravenous (i.v.)					
Ferumoxides AMI-25	Endorem Feridex	Dextran	SPIO: 50–100	10 min	[168–170]
Ferucarbotran SHU555A	Resovist# (Japan)	Carboxy-dextran	SPIO: 60–80 nm	12 min	
	Resovist S Supravist	Carboxy-dextran	USPIO: 20–25 nm	6–8 h	
Ferumoxtran-10 AMI-227	Sinerem# (Europe)	Dextran	USPIO: 20–50 nm	24 h	
Feruglose NC100150	Clariscan	Carbohydrate-polyethylene glycol	USPIO: 11–15 nm	2 h	
Ferumoxytol AMI-7228	Feraheme# (USA)	Carboxy-methyldextran	USPIO: 20–30 nm	10–14 h	
oral (p.o.)					
Ferumoxsil AMI-121	GastroMARK# (USA) Lumirem# (Europe)	Siloxane	SPIO: 10–300 nm		[171, 172]

Contrast media that are currently available for use in patients in countries listed. Other media have already been withdrawn from the market in certain countries

intravenous administration for indications including MR angiography, tissue perfusion studies, and atherosclerotic plaque and tumor imaging (Table 12.3) [169].

The FDA approval of *Ferumoxytol* as an intravenous iron drug in 2009 and 2017 has led to a renaissance of "off-label" use of SPION also for a variety of MRI imaging applications, in particular because these nanoparticles are regarded as safer contrast agents compared to the frequently used gadolinium chelates, especially in patients with renal insufficiency [170].

To date, *Ferumoxsil* is the only FDA-approved SPION for oral administration. This contrast agent is composed of iron particles of about 10 nm, coated with a non-biodegradable and insoluble matrix (siloxane) and suspended in viscosity-increasing agents such as starch and cellulose. After oral administration, *Ferumoxsil* fills the stomach and intestines and the T2 relaxation is enhanced, thereby darkening the contrast agent-containing portion of the gastrointestinal tract, distinguishing bowel from organs and tissues adjacent to the upper regions of the gastrointestinal tract. Routine indication for *Ferumoxsil* is the Magnetic Resonance Cholangiopancreatography (MRCP), the non-invasive imaging technique of choice to evaluate the pancreatobiliary system, where the fluid in the biliary and pancreatic ducts is visualized with heavily T2-weighted sequences [171]. In T2-weighted bowel MR imaging, *Ferumoxil* is also routinely used to highlight Crohn's disease lesions, and can detect mural and transmural inflammation with the same accuracy as gadolinium-enhanced T1-weighted MR [172].

Magnetic particle imaging (MPI) is a new imaging modality with extraordinary contrast and sensitivity using superparamagnetic iron nanoparticles [173, 174]. Recently, first gastrointestinal applications for this method using a designed nanodevice have been published [175]. MPI-tailored, long-circulating SPIONs were injected through the tail vein in a mouse model of induced acute gastrointestinal bleeding. The captured tracer accumulation in the lower GI tract was monitored with excellent contrast showing a bleed rate between 1 and 5 μL/min in this model. This could be an important clinical translation in the future, because unclear gastrointestinal bleeding is a major concern in internal medicine.

However, with all the described applications of nanoparticles as oral contrast media for MRI, a possible uptake of intact or partly digested particles or particles materials was not addressed so far, always relying on the expected low toxicity of iron as essential trace element.

12.6 Fate of Ingested Particulate Matter: Beeline or Detour

Cells interact with their surroundings and will, upon contact, also try to incorporate nanoparticles. It is well known from cell-culture studies that almost all mammalian cells take up nanoparticles to some extent using a variety of uptake mechanisms which are mostly nonspecific. Extensive in vitro studies have explored the features of nanoparticles (size and physical properties) that influence their cellular uptake

and intracellular processing resulting in elimination, degradation or storage of the particles in the respective cell.

However, it should be asked at this point, how relevant these results are for the in vivo situation. Despite their general capability to take up larger molecules and particles, normal cells in the body will routinely not be involved in this process because, in a close interaction between tissues, incorporated nanoparticles are recognized as exogenous materials and sequestered by mononuclear phagocytic system (MPS) cells mainly in liver or spleen. When dealing with particles that are taken up via the digestive tract, the central question is certainly how many of them are capable of crossing the epithelial barrier at all before they can encounter any other cells inside the body.

12.6.1 Measuring Gastrointestinal Particle Uptake in Model Systems

The question of particle uptake/translocation at mucosal surfaces has incented numerous investigations using different model systems which reflect the in vivo situation and the compartments involved in particle-host-interaction to various degrees. In vitro, ex vivo and in vivo systems for particle uptake studies have been developed which shall address different aspects of the process [176].

In vitro cell culture systems use intestinal epithelial cells of various origin, with the human colon carcinoma-derived cell line Caco-2 being the favourite tool. In culture, Caco-2 cells differentiate into a columnar epithelial cell type upon reaching confluence, with several biochemical and morphological characteristics of small intestinal enterocytes, e.g. microvilli and tight junctions [177–179]. Co-culture models, where Caco-2 cells are mixed with other cell types, try to more closely imitate the in vivo situation: Co-culture with the human adenocarcinoma cell line HT29 will introduce a goblet-cell-type into the cell layer and lead to the formation of a mucus layer on the apical cell surface [180, 181], induction of M cell formation is attempted by co-culturing Caco-2 cells with lymphocytes isolated from murine intestinal Peyer's patches [182] or human Burkitt's lymphoma B cells [183–185], and triple cultures attempt to combine all these features [186]. Studies on particle uptake and translocation are usually performed using a so-called transwell-system where the intestinal epithelial cells are grown on permeable filter supports inserted into the wells of culture dishes. This way, both sides of a polarized cell monolayer are in contact with a separate fluid compartment which shall render the system particularly versatile for transport studies [178, 187]. In the different experimental set-ups, particle uptake and/or translocation was observed to various degrees, obviously depending on factors such as particle type, size, and surface modification. In most cases, particle acquisition by the Caco-2 layer appears to follow an endocytosis/transcytosis mechanism, with clathrin- or receptor-mediated endocytosis, macropinocytosis and lipid raft/caveolae all contributing to the process [188–191]. Only rarely, opening of tight junctions

and paracellular transport have been described [191]. With lipidic nanoparticulate formulations which may desintegrate in the cells substantial uptake of a hydrophilic fluorescent dye encapsulated in the particles could be observed [192] but in studies where actual translocation in a transwell system was tested free cargo was transported at least as good or even better than cargo formulated in lipidic nanoparticles [193]. In the few examples were stable particles were used and quantification was given at all, translocation rate of particles was almost always well below 1% of all particles offered [189, 190, 194]. The presence of M-like cells in the Caco-2 co-culture models greatly increased translocation of plain latex or polystyrene particles between 100 and 1000 nm in size [183–185, 194]. However, the significance of these findings for the in vivo situation must be regarded with caution, since the M cell concentration in the human intestine (<5% in the FAE and <1‰ in the total intestine) is far below the value described in these studies (up to 30%). Also, co-culture with M-like cells did not enhance transport of polyacrylic acid-coated iron oxide or silver nanoparticles of <10 nm core size [194]. Thus, for in vitro systems uptake was reported quite often but the taken-up particles seem to become trapped inside the cultured cells. This is bad news with regard to drug delivery and good news in terms of nanoparticle safety since the particle-laden cells would be sloughed off into the gastrointestinal lumen within a few days. What still remains entirely unconsidered in those systems is the question whether nanoparticles would actually gain access to the epithelial cell membrane where they can be taken up. The apical cell membrane of Caco-2 cells is far better accessible for particles than that of enterocytes in vivo because Caco-2 cell layers display only a rudimentary often gappy glycocalyx of max. 30–50 nm hight [29, 193, 195, 196] as compared to the continuous enterocyte glycocalyx in vivo which is several hundred nanometers thick [24]. In co-culture studies with mucus producing cells the important filter- and sink-like effect of an apical surface coat, in this case mucus, was reported [160, 191, 197, 198]. Considering all of the above, it seems questionable whether in vitro cell culture systems are able to yield robust and valid data concerning nanoparticle translocation at the gastrointestinal mucosa.

In an attempt to more closely reflect the in vivo situation, some work has been performed using ex vivo animal experiments. In the everted sac model, a segment of the rat small intestine is dissected, flushed, turned inside-out, and the ends are ligated. Uptake from the—former luminal—outside into the serosal compartment is analyzed after incubation of the sac in the medium of interest. Using this method, uncoated silica particles between 70 and 1000 nm were not absorbed through the intestine in a measurable extent, but after surface modification with amine- or carboxyl groups, uptake of 70-nm particles was demonstrated [199]. In the same system, polyamidoamine dendrimers of approximately 3–7 nm diameter were clearly crossing into the serosal compartment, whereby anionic nanoparticles displayed a higher transfer rate than cationic ones [200].

Still more close to the in vivo situation is the isolated perfused rat intestine model. Here, the complete small intestine of a rat including mesenteric artery and portal vein is explanted. The organ is placed in a perfusion chamber where a constant supply of nutrients and oxygen can be provided via the cannulated vessels (Fig. 12.8). In this system, import into as well as efflux out of the gut lumen can be investigated

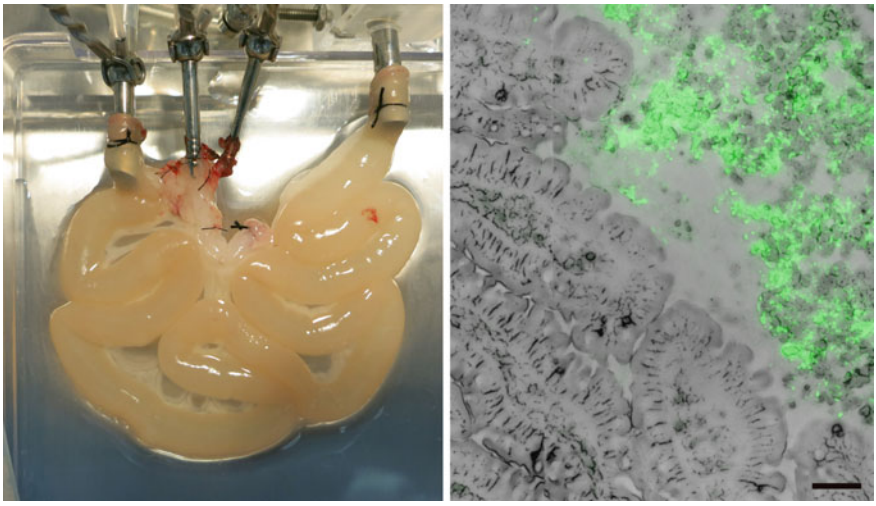

Fig. 12.8 Nanoparticle uptake studies in an ex vivo model. Left: Isolated rat intestinal explant in the perfusion chamber. Via the cannulated artery, the explant is supplied with artificial blood plasma; particle samples can be applied luminally, and their final location in different compartments (luminal, lymph, tissue) can be analyzed. Right: Entrapment of NPs in intestinal mucus. Cryostat section of gut tissue after luminal application of fluorescent 20 nm particles into the lumen of the isolated rat intestine. Bar: 50 μm

by separately collecting and analyzing the fluid from lumen, vascular perfusate and lymph [201]. In particle uptake studies using the isolated perfused rat intestine and luminally applied fluorescent polystyrene particles of 20, 40 and 200 nm in size, no particles could be found in either vascular or lymphatic effluate samples, nor were any particles detected by histological examination in the gut tissue. Instead, a large fraction of the NPs was trapped in the mucus layer lining the intestinal wall (Fig. 12.8) [202].

The few data obtained from the organ explant/ex vivo studies indicate only very sparse, if any, uptake of nanoparticles in the size range between 20 nm and 100 nm in the intestine, but particulate matter below 10 nm in size can cross the epithelial barrier to a certain extent.

No matter how well an in vitro/ex vivo model performs, the most reliable answers will be obtained by in vivo evaluations. With regard to the fate of ingested nanoparticles, several animal models have been established and used in uptake and toxicity experiments [203, 204]. Application of particles is usually done via feeding or intragastric delivery, but the different experimental set-ups vary in their methodology to detect and quantify uptake of nanoparticles into the body. An in vivo study can be performed in two ways: “in situ”, i.e. by continuous or time-lapsed recording of the consequences of a set trigger, or “ex situ”, i.e. by analyzing the outcome of a set trigger once after a defined period of time.

In situ analyses of nanoparticle-host-interaction require cutting-edge technologies which allow detection of the particles inside the body in a time- and spatially-resolved manner, ideally on microscopic scale [205]. Due to this demanding instrumental requirements few studies have been conducted as yet on the in situ level, either via intravital optical imaging after application of fluorescent probes, or by whole body scintigraphy if radioactive particles were used. In general, the in situ acquired data could only give a very limited overview of particle distribution; to date, this approach is neither sensitive nor accurate enough for quantitative interpretations. For example, when silica-based rhodamine-derivatized nanoparticles (20 and 100 nm) or polymer-coated quantum dots (33–36 nm) were applied orally to mice and tissue distribution was monitored at different time points with intravital imaging systems, fluorescence signals were mainly visualized in the gastrointestinal region [68, 206]. Only subsequent ex situ imaging of dissected organs allowed some quantification of the fluorescence signals in one study and proved dissemination of particles over the body, with accumulation in lung, liver and kidney [206]. At the starting point of the particle uptake process, intravital confocal microscopy revealed uptake of 20- and 40-nm particles by intestinal epithelial cells and transport into the serosa and the lymphatics of the intestine, but larger particles (100–2000 nm) were scarcely internalized [207]. Here again, quantification of results was only relative, and no absolute uptake rates could be deduced.

Ex situ analysis is the methodology of choice in most particle uptake studies, simply because one needs less sophisticated instrumentation to analyse a faeces or urine sample or post mortem resected tissue. In case of intestinal tissue which ought to be most informative about the sites and degrees of particle entry the specimen is either fixed, cut and investigated by classical microscopy (light or electron microscopically). Alternatively, bulk analyses are performed where the whole tissue, blood or excretion is digested selectively in solutions that either leave particles intact or disintegrate them, too, with particle constituents becoming solutes. Intact particles are then counted on filters or in Neubauer chambers, constituents from dissolved particles are determined radiologically or spectroscopically, e.g. by fluorescence or inductively-coupled plasma mass spectroscopy (ICP-MS) [203]. Each of these ex situ techniques has its strengths and weaknesses. Only a careful combination of histological and bulk analysis will provide the desired information about site and degree of particle uptake [16]. Looking histologically, numerous studies report uptake of particulate matter in the Peyer's patches/follicle associated epithelium, presumably via M cells, though most of those studies use particles above 1 μm in size [97, 99], but location in/transport via villus regions was also observed with particles below 100 nm [207, 208]. Performing bulk analyses of tissues, organs and excretions after oral nanoparticle exposure still reveals conflicting results. While in several studies nanoparticles up to 100 nm in size were found—to various extents—in spleen, liver, kidney, blood and urine [203, 204, 209], others describe that no measurable amounts of nanoparticle material could be detected in any of these tissues [210, 211].

Ex situ bulk analyses is also the method of choice for the archetype food-borne nanoparticle, titanium dioxide (titania), because the oxide is believed to be sufficiently resistant to luminal content of the gut. Numerous studies have been con-

ducted in rodents with adverse effects to inner organs been detected [212]. In these studies titania doses ranging from 1.5 to 5000 mg/kg body weight were used, with a mean dosage of 453 mg/kg and a median dosage of 27.5 mg/kg. Even studies on human volunteers were performed with the titanium content of blood samples being determined [213–215]. In two of the three human studies elevated serum titanium levels were found; whether or not those elevated levels caused any pathology was not checked. In these three studies titania doses ranging from 0.31–5 mg/kg body weight were used with a mean dosage of 2.26 mg/kg and a median dosage of 1.34 mg/kg. The latter doses were 200-fold (mean) or 20-fold (median) lower than those used in rodents where pathology was observed. Yet, dosing in human beings was still about 4100-fold (mean) or 2400-fold (median) higher than the estimated daily titania nanoparticle intake of 7–69 year old humans (0.55 μg/kg body weight) in the Netherlands [216]. The daily Dutch titanium dioxide nanoparticle intake differs considerably from calculations for North-American citizens who are believed to ingest about 1 mg titania nanoparticles per kg body weight and day [217]. Why a North-American may ingest about 180-times more titania nanoparticles than the average European cannot be clarified at this point. Even then is the dosing in the rodent studies in which adverse effects of titania on inner organs were observed on average 27-fold (median) to 450-fold (mean) higher than the food-borne titania nanoparticle intake of North-Americans. For Europeans the animal models are overloaded by a factor of about 5000 (median) to 800,000 (mean). In light of this, it seems questionable whether those models can faithfully predict possible risks for human beings. In some of the (overloaded) rodent studies deposition rates in certain inner organs were reported. In most cases the amount of titanium found was less than 1% of the administered dose. Only in one study [218] a comprehensive analysis of 11 tissues was conducted. Here a cumulative TiO_2 deposition of about 5% of the administered amount was reported. Deposition was highest in Peyer's patches and the mesentery network which is in line with the particle uptake mechanisms presented above. Exogenous pigment was also found consistently in Peyer's patches of human beings beyond preschool age [219, 220]. The amount of those aluminium, silicon and titania-containing deposits seems to increase with age.

Taken together, the majority of ex vivo and in vivo studies which most closely reflect the situation in the human gastrointestinal tract show that incorporation of digestion-resistant nanoparticles occurs in the alimentary tract but at a low rate and preferentially at sites dedicated for collection of luminal matter. Nanoparticles may be an inevitable by-catch and may ever have been. Whether or not the deposition rate is higher nowadays than it used to be in the preindustrial age is open to debate.

12.6.2 Deposition, Breakdown and Excretion of Incorporated Matter

The biological and toxical effects of nanoparticles depend on their ability to reach and to interact with cells and organs in the body. The first step is uptake into the body, which usually implies nanoparticle contact with and penetration of either of the

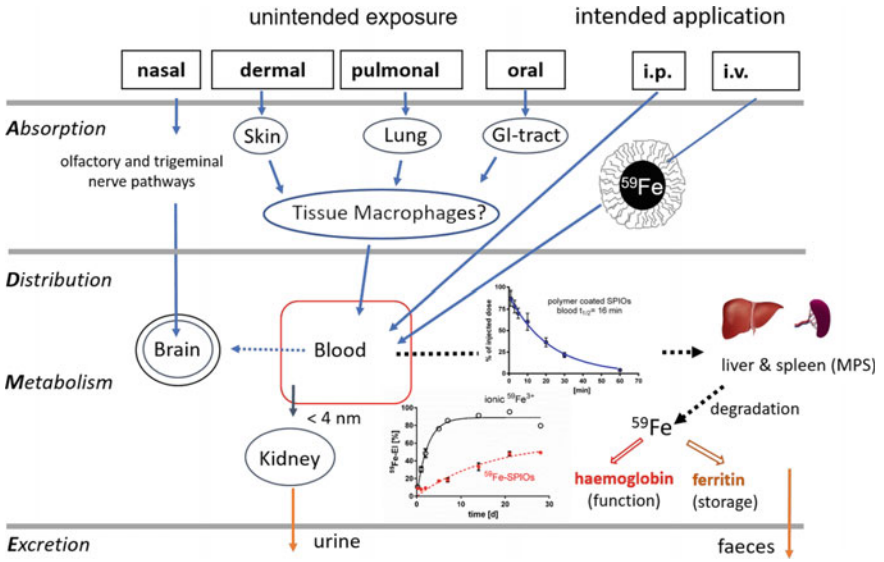

Fig. 12.9 Uptake into and fate of nanoparticles in the body. The acronym ADME stands for the pharmacokinetic processes involved: absorption, distribution, metabolism and elimination. The fate of an iron-based nanoparticle (polymer-coated ^{59}Fe-labelled-SPION, 25 nm hydrodynamic diameter) is depicted as an example showing the fast clearance from the blood (half-life 16 min), degradation in the liver and use of iron in newly formed haemoglobin or storage in form of ferritin. MPS, mononuclear phagocytic system (figure adapted from [221])

three main body barriers, namely the skin, the lung, and the mucous membrane of the gastrointestinal tract (Fig. 12.9). The evaluation of the respective uptake mechanisms is of great importance for basic science but also for developing effective and safe nanomedical applications.

Different exposure conditions are not only of considerable impact on how much nanoparticles are incorporated into the body, but also on how they may be distributed, excreted or metabolised. In most currently used or planned nanomedical applications, the respective nanodevice is administered by intravenous injection. Here, the complete bolus dose is given into a peripheral vein. The particles are swirled around with thousands of plasma proteins throughout the venous systems, are facing billions of moving cells and the large surface area of vascular endothelial cells.

The intravenous application of nanoparticles can be regarded as a model of how natural defense mechanisms cope with natural or xenobiotic particles larger than the normal molecules [221]. Such particles are almost immediately covered by a protein corona including specialized plasma proteins, so-called opsonins. Opsonins serve as a signal for a fast clearance of these particles from the blood by uptake into cells of the so-called Mononuclear-Phagocyte-System (MPS) mainly based in the liver and spleen. Again, using a radiolabelled polymer-coated SPION (compare also Fig. 12.5), it was shown that these particles upon injection in mice were rapidly taken up into

the liver mainly by macrophages (Kupffer cell), but surprisingly also by sinus-lining endothelial cells [222]. After some days, the ^{59}Fe-label was incorporated into newly formed erythrocytes, indicating a complete degradation of the iron oxide cores and use of the iron in the iron metabolism of the mice.

The intraperitoneal injection (i.p.) could also be an application modus for future nanodevices in nanomedicine. In this context, it was shown that ^{59}Fe-labeled SPION, which were embedded into the lipid core of triglyceride-rich lipoproteins (TRL-^{59}Fe-SPION) (nanosomes, similar to shown in Fig. 12.5) and injected i.p. into mice did appear in the blood to a substantial extent ($10.1 \pm 0.91\%$ of the injected doses after 24 h), whereas polymer-coated SPION were not able to escape the abdominal cavity barrier [223].

However, the situation after intravenous or intraperitoneal injection is different from dermal, pulmonal, or oral exposure which can deliver at most only the minor fraction of particles that is able to cross the barrier by different mechanisms and reaches specific cells in deeper layers of the skin, lung, or gastrointestinal tract before these particles can enter small capillary blood vessels.

To detect and—even better—to quantify these small amounts of nanoparticles in the body is a very tough experimental challenge. To quantify nanoparticles in biological surroundings, a number of methods have been used which attempt to exploit specific chemical properties of the respective class of matter. Quantum dots, for example, show a very strong and stable fluorescence which renders these particles identifiable even in living tissue by confocal microscopy. In order to better separate the signals from nonspecific noise, the wavelength of the fluorescence maxima can even be tuned by simply changing the size of the particles. A number of drug delivery nanodevices have been used to document an apparent successful overcoming of multiple gastrointestinal barriers using Quantum dots composite particles [224]. However, as discussed before, a true and sensitive quantification by fluorescence alone is almost impossible, especially when only a small uptake of particles can be expected.

As shown already in Fig. 12.5, radiolabelling of nanoparticles is a sensitive and reliable technique and represents so far the methodological gold standard to investigate the translocation of nanoparticles into the body. But also with radiolabelled probes, the detection of minor translocation processes requires special techniques and equipment. ^{59}Fe is an isotope radiating hard γ-rays which makes it easy to measure small amounts of incorporated ^{59}Fe in living mice by whole-body-counting. This technique has been used successfully to measure particokinetics and biodistribution in nanoscience. Consequently, much is known about iron-oxide based nanoparticles including the uptake from the intestinal tract [58, 222, 225, 226].

The group of Wolfgang Kreyling has studied the translocation of radiolabelled gold nanoparticles. The advantage of AuNP is the availability of a wide range of possible core diameters and Au can easily be neutron-activated to form ^{198}Au, a weak γ-radiating isotope with rather short half-life (2.7 d). These characteristics make, however, the analysis of low activities of ^{198}Au in tissue samples less sensitive compared to ^{59}Fe, especially when no whole body counter is available. When the translocation of intratracheally instilled ^{198}Au-particles with different size (1.4–200 nm)

was analysed in rats, low ^{198}Au activities were measured in different tissues such as liver and spleen, with a significant translocation into organs only for the small particles (1.4 and 2.8 nm) [227]. It appears likely that the small particles can cross the very thin air-blood-barrier to the circulation. A similar approach was performed with intra-esophagal installation of ^{198}AuNP. Again, only small amounts of ^{198}AuNP reached the circulation (after 24 h, $0.37 \pm 0.10\%$ for 1.4 nm particles; $0.12 \pm 0.02\%$ for 18 nm particles) with some methodological problems to properly define a reliable 100%-reference value for the short investigation time span of 24 h. The highest amount of the applied particles was detected in the remaining carcass, which encompassed adipose tissue, bones, muscles and skin [228]. Thus, from the studies with radiolabelled iron- or gold particles it can be concluded that nanoparticles with a typical size of 10–20 nm are almost not absorbed from the normal intestinal tract of rodents. The very small ^{198}Au-particles (1.4 nm) may be different, but also here is the absorption very limited.

What remains unclear is the biodistribution of particles that can cross the epithelial layer in the gastrointestinal tract. A true translocation of a particle through a cell has so far not been unequivocally established. As mentioned above, many cells can engulf particulate matter to a certain extent, and they will always try to degrade xenobiotic particles with different success, macrophages being most potent for this. As for the epithelial cells in the intestinal lining, intracellularly located particles may face another fate altogether: Considering that the cells in the gastrointestinal tract have a short half-life of several days only, a major fraction of particles taken up directly by epithelial cells in the gut will not reach the circulation, but be expelled by exfoliation. Another fraction may be taken up by tissue-based macrophages via phagocytosis and will be metabolised within these cells, and components (including e.g. radiolabels) will then reach the circulation. Some cells which have taken up highly toxic nanoparticles could also die of apoptosis and remnant particles, located in siderosomes may be taken up by other cells. Thus, the biodistribution will be very much different from experiments with intravenous application with a more local distribution apart from the MPS system.

12.7 Conclusions

Nanoparticles, most abundant from natural sources, but increasingly also as engineered substances, are perpetually present in the environment and in the food chain. Ingestion and uptake from the gastrointestinal tract represents one of the most relevant routes for unintended exposure to nanoparticles.

A main process that nanoparticles have to face in stomach and intestine is digestion mediated by hydrochloric acid as well as hydrolytic enzymes. Many nanoparticles with limited colloidal stability will thereby shrink and liberate all kinds of ingredients. Released low molecular weight components may then be taken up by the intestinal epithelial cells utilizing transport mechanisms originally intended for uptake of nutrients. As far as dietary useful components are concerned, their release

from nanoparticles would simply contribute to nutrition. With any toxic ingredient such as Cd^{2+} from Quantum dots, or Ag^{+} from AgNP, release from nanoparticles would fall into the field of toxicology of bulk materials. Yet, within all logical considerations, the expected concentration should be well below any permissive limit values.

It may be that very small sized nanoparticles can directly cross the intestinal barriers to a small but significant degree, reach the circulation, but then are mostly excreted by the kidneys due to their small size (<4–5 nm). A controlled uptake of rather large particles in the micrometer size range can be mediated by M cells and dendritic cells. However, due to the low number of these cells, this represents only a limited uptake possibility for nanoparticles. Another uptake route for nanoparticles could be the consequence of recurring epithelial leakages resulting in paracellular influx. Inflammatory bowel diseases may trigger the formation of spontaneous fissures, and—discussed at the moment—the gut epithelium may partly lose its barrier function towards nanoparticles in diseases such as Crohn's disease.

A matter of high relevance when looking at the fate of nanoparticles in the gastrointestinal tract is the issue of drug delivery since the oral application route is highly attractive for the administration of drugs and mucosal vaccines. In this field, many studies using larger drug-delivery nanoconstructs have been published. However, successful achievement on the oral route was more often proclaimed than conclusive experimental data was given. Clearly more in vivo studies in health and disease using better quantification techniques are needed, because the relevance of cell culture studies for this purpose is more than questionable.

So far, the experimental evidence shows that the uptake of nanoparticles from the intestine in vivo is very limited. It appears that the gastrointestinal tract is a very complex organ that can quite well discriminate between valuable dietary ingredients, which are taken up by a battery of specific uptake mechanisms, and worthless particles, which hardly can overcome effective barriers in the healthy gut.

Therefore, despite the risk of increasing amounts of engineered nanomaterial in the food chain, a harmful acute or chronic poisoning with ingested nanoparticles seems to be highly unlikely at this point in time.

References

1. Piccinno, F., Gottschalk, F., Seeger, S., Nowack, B.: Industrial production quantities and uses of ten engineered nanomaterials in Europe and the world. J. Nanopart. Res. **14**, 1109 (2012). https://doi.org/10.1007/s11051-012-1109-9
2. Ostiguy, C., Roberge, B., Woods, C., Soucy, B.: Engineered Nanoparticles: Current Knowledge about OHS Risks and Prevention Measures, 2nd edn. Institut de recherche Robert-Sauvé en santé et en sécurité du travail (2010). ISBN 2896314792, 9782896314799. https://www.irsst.qc.ca/en/publications-tools/publication/i/100529/n/engineered-nanoparticles-current-knowledge-about-occupational-health-and-safety-risks-and-prevention-measures-second-edition-r-656/redirected/1

3. Griffin, S., Masood, M.I., Nasim, M.J., Sarfraz, M., Ebokaiwe, A.P., Schäfer, K.-H., Keck, C.M., Jacob, C.: Natural nanoparticles: a particular matter inspired by Nature. Antioxidants **7**, 3 (2018). https://doi.org/10.3390/antiox7010003
4. Boccuni, F., Ferrante, R., Tombolini, F., Lega, D., Antonini, A., Alvino, A., Pingue, P., Beltram, F., Sorba, L., Piazza, V., Gemmi, M., Porcari, A., Iavicoli, S.: Workers' exposure to nano-objects with different dimensionalities in R&D laboratories: measurement strategy and field studies. Int. J. Mol. Sci. **19**, 349–377 (2018). https://doi.org/10.3390/ijms19020349
5. Kirch, J., Guenther, M., Doshi, N., Schaefer, U.F., Schneider, M., Mitragotri, S., Lehr, C.-M.: Mucociliary clearance of micro- and nanoparticles is independent of size, shape and charge—an ex vivo and in silico approach. J. Control Rel. **159**, 128–134 (2012). https://doi.org/10.1016/j.jconrel.2011.12.015
6. Pan, K., Zhong, Q.: Organic nanoparticles in foods: fabrication, characterization and utilization. Annu. Rev. Food Sci. Technol. **7**, 245–266 (2016). https://doi.org/10.1146/annurev-food-041715-033215
7. Sekhon, B.S.: Food nanotechnology—an overview. Nanotechnol. Sci. Appl. **3**, 1–15 (2010). https://doi.org/10.2147/NSA.S8677
8. Herbst, E.F.G.: Das Lymphgefäßsystem und seine Verrichtungen, pp. 333–337, Göttingen (1844)
9. Hirsch, R.: Über das Vorkommen von Stärkekörnern im Blut und im Urin. Z. Exp. Path. Ther. **3**, 390 (1906)
10. Volkheimer, G.: Detection of starch in tissue and urine after oral starch intake. Dtsch Gesundheitsw **15**, 1298–1302 (1960)
11. Jani, P.U., Florence, A.T., McCarthy, D.E.: Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat. Int. J. Pharm. **84**, 245–252 (1992). https://doi.org/10.1016/0378-5173(92)90162-U
12. Alpar, H.O., Field, W.N., Hyde, R., Lewis, D.A.: The transport of microspheres from the gastro-intestinal tract to inflammatory air pouches in the rat. J. Pharm. Pharmacol. **41**, 194–196 (1989). https://doi.org/10.1111/j.2042-7158.1989.tb06429.x
13. Payne, J.M., Sansom, B.F., Garner, R.J., Thomson, A.R., Miles, B.J.: Uptake of small resin particles (1-5 μ diameter) by the alimentary canal of the calf. Nature **188**, 586–587 (1960). https://doi.org/10.1038/188586a0
14. Pontefract, R.D., Cunningham, H.M.: Penetration of asbestos through the digestive tract of rats. Nature **243**, 352–353 (1973). https://doi.org/10.1038/243352a0
15. Sanders, E., Ashworth, C.T.: A study of particulate intestinal absorption and hepatocellular uptake: Use of polystyrene latex particle. Exp. Cell Res. **22**, 137–145 (1961). https://doi.org/10.1016/0014-4827(61)90092-1
16. Hodges, G.M., Carr, E.A., Hazzard, R.A., O'Reilly, C., Carr, K.E.: A commentary on morphological and quantitative aspects of microparticle translocation across the gastrointestinal mucosa. J. Drug Target. **3**, 57–60 (1995). https://doi.org/10.3109/10611869509015934
17. Ebel, J.P.: A method for quantifying particle absorption from the small intestine of the mouse. Pharm. Res. **7**, 848–851 (1990). https://doi.org/10.1023/A:1015964916486
18. Limpanussorn, J., Simon, L., Dayan, A.D.D.: Transepithelial transport of large particles in rat: a new model for the quantitative study of particle uptake. J. Pharm. Pharmacol. **50**, 753–760 (1998). https://doi.org/10.1111/j.2042-7158.1998.tb07136.x
19. Powell, J.J., Faria, N., Thomas-McKay, E., Pele, L.C.: Origin and fate of dietary nanoparticles and microparticles in the gastrointestinal tract. J. Autoimmun. **34**, J226–J233 (2010). https://doi.org/10.1016/j.jaut.2009.11.006
20. Fröhlich, E., Mercuri, A., Wu, S., Salar-Behzadi, S.: Measurements of deposition, lung surface area and lung fluid for simulation of inhaled compounds. Front. Pharmacol. **7**, 181 (2016). https://doi.org/10.3389/fphar.2016.00181
21. Squier, C.A., Kremer, M.J.: Biology of oral mucosa and esophagus. J. Natl. Cancer Inst. Monogr. **29**, 7–15 (2001). https://doi.org/10.1093/oxfordjournals.jncimonographs.a003443
22. Squier, C.A.: The permeability of keratinized and nonkeratinized oral epithelium to horseradish peroxidase. J. Ultrastruct. Res. **43**, 160–177 (1973). https://doi.org/10.1016/S0022-5320(73)90076-2

23. Squier, C.A.: The permeability of oral mucosa. Crit. Rev. Oral Biol. Med. **2**, 13–32 (1991)
24. Ramaker, K., Bade, S., Röckendorf, N., Meckelein, B., Vollmer, E., Schulz, H., Fröschle, G.-W., Frey, A.: Absence of the epithelial glycocalyx as potential tumor marker for the early detection of colorectal cancer. PLoS ONE **11**, e0168801 (2016). https://doi.org/10.1371/journal.pone.0168801
25. Bullen, T.F., Forrest, S., Campbell, F., Dodson, A.R., Hershman, M.J., Pritchard, D.M., Turner, J.R., Montrose, M.H., Watson, A.J.M.: Characterization of epithelial cell shedding from human small intestine. Lab. Invest. **86**, 1052–1063 (2006). https://doi.org/10.1038/labinvest.3700464
26. Madara, J.L.: Maintenance of the macromolecular barrier at cell extrusion sites in intestinal epithelium: physiological rearrangement of tight junctions. J. Mem. Biol. **116**, 177–184 (1990)
27. Marchiando, A.M., Shen, L., Graham, W.V., Edelblum, K.L., Duckworth, C.A., Guan, Y., Montrose, M.H., Turner, J.R., Watson, A.J.M.: The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. Gastroenterology **140**, 1208–1218 (2011). https://doi.org/10.1053/j.gastro.2011.01.004
28. Watson, A.J.M., Chu, S., Sieck, L., Gerasimenko, O., Bullen, T., Campbell, F., McKenna, M., Rose, T., Montrose, M.H.: Epithelial barrier function in vivo is sustained despite gaps in epithelial layers. Gastroenterology **129**, 902–912 (2005). https://doi.org/10.1053/j.gastro.2005.06.015
29. Frey, A., Giannasca, K.T., Weltzin, R., Giannasca, P.J., Reggio, H., Lencer, W.I., Neutra, M.R.: 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. **184**, 1045–1059 (1996). https://doi.org/10.1084/jem.184.3.1045
30. Pelasayed, T., Bergström, J.H., Gustafsson, J.K., Ermund, A., Birchenough, G.M.H., Schütte, A., van der Post, S., Svensson, F., Rodríguez-Piñeiro, A.M., Nyström, E.E.L., Wising, C., Johansson, M.E.V., Hansson, G.C.: The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. Immunol. Rev. **260**, 8–20 (2014). https://doi.org/10.1111/imr.12182
31. Johansson, M.E.V., Sjövall, H., Hansson, G.C.: The gastrointestinal mucus system in health and disease. Nat. Rev. Gastroenterol. Hepatol. **10**, 352–361 (2013). https://doi.org/10.1038/nrgastro.2013.35
32. Neutra, M.R., Forstner, J.F.: Gastrointestinal mucus: synthesis, secretion, and function. In: Johnson, L.R. (ed.) Physiology of the Gastrointestinal Tract. Raven Press: New York, NY, U.S.A, (1987)
33. Johansson, M.E.V., Phillipson, M., Petersson, J., Velcich, A., Holm, L., Hansson, G.C.: The inner of the two MUC2 mucin-dependent mucus layers in colon is devoid of bacteria. Proc. Natl. Acad. Sci. U.S.A. **105**, 15064–15069 (2008). https://doi.org/10.1073/pnas.0803124105
34. Busch, A.E., Herzer, T., Waldegger, S., Schmidt, F., Palacin, M., Biber, J., Markovich, D., Murer, H., Lang, F.: Opposite directed currents induced by the transport of dibasic and neutral amino acids in Xenopus oocytes expressing the protein rBAT. J. Biol. Chem. **269**, 25581–25586 (1994)
35. Palacín, M., Kanai, Y.: The ancillary proteins of HATs: SLC3 family of amino acid transporters. Pflugers Arch. Eur. J. Physiol. **447**, 490–494 (2004). https://doi.org/10.1007/s00424-003-1062-7
36. Howard, A., Hirst, B.H.: The glycine transporter GLYT1 in human intestine: expression and function. Biol. Pharm. Bull. **34**, 784–788 (2011). https://doi.org/10.1248/bpb.34.784
37. Pramod, A.B., Foster, J., Carvelli, L., Henry, L.K.: SLC6 transporters: structure, function, regulation, disease association and therapeutics. Mol. Asp. Med. **34**, 197–219 (2013). https://doi.org/10.1016/j.mam.2012.07.002
38. Bröer, A., Klingel, K., Kowalczuk, S., Rasko, J.E.J., Cavanaugh, J., Bröer, S.: Molecular cloning of mouse amino acid transport system B0, a neutral amino acid transporter related to Hartnup Disorder. J. Biol. Chem. **279**, 24467–24476 (2004). https://doi.org/10.1074/jbc.M400904200

39. Takanaga, H., Mackenzie, B., Suzuki, Y., Hediger, M.A.: Identification of mammalian proline transporter SIT1 (SLC6A20) with characteristics of classical System Imino. J. Biol. Chem. **280**, 8974–8984 (2005). https://doi.org/10.1074/jbc.M413027200
40. Thwaites, D.T., Anderson, C.M.H.: The SLC36 family of proton-coupled amino acid transporters and their potential role in drug transport. Br. J. Pharmacol. **164**, 1802–1816 (2011). https://doi.org/10.1111/j.1476-5381.2011.01438.x
41. Douard, V., Ferraris, R.P.: Regulation of the fructose transporter GLUT5 in health and disease. Am. J. Physiol. **295**, E227–E237 (2008). https://doi.org/10.1152/ajpendo.90245.2008
42. Wright, E.M.: Glucose transport families SLC5 and SLC50. Mol. Asp. Med. **34**, 183–196 (2013). https://doi.org/10.1016/j.mam.2012.11.002
43. Wright, E.M., Turk, E.: The sodium/glucose cotransport family SLC5. Pflugers Arch. Eur. J. Physiol. **447**, 510–518 (2004). https://doi.org/10.1007/s00424-003-1063-6
44. Barley, N.F., Howard, A., O'Callaghan, D., Legon, S., Walters, J.R.F.: Epithelial calcium transporter expression in human duodenum. Am. J. Physiol. **280**, G285–G290 (2001). https://doi.org/10.1152/ajpgi.2001.280.2.G285
45. Vesey, D.A.: Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. Toxicol. Lett. **198**, 13–19 (2010). https://doi.org/10.1016/j.toxlet.2010.05.004
46. Mackenzie, B., Hediger, M.A.: SLC11 family of H + -coupled metal-ion transporters NRAMP1 and DMT1. Pflugers Arch. Eur. J. Physiol. **447**, 571–579 (2004). https://doi.org/10.1007/s00424-003-1141-9
47. Hashimoto, A., Kambe, T.: Mg, Zn and Cu transport proteins: a brief overview from physiological and molecular perspectives. J. Nutr. Sci. Vitaminol. **61**, S116–S118 (2015). https://doi.org/10.3177/jnsv.61.S116
48. Voets, T., Nilius, B., Hoefs, S., van der Kemp, A.W.C.M., Droogmans, G., Bindels, R.J.M., Hoenderop, J.G.J.: TRPM6 forms the Mg^{2+} influx channel involved in intestinal and renal Mg^{2+} absorption. J. Biol. Chem. **279**, 19–25 (2004). https://doi.org/10.1074/jbc.M311201200
49. Reboul, E.: Vitamin E bioavailability: mechanisms of intestinal absorption in the spotlight. Antioxidants **6**, 95 (2017). https://doi.org/10.3390/antiox6040095
50. Reboul, E., Borel, P.: Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mamalian enterocytes. Prog. Lipid Res. **50**, 388–402 (2011). https://doi.org/10.1016/j.plipres.2011.07.001
51. Anderson, C.M., Stahl, A.: SLC27 fatty acid transport proteins. Mol. Asp. Med. **34**, 516–528 (2013). https://doi.org/10.1016/j.mam.2012.07.010
52. Daniel, H., Kottra, G.: The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. Pflugers Arch. Eur. J. Physiol. **447**, 610–618 (2004). https://doi.org/10.1007/s00424-003-1101-4
53. May, J.M.: The SLC23 family of ascorbate transportes: ensuring that you get and keep your daily dose of vitamin C. Br. J. Pharmacol. **164**, 1793–1801 (2011). https://doi.org/10.1111/j.1476-5381.2011.01350.x
54. Yonezawa, A., Inui, K.: Novel riboflavin transporter family RFVT/SLC52: identification, nomenclature, functional characterization and genetic diseases of RFVT/SLC52. Mol. Asp. Med. **34**, 693–701 (2013). https://doi.org/10.1016/j.mam.2012.07.014
55. Zhao, R., Goldman, I.D.: Folate and thiamine transporters mediated by facilitative carriers (SLC19A1-3 and SLC46A1) and folate receptors. Mol. Asp. Med. **34**, 373–385 (2013). https://doi.org/10.1016/j.mam.2012.07.006
56. Ganapathy, V., Smith, S.B., Prasad, P.D.: SLC19: the folate/thiamine transporter family. Pflugers Arch. Eur. J. Physiol. **447**, 641–646 (2004). https://doi.org/10.1007/s00424-003-1068-1
57. Roth, M., Obaidat, A., Hagenbuch, B.: OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Brit. J. Pharmacol. **165**, 1260–1287 (2012). https://doi.org/10.1111/j.1476-5381.2011.01724.x

58. Bargheer, D., Giemsa, A., Freund, B., Heine, M., Waurisch, C., Stachowski, G.M., Hickey, S.G., Eychmüller, A., Heeren, J., Nielsen, P.: The distribution and degradation of radiolabeled superparamagnetic iron oxide nanoparticles and quantum dots in mice. Beilstein J. Nanotechnol. **6**, 111–123 (2015). https://doi.org/10.3762/bjnano.6.11
59. Heinrich, H.C.: Diagnostik, Ätiologie und Therapie des Eisenmangels unter besonderer Berücksichtigung der 59Fe-Retentionsmessung im Gesamtkörper-Radioaktivitätsdetektor. Der Nuklearmediziner **137**, 137–269 (1983)
60. Bruns, O.T., Ittrich, H., Peldschus, K., Kaul, M.G., Tromsdorf, U.I., Lauterwasser, J., Nikolic, M.S., Mollwitz, B., Merkel, M., Bigall, N.C., Sapra, S., Reimer, R., Hohenberg, H., Weller, H., Eychmüller, A., Adam, G., Beisiegel, U., Heeren, J.: Real-time magnetic resonance imaging and quantification of lipoprotein metabolism in vivo using nanocrystals. Nat. Nanotechnol. **4**, 193–201 (2009). https://doi.org/10.1038/nnano.2008.405
61. Kottwitz, K., Laschinsky, N., Fischer, R., Nielsen, P.: Absorption, excretion and retention of ^{51}Cr from labelled Cr-(III)-picolinate in rats. Biometals **22**, 289–295 (2009). https://doi.org/10.1007/s10534-008-9165-4
62. Chen, N., He, Y., Su, Y., Li, X., Huang, Q., Wang, H., Zhang, X., Tai, R., Fan, C.: The cytotoxicity of cadmium-based quantum dots. Biomaterials **33**, 1238–1244 (2012). https://doi.org/10.1016/j.biomaterials.2011.10.070
63. Cho, S.J., Maysinger, D., Jain, M., Röder, B., Hackbarth, S., Winnik, F.M.: Long-term exposure of CdTe quantum dots causes functional impairments in live cells. Langmuir **23**, 1974–1980 (2007). https://doi.org/10.1021/la060093j
64. Hardman, R.: A toxicological review of quantum dots: toxicity depends on physicochemical and environmental factors. Environ. Health Perspect. **114**, 165–172 (2006). https://doi.org/10.1289/ehp.8284
65. Hoshino, A., Hanada, S., Yamamoto, K.: Toxicity of nanocrystal quantum dots: the relevance of surface modifications. Arch. Toxicol. **85**, 707–720 (2011). https://doi.org/10.1007/s00204-011-0695-0
66. Winnik, F.M., Maysinger, D.: Quantum dot cytotoxicity and ways to reduce it. Acc. Chem. Res. **46**, 672–680 (2013). https://doi.org/10.1021/ar3000585
67. Zheng, X., Tian, J., Weng, L., Wu, L., Jin, Q., Zhao, J., Wang, L.: Cytotoxicity of cadmium-containing quantum dots based on a study using a microfluidic chip. Nanotechnology **23**, 055102 (2012). https://doi.org/10.1088/0957-4484/23/5/055102
68. Loginova, Y.F., Dezhurov, S.V., Zherdeva, V.V., Kazachkina, N.I., Wakstein, M.S., Savitsky, A.P.: Biodistribution and stability of CdSe core quantum dots in mouse digestive tract following per os administration: Advantages of double polymer/silica coated nanocrystals. Biochem. Biophys. Res. Commun. **419**, 54–59 (2012). https://doi.org/10.1016/j.bbrc.2012.01.123
69. Mohs, A.M., Duan, H., Kairdolf, B.A., Smith, A.M., Nie, S.: Proton-resistant quantum dots: stability in gastrointestinal fluids and implications for oral delivery of nanoparticle agents. Nano Res. **2**, 500–508 (2009). https://doi.org/10.1007/s12274-009-9046-3
70. Mancini, M.C., Kairdolf, B.A., Smith, A.M., Nie, S.: Oxidative quenching and degradation of polymer-encapsulated quantum dots: new insights into the long term fate and toxicity of nanocrystals in-vivo. J. Am. Chem. Soc. **130**, 10836–10837 (2008). https://doi.org/10.1021/ja8040477
71. Smith, A.M., Duan, H., Rhyner, M.N., Ruan, G., Nie, S.: A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. Phys. Chem. Chem. Phys. **8**, 3895–3903 (2006). https://doi.org/10.1039/b606572b
72. Min, K.S., Sano, E., Ueda, H., Sakazaki, F., Yamada, K., Takano, M., Tanaka, K.: Dietary deficiency of calcium and/or iron, an age-related risk factor for renal accumulation of cadmium in Mice. Biol. Pharm. Bull. **38**, 1557–1563 (2015)
73. Hauck, T.S., Anderson, R.E., Fischer, H.C., Newbigging, S., Chan, W.C.W.: In vivo quantum-dot toxicity assessment. Small **6**, 138–144 (2010). https://doi.org/10.1002/smll.200900626
74. Rzigalinski, B.A., Strobl, J.S.: Cadmium-containing nanoparticles: perspectives on pharmacology and toxicology of quantum dots. Toxicol. Appl. Pharmacol. **238**, 280–288 (2009). https://doi.org/10.1016/j.taap.2009.04.010

75. Tsoi, K.M., Dai, Q., Alman, B.A., Chan, W.C.: Are quantum dots toxic? Exploring the discrepancy between cell culture and animal studies. Acc. Chem. Res. **46**, 662–671 (2013). https://doi.org/10.1021/ar300040z
76. Liu, Y., Li, Y., Liu, K., Shen, J.: Exposing to cadmium stress cause profound toxic effect on microbiota of the mice intestinal tract. PLoS ONE **9**, e85323 (2014). https://doi.org/10.1371/journal.pone.0085323
77. Breton, J., Daniel, C., Dewulf, J., Pothion, S., Froux, N., Sauty, M., Thomas, P., Pot, B., Foligné, B.: Gut microbiota limits heavy metals burden caused by chronic oral exposure. Toxicol. Lett. **222**, 132–138 (2013). https://doi.org/10.1016/j.toxlet.2013.07.021
78. Zhai, Q., Yin, R., Yu, L., Wang, G., Tian, F., Yu, R., Zhao, J., Liu, X., Chen, Y.Q., Zhang, H., Chen, W.: Screening of lactic acid bacteria with potential protective effects against cadmium toxicity. Food Control **54**, 23–30 (2015). https://doi.org/10.1016/j.foodcont.2015.01.037
79. Breton, J., Massart, S., Vandamme, P., De Brandt, E., Pot, B., Foligné, B.: Ecotoxicology inside the gut: impact of heavy metals on the mouse microbiome. BMC Pharmacol. Toxicol. **14**, 62 (2013). https://doi.org/10.1186/2050-6511-14-62
80. Tiwari, R., Singh, R.D., Khan, H., Gangopadhyay, S., Mittal, S., Singh, V., Arjaria, N., Shankar, J., Roy, S.K., Singh, D., Srivastava, V.: Oral subchronic exposure to silver nanoparticles causes renal damage through apoptotic impairment and necrotic cell death. Nanotoxicology **11**, 671–686 (2017). https://doi.org/10.1080/17435390.2017.1343874
81. Nielsen, P.: Chelation therapy for heavy metals. In: Crichton, R., Ward, R.J., Hider, R.C., (eds.) Metal Chelation in Medicine. The Royal Society of Chemistry (2016). https://doi.org/10.1039/9781782623892
82. Lo, D.D.: Vigilance or subversion? Constitutive and inducible M cells in mucosal tissues. Trends Immunol. **39**, 185–195 (2017). https://doi.org/10.1016/j.it.2017.09.002
83. Mantis, N.J., Frey, A., Neutra, M.R.: Accessibility of glycolipid and oligosaccharide epitopes on rabbit villus and follicle-associated epithelium. Am. Physiol. **278**, G915–G923 (2000). https://doi.org/10.1152/ajpgi.2000.278.6.G915
84. Bonnardel, J., Da Silva, C., Henri, S., Tamoutounour, S., Chasson, L., Montaña-Sanchis, F., Gorvel, J.-P., Lelouard, H.: Innate and adaptive immune functions of Peyer's patch monocyte-derived cells. Cell Rep. **11**, 770–784 (2015). https://doi.org/10.1016/j.celrep.2015.03.067
85. Neutra, M.R., Frey, A., Kraehenbuhl, J.-P.: Epithelial M cells: gateways for mucosal infection and immunization. Cell **86**, 345–348 (1996). https://doi.org/10.1016/S0092-8674(00)80106-3
86. Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhl, J.-P., Ricciardi-Castagnoli, P.: Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat. Immunol. **2**, 361–367 (2001). https://doi.org/10.1038/86373
87. Jung, C., Hugot, J.-P., Barreau, F.: Peyer's patches: the immune sensors of the intestine. Int. J. Inflamm. **2010**, 823710 (2010). https://doi.org/10.4061/2010/823710
88. Knoop, K.A., Kumar, N., Butler, B.R., Sakthivel, S.K., Taylor, R.T., Nochi, T., Akiba, H., Yagita, H., Kiyono, H., Williams, I.R.: RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epitelium. J. Immunol. **183**, 5738–5747 (2009). https://doi.org/10.4049/jimmunol.0901563
89. Kanaya, T., Ohno, H.: The mechanisms of M cell differentiation. Biosci. Microbiota Food Health **33**, 91–97 (2014). https://doi.org/10.12938/bmfh.33.91
90. Jang, M.H., Kweon, M.-N., Iwatani, K., Yamamoto, M., Terahara, K., Sasakawa, C., Suzuki, T., Nochi, T., Yokota, Y., Rennert, P.D., Hiroi, T., Tamagawa, H., Iijima, H., Kunisawa, J., Yuki, Y., Kiyono, H.: Intestinal villous M cells: an antigen entry site in the mucosal epithelium. Proc. Natl. Acad. Sci. U.S.A **101**, 6110–6115 (2004). https://doi.org/10.1073/pnas.0400969101
91. Lelouard, H., Fallet, M., De Boris, B., Méresse, S., Gorvel, J.-P.: Peyer's patch dendritic cells sample antigens by extending dendrites through M-cell specific transcellular pores. Gastroenterology **142**, 592–601 (2012). https://doi.org/10.1053/j.gastro.2011.11.039
92. Pelasayed, T., Gustafsson, J.K., Gustafsson, I.J., Ermund, A., Hansson, G.C.: Carbachol-induced MUC-17 endocytosis is concomitant with NHE3 internalization and CFTR membrane

recruitment in enterocytes. Am. J. Physiol. **305**, C457–C467 (2013). https://doi.org/10.1152/ajpcell.00141.2013
93. Silveira, J.R., Raymond, G.J., Hughson, A.G., Race, R.E., Sim, V.L., Hayes, S.F., Caughy, B.: The most infectious prion particles. Nature **437**, 257–261 (2005). https://doi.org/10.1038/nature03989
94. Bade, S., Frey, A.: Potential of active and passive immunizations for the prevention and therapy of transmissible spongiform encephalopathies. Expert Rev. Vaccines **6**, 153–168 (2007). https://doi.org/10.1586/14760584.6.2.153
95. Donaldson, D.S., Kobayashi, A., Ohno, H., Yagita, H., Williams, I.R., Mabbott, N.A.: M cell-depletion blocks oral prion disease pathogenesis. Mucosal Immunol. **5**, 216–225 (2012). https://doi.org/10.1038/mi.2011.68
96. Donaldson, D.S., Sehgal, A., Rios, D., Williams, I.R., Mabbott, N.A.: Increased abundance of M cells in the gut epithelium dramatically enhances oral prion disease susceptibility. PLoS Pathog. **12**, e1006075 (2016). https://doi.org/10.1371/journal.ppat.1006075
97. Ermak, T.H., Dougherty, E.P., Bhagat, H.R., Kabok, Z., Papp, J.: Uptake and transport of copolymer biodegradable microspheres by rabbit Peyer's patch M cells. Cell Tissue Res. **279**, 433–436 (1995). https://doi.org/10.1007/BF00318501
98. Foged, C., Brodin, B., Frokjaer, S., Sundblad, A.: Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model. Int. J. Pharm. **298**, 315–322 (2005). https://doi.org/10.1016/j.ijpharm.2005.03.035
99. Gebert, A., Steinmetz, I., Fassbender, S., Wendlandt, K.-H.: Antigen transport into Peyer's patches: Increased uptake by constant numbers of M cells. Am. J. Pathol. **164**, 65–72 (2004). https://doi.org/10.1016/s0002-9440(10)63097-0
100. Jepson, M., Simmons, N.L., O'Hagan, D.T., Hirst, B.H.: Comparison of poly(DL-lactide-co-glycolide) and polystyrene microsphere targeting to intestinal M cells. J. Drug Target. **1**, 245–249 (1993). https://doi.org/10.3109/10611869308996082
101. Jepson, M.A., Simmons, N.L., Savidge, T.C., James, P.S., Hirst, B.H.: Selective binding and transcytosis of latex microspheres by rabbit intestinal M cells. Cell Tissue Res. **271**, 399–405 (1993). https://doi.org/10.1007/BF02913722
102. Pappo, J., Ermak, T.H.: Uptake and translocation of fluorescent latex particles by rabbit Peyer's patch follicle epithelium: a quantitative model for M cell uptake. Clin. Exp. Immunol. **76**, 144–148 (1989)
103. Berg, R.D.: Bacterial translocation from the gastrointestinal tract. Trends Microbiol. **3**, 149–154 (1995). https://doi.org/10.1016/S0966-842X(00)88906-4
104. Gewirtz, A.T., Navas, T.A., Lyons, S., Godowski, P.G., Madara, J.L.: Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. J. Immunol. **167**, 1882–1885 (2001). https://doi.org/10.4049/jimmunol.167.4.1882
105. Husebye, E.: The pathogenesis of gastrointestinal bacterial overgrowth. Chemotherapy **51**(Suppl1), 1–22 (2005). https://doi.org/10.1159/000081988
106. Berkes, J., Viswanathan, V.K., Savkovic, S.D., Hecht, G.: Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. Gut **52**, 439–451 (2003). https://doi.org/10.1136/gut.52.3.439
107. Mukiza, C.N., Dubreuil, J.D.: Escherichia coli heat-stable toxin b impairs intestinal barrier function by altering tight junction proteins. Infect. Immun. **81**, 2819–2827 (2013). https://doi.org/10.1128/IAI.00455-13
108. Ugalde-Silva, P., Gonzalez-Lugo, O., Navarro-Garcia, F.: Tight junction disruption induced by type 3 secretion system effectors injected by enteropathogenic and enterohemorrhagic *Escherichia coli*. Front. Cell Infect. Mirobiol. **6**, 87 (2016). https://doi.org/10.3389/fcimb.2016.00087
109. Freeman, H.J.: Spontaneous free perforation of the small intestine in adults. World J. Gastroenterol. **20**, 9990–9997 (2014). https://doi.org/10.3748/wjg.v20.i29.9990
110. Laukoetter, M.G., Nava, P., Nusrat, A.: Role of the intestianal barrier in inflammatory bowel disease. World J. Gastroenterol. **14**, 401–407 (2008). https://doi.org/10.3748/wjg.14.401

111. Schmitz, H., Barmeyer, C., Fromm, M., Runkel, N., Foss, H.-D., Bentzel, C.J., Rieken, E.-O., Schulzke, J.-D.: Altered tight junction structure contributes to the impaired epithelial barrier function in ulcerative colitis. Gastroenterology **116**, 301–309 (1999). https://doi.org/10.1016/S0016-5085(99)70126-5
112. Lechuga, S., Ivanov, A.I.: Disruption of the epithelial barrier during intestinal inflammation: quest for new molecules and mechanisms. Biochim. Biophys. Acta **1864**, 1183–1194 (2017). https://doi.org/10.1016/j.bbamcr.2017.03.007
113. Lautenschläger, C., Schmidt, C., Lehr, C.-M., Fischer, D., Stallmach, A.: PEG-functionalized microparticles selectively target inflamed mucosa in inflammatory bowel disease. Eur. J. Pharm. Biopharm. **85**, 578–586 (2013). https://doi.org/10.1016/j.ejpb.2013.09.016
114. Champion, J.A., Mitragotri, S.: Role of target geometry in phagocytosis. Proc. Natl. Acad. Sci. U.S.A. **103**, 4930–4934 (2006). https://doi.org/10.1073/pnas.0600997103
115. Docter, D., Westmeier, D., Markiewicz, M., Stolte, S., Knauer, S.K., Staubert, R.H.: The nanoparticle biomolecule corona: lessons learned—challenge accepted? Chem. Soc. Rev. **44**, 6094–6121 (2015). https://doi.org/10.1039/c5cs00217f
116. Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., Dawson, K.A.: Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proc. Natl. Acad. Sci. U.S.A. **105**, 14265–14270 (2008). https://doi.org/10.1073/pnas.0805135105
117. Saptarshi, S.R., Duschi, A., Lopata, A.L.: Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle. J. Nanobiotech. **11**, 26 (2013). https://doi.org/10.1186/1477-3155-11-26
118. Parsons, B.N., Campbell, B.J., Wigley, P.: Soluble plantain nonstarch polysaccharides, although increasing caecal load, reduce systemic invasion of *Salmonella* gallinarum in the chicken. Lett. Appl. Microbiol. **60**, 347–351 (2014). https://doi.org/10.1111/lam.12377
119. Parsons, B.N., Wigley, P., Simpson, H.L., Williams, J.M., Humphrey, S., Salisbury, A.-M., Watson, A.J.M., Fry, S.C., O'Brien, D., Roberts, C.L., O'Kennedy, N., Keita, A.V., Söderholm, J.D., Rhodes, J.M., Campbell, B.J.: Dietary Supplementation with soluble plantain non-starch polysaccharides inhibits intestinal invasion of *Salmonella* typhimurium in the chicken. PLoS ONE **9**, e87658 (2014). https://doi.org/10.1371/journal.pone.0087658
120. Roberts, C.L., Keita, A.V., Duncan, S.H., O'Kennedy, N., Söderholm, J.D., Rhodes, J.M., Campbell, B.J.: Translocation of Crohn's disease Escherichia coli across M-cells: contrasting effects of soluble plant fibres and emulsifiers. Gut **59**, 1331–1339 (2010). https://doi.org/10.1136/gut.2009.195370
121. Roberts, C.L., Keita, A.V., Parsons, B.N., Prorok-Hamon, M., Knight, P., Winstanley, C., O'Kennedy, N., Söderholm, J.D., Rhodes, J.M., Campbell, B.J.: Soluble plantain fibre blocks adhesion and M-cell translocation of intestinal pathogens. J. Nutr. Biochem. **24**, 97–103 (2013). https://doi.org/10.1016/j.jnutbio.2012.02.013
122. Hochella Jr., M.F., Spencer, M.G., Jones, K.L.: Nanotechnology: nature's gift or scientists' brainchild? Environ. Sci. Nano **2**, 114–119 (2015). https://doi.org/10.1039/c4en00145a
123. Sharma, V.K., Filip, J., Zboril, R., Varma, R.S.: Natural inorganic nanoparticles—formation, fate, and toxicity in the environment. Chem. Soc. Rev. **44**, 8410–8423 (2015). https://doi.org/10.1039/c5cs00236b
124. Griffin, S., Masood, M.I., Nasim, M.J., Sarfraz, M., Ebokaiwe, A.P., Schäfer, K.-H., Keck, C.M., Jacob, C.: Natural nanoparticles: a particular matter inspired by Nature. Antioxidants **7**, 3 (2018). https://doi.org/10.3390/antiox7010003
125. Strambeanu, N., Demetrovici, L., Dragos, D.: Natural sources of nanoparticles. In: Lungu, M. et al. (eds.) Nanoparticles' Promises and Risks. Springer International Publishing Switzerland (2015). https://doi.org/10.1007/978-3-319-11728-7
126. Dykman, L.A., Khlebtsov, N.G.: Gold nanoparticles in biology and medicine: recent advances and prospects. Acta Naturae **3**, 34–55 (2011)
127. Zhang, X.F., Liu, Z.G., Shen, W., Gurunathan, S.: Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int. J. Mol. Sci. **17**, 1534 (2016). https://doi.org/10.3390/ijms17091534

128. Ali, A., Zafar, H., Zia, M., ul Haq, I., Phull, A.R., Ali, J.S., Hussain, A.: Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnol. Sci. Appl. **9**, 49–67 (2016). https://doi.org/10.2147/NSA.S99986
129. Valizadeh, A., Mikaeili, H., Samiei, M., Farkhani, S.M., Zarghami, N., Kouhi, M., Akbarzadeh, A., Davaran, S.: Quantum dots: synthesis, bioapplications, and toxicity. Nanoscale Res. Lett. **7**, 480 (2012). https://doi.org/10.1186/1556-276X-7-480
130. Pan, K., Zhong, Q.: Organic nanoparticles in foods: fabrication, characterization and utilization. Annu. Rev. Food Sci. Technol. **7**, 245–266 (2016). https://doi.org/10.1146/annurev-food-041715-033215
131. Marcaccio, M., Paolucci, F., eds.: Making and exploiting fullerenes, graphene, and carbon nanotubes. Springer, Berlin, Heidelberg (2014). https://doi.org/10.1007/978-3-642-55083-6
132. Nasir, S., Hussein, M.Z., Zainal, Z., Yusof, N.A.: Carbon-based nanomaterials/allotropes: a glimpse of their synthesis, properties and some applications. Materials **11**, 295 (2018). https://doi.org/10.3390/ma11020295
133. Feltracco, M., Barbaro, E., Contini, D., Zangrando, R., Toscano, G., Battistel, D., Barbante, C., Gambaro, A.: Photo-oxidation products of α-pinene in coarse, fine and ultrafine aerosol: a new high sensitive HPLC-MS/MS method. Atmos. Environ. **180**, 149–155 (2018). https://doi.org/10.1016/j.atmosenv.2018.02.052
134. Kerminen, V.-M.: Roles of SO_2 and secondary organics in the growth of nanometer particles in the lower atmosphere. J. Aerosol Sci. **30**, 1069–1078 (1999). https://doi.org/10.1016/S0021-8502(98)00775-7
135. Tu, P., Johnston, M.V.: Particle size dependence of biogenic secondary organic aerosol molecular composition. Atmos. Chem. Phys. **17**, 7593–7603 (2017). https://doi.org/10.5194/acp-17-7593-2017
136. Lindner, K., Ströbele M., Schlick, S., Webering, S., Jenckel, A., Kopf, J., Danov, O., Sewald, K., Buj, C., Creutzenberg, O., Tillmann, T., Pohlmann, G., Ernst, H., Ziemann, C., Hüttmann, G., Heine, H., Bockhorn, H., Hansen, T., König, P., Fehrenbach, H.: Biological effects of carbon black nanoparticles are changed by surface coating with polycyclic aromatic hydrocarbons. Part. Fibre Toxicol. **14**, 8 (2017). https://doi.org/10.1186/s12989-017-0189-1
137. Ginoux, P., Chin, M., Tegen, I., Prospero, J.M., Holben, B., Dubovik, O., Lin, S.-J.: Sources and distributions of dust aerosols simulated with GOCART model. J. Geophys. Res. **106**, 20255–20273 (2001). https://doi.org/10.1029/2000JD000053
138. D'Andrea, S.D., Häkkinen, S.A.K., Westervelt, D.M., Kuang, C., Levin, E.J.T., Kanawade, V.P., Leaitch, W.R., Spracklen, D.V., Riipinen, I., Pierce, J.R.: Understanding global secondary organic aerosol amount and size-resolved condensational behavior. Atmos. Chem. Phys. **13**, 11519–11534 (2013). https://doi.org/10.5194/acp-13-11519-2013
139. Taghavi, S.M., Momenpour, M., Azarian, M., Ahmadian, M., Souri, F., Taghavi, S.A., Sadeghain, M., Karchani, M.: Effects of nanoparticles on the environment and outdoor workplaces. Electron. Physician **5**, 706–712 (2013). https://doi.org/10.14661/2013.706-712
140. Rivero, P.J., Urrutia, A., Goicoechea, J., Arregui, F.J.: Nanomaterials for functional textiles and fibers. Nanoscale Res. Lett. **10**, 501 (2015). https://doi.org/10.1186/s11671-015-1195-6
141. Blackford, D.B., Simons, G.R.: Particle size analysis of carbon black. Part. Charact. **4**, 112–117 (1987). https://doi.org/10.1002/ppsc.19870040123
142. ICBA International Carbon Black Association. Carbon Black User's Guide. www.carbon-black.org (2016)
143. SCCS Scientific Committee on Consumer Safety and Chaudhry Q. Opinion of the Scientific Committee on Consusmer Safety (SCCS)—Second revision of the opinion on carbon black, nano-form, in cosmetic products. Regul. Toxicol. Pharmacol. **79**, 103–104 (2016). https://doi.org/10.1016/j.yrtph.2016.02.021
144. Suryanto, B.H.R., Zhao, C.: Surface-oxidized carbon black as a catalyst for the water oxidation and alcohol oxidation reactions. Chem. Commun. **52**, 6439–6442 (2016). https://doi.org/10.1039/c6cc01319h
145. Yuan, L., Lu, X.-H., Xiao, X., Zhai, T., Dai, J., Zhang, F., Hu, B., Wang, X., Gong, L., Chen, J., Hu, C., Tong, Y., Zhou, J., Wang, Z.L.: Flexible solid-state supercapacitors based on carbon

nanoparticles/MnO_2 nanorods hybrid structure. ACS Nano **6**, 656–661 (2012). https://doi.org/10.1021/nn2041279
146. Yuan, L., Tao, Y., Chen, J., Dai, J., Song, T., Ruan, M., Ma, Z., Gong, L., Liu, K., Zhang, X., Hu, X., Zhou, J., Wang, Z.L.: Carbon nanoparticles on carbon fabric for flexible and high-performance field emitters. Adv. Funct. Mater. **21**, 2150–2154 (2011). https://doi.org/10.1002/adfm.201100172
147. Posthuma-Trumpie, G.A., Wichers, J.H., Koets, M., Berendsen, L.B.J.M., van Amerongen, A.: Amorphous carbon nanoparticles: a versatile label for diagnostic (immuno)assays. Anal. Bioanal. Chem. **402**, 593–600 (2012). https://doi.org/10.1007/s00216-011-5340-5
148. Rosic, J.S., Conte, M., Muncan, J., Matija, L., Koruga, D.: Characterization of fullerenes thin film on glasses by UV/VIS/NIR and opto-magnetic imaging spectroscopy. FME Trans. **42**, 172–176 (2014). https://doi.org/10.5937/fmet1402172S
149. Gatti, T., Menna, E., Meneghetti, M., Maggini, M., Petrozza, A., Lamberti, F.: The renaissance of fullerenes with perovskite solar cells. Nano Energy **41**, 84–100 (2017). https://doi.org/10.1016/j.nanoen.2017.09.016
150. Liu, L., Niu, Z., Chen, J.: Unconventional supercapacitors from nanocarbon-based electrode materials to device configurations. Chem. Soc. Rev. **45**, 4340–4363 (2016). https://doi.org/10.1039/c6cs00041j
151. Lv, T., Liu, M., Zhu, D., Gan, L., Chen, T.: Nanocarbon-based materials for flexible all-solid-state supercapacitors. Adv. Mater. **2018**, 1705489 (2018). https://doi.org/10.1002/adma.201705489
152. Yong, Y., Zhou, Q., Li, X., Lv, S.: The $H_{60}Si_6C_{54}$ heterofullerene as high-capacity storage medium. AIP Adv. **6**, 075321 (2016). https://doi.org/10.1063/1.4960330
153. Yoon, M., Yang, S., Hicke, C., Wang, E., Geohegan, D., Zhang, Z.: Calcium as the superior coating metal in functionalization of carbon fullerenes for high-capacity hydrogen storage. Phys. Rev. Lett. **100**, 206806 (2008). https://doi.org/10.1103/physrevlett.100.206806
154. Yoon, M., Yang, S., Wang, E., Zhang, Z.: Charged fullerenes as high-capacity hydrogen storage media. Nano Lett. **7**, 2578–2583 (2007). https://doi.org/10.1021/nl070809a
155. Al-Jumaili, A., Alancherry, S., Bazaka, K., Jacob, M.V.: Review on the antimicrobial properties of carbon nanostructures. Materials **10**, 1066 (2017). https://doi.org/10.3390/ma10091066
156. Teradal, N.L., Jelinek, R.: Carbon nanomaterials in biological studies and biomedicine. Adv. Healthc. Mater. **6**, 1700574 (2017). https://doi.org/10.1002/adhm.201700574
157. De Smet, R., Demoor, T., Verschuere, S., Dullaers, M., Ostroff, G.R., Leclerq, G., Allais, L., Pilette, C., Dierendonck, M., De Geest, B.G., Cuvelier, C.A.: β-Glucan microparticles are good candidates for mucosal antigen delivery in oral vaccination. J. Control Rel. **172**, 671–678 (2013). https://doi.org/10.1016/j.jconrel.2013.09.007
158. des Rieux, A., Fievez, A., Garinot, M., Schneider, Y.-J., Préat, V.: Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J. Control Rel. **116**, 1–27 (2006). https://doi.org/10.1016/j.jconrel.2006.08.013
159. Zhu, Q., Talton, J., Zhang, G., Cunningham, T., Wang, Z., Waters, R.C., Kirk, J., Eppler, B., Klinman, D.M., Sui, Y., Gagnon, S., Belyakov, I.M., Mumper, R.J., Berzofsky, J.A.: Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. Nat. Med. **18**, 1291–1297 (2012). https://doi.org/10.1038/nm.2866
160. Fonte, P., Nogueira, T., Gehm, C., Ferreira, D., Sarmento, B.: Chitosan-coated solid lipid nanoparticles enhance the oral absorption of insulin. Drug Deliv. Transl. Res. **1**, 299–308 (2011). https://doi.org/10.1007/s13346-011-0023-5
161. Ren, T., Wang, Q., Xu, Y., Cong, L., Gou, J., Tao, X., Zhang, Y., He, H., Yin, T., Zhang, H., Zhang, Y., Tang, X.: Enhanced oral absorption and anticancer efficacy of cabazitaxel by overcoming intestinal mucus and epithelium barriers using surface polyethylene oxide (PEO) decorated positively charged polymer-lipid hybrid nanoparticles. J. Control Rel. **269**, 423–438 (2018). https://doi.org/10.1016/j.jconrel.2017.11.015
162. Sun, S., Liang, N., Gong, X., An, W., Kawashima, Y., Cui, F., Yan, P.: Multifunctional composite microcapsules for oral delivery of insulin. Int. J. Mol. Sci. **18**, 54 (2017). https://doi.org/10.3390/ijms18010054

163. Niu, Z., Conejos-Sánchez, I., Griffin, B.T., O'Driscoll, C.M., Alonso, M.J.: Lipid-based nanocarriers for oral peptide delivery. Adv. Drug Deliv. Rev. **106 Part B**, 337–354 (2016). https://doi.org/10.1016/j.addr.2016.04.001
164. Sheng, Y., He, H., Zou, H.: Poly(lactic acid) nanoparticles coated with combined WGA and water-soluble chitosan for mucosal delivery of β-galactosidase. Drug Deliv. **21**, 370–378 (2014). https://doi.org/10.3109/10717544.2014.905653
165. Yin, Y.S., Chen, D.W., Qiao, M.X., Wei, X.Y., Hu, H.Y.: Lectin-conjugated PLGA nanoparticles loaded with thymopentin: ex vivo bioadhesion and in vivo biodistribution. J. Control Rel. **123**, 27–38 (2007). https://doi.org/10.1016/j.jconrel.2007.06.024
166. Menzel, C., Bernkop-Schnürch, A.: Enzyme decorated drug carriers: targeted swords to cleave and overcome the mucus barrier. Adv. Drug Deliv. Rev. **124**, 164–174 (2018). https://doi.org/10.1016/j.addr.2017.10.004
167. Frøkjær, J.B., Drewes, A.M., Gregersen, H.: Imaging of the gastrointestinal tract-novel technologies. World J. Gastroenterol. **15**, 160–168 (2009). https://doi.org/10.3748/wjg.15.160
168. Stark, D.D., Weissleder, R., Elizondo, G., Hahn, P.F., Saini, S., Todd, L.E., Wittenberg, J., Ferrucci, J.T.: Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. Radiology **168**, 297–301 (1988). https://doi.org/10.1148/radiology.168.2.3393649
169. Shokrollahi, H.: Contrast agents for MRI. Mater. Sci. Eng. C **33**, 4485–4497 (2013). https://doi.org/10.1016/j.msec.2013.07.012
170. Li, W., Tutton, S., Vu, A.T., Pierchala, L., Li, B.S.Y., Lewis, J.M., Prasad, P.V., Edelman, R.R.: First-pass contrast-enhanced magnetic resonance angiography in humans using ferumoxytol, a novel ultrasmall superparamagnetic iron oxide (USPIO)-based blood pool agent. J. Magn. Reson. Imaging **21**, 46–52 (2005). https://doi.org/10.1002/jmri.20235
171. Frisch, A., Walter, T.C., Hamm, B., Denecke, T.: Efficacy of oral contrast agents for upper gastrointestinal signal suppression in MRCP: A systematic review of the literature. Acta Radiol. Open **6**, 2058460117727315 (2017). https://doi.org/10.1177/2058460117727315
172. Maccioni, F., Bruni, A., Viscido, A., Colaiacomo, M.C., Cocco, A., Montesani, C., Caprilli, R., Marini, M.: MR imaging in patients with Crohn disease: value of T2- versus T1-weighted gadolinium-enhanced MR sequences with use of an oral superparamagnetic contrast agent. Radiology **238**, 517–530 (2006). https://doi.org/10.1148/radiol.2381040244
173. Gleich, B., Weizenecker, J.: Tomographic imaging using the nonlinear response of magnetic particles. Nature **435**, 1214–1217 (2005). https://doi.org/10.1038/nature03808
174. Salamon, J., Hofmann, M., Jung, C., Kaul, M.G., Werner, F., Them, K., Reimer, R., Nielsen, P., vom Scheidt, A., Adam, G., Knopp, T., Ittrich, H.: Magnetic particle/magnetic resonance imaging: In-Vitro MPI-guided real time catheter tracking and 4D angioplasty using a road map and blood pool tracer approach. PLoS ONE **11**, e0156899 (2016). https://doi.org/10.1371/journal.pone.0156899
175. Yu, E.Y., Chandrasekharan, P., Berzon, R., Tay, Z.W., Zhou, X.Y., Khandhar, A.P., Ferguson, R.M., Kemp, S.J., Zheng, B., Goodwill, P.W., Wendland, M.F., Krishnan, K.M., Behr, S., Carter, J., Conolly, S.M.: Magnetic particle imaging for highly sensitive, quantitative, and safe in vivo gut bleed detection in a murine model. ACS Nano **11**, 12067–12076 (2017). https://doi.org/10.1021/acsnano.7b04844
176. Gamboa, J.M., Leong, K.W.: In vitro and in vivo models for the study of oral delivery of nanoparticles. Adv. Drug Deliv. Rev. **65**, 800–810 (2013). https://doi.org/10.1016/j.addr.2013.01.003
177. Buhrke, T., Lengler, I., Lampen, A.: Analysis of proteomic changes induced upon cellular differentiation of the human intestinal cell line Caco-2. Dev. Growth Differ. **53**, 411–426 (2011). https://doi.org/10.1111/j.1440-169X.2011.01258.x
178. Hidalgo, I.J., Raub, T.J., Borchardt, R.T.: Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology **96**, 736–749 (1989)
179. Sinnecker, H., Ramaker, K., Frey, A.: Coating with luminal gut-constituents alters adherence of nanoparticles to intestinal epithelial cells. Beilstein J. Nanotechnol. **5**, 2308-2315 (2014). https://doi.org/10.3762/bjnano.5.239

180. Béduneau, A., Tempesta, C., Fimbel, S., Pellequer, Y., Jannin, V., Demarne, F., Lamprecht, A.: A tunable Caco-2/HT29-MTX co-culture model mimicking variable permeabilities of the human intestine obtained by an original seeding procedure. Eur. J. Pharm. Biopharm. **87**, 290–298 (2014). https://doi.org/10.1016/j.ejpb.2014.03.017
181. Mahler, G.J., Shuler, M.L., Glahn, R.P.: Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. J. Nutr. Biochem. **20**, 494–502 (2009). https://doi.org/10.1016/j.jnutbio.2008.05.006
182. Kernéis, S., Bogdanova, A., Kraehenbuhl, J.-P., Pringault, E.: Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science **277**, 949–952 (1997). https://doi.org/10.1126/science.277.5328.949
183. Ahmad, T., Gogarty, M., Walsh, E.G., Brayden, D.J.: A comparison of three Peyer's patch "M-like" cell culture models: particle uptake, bacterial interaction and epithelial histology. Eur. J. Pharm. Biopharm. **119,** 426–436 (2017). https://doi.org/10.1016/j.ejpb.2017.07.013
184. des Rieux, A., Fievez, V., Théate, I., Mast, J., Préat, V., Schneider, Y.-J.: An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle transport by M cells. Eur. J. Pharm. Sci. **30**, 380–391 (2007). https://doi.org/10.1016/j.ejps.2006.12.006
185. Gullberg, E., Leonard, M., Karlsson, J., Hopkins, A.M., Brayden, D., Baird, A.W., Artursson, P.: Expression of specific markers and particle transport in a new human intestinal M cell model. Biochim. Biophys. Res. Commun. **279**, 808–813 (2000). https://doi.org/10.1006/bbrc.2000.4038
186. Schimpel, C., Teubl, B., Absenger, M., Meindl, C., Fröhlich, E., Leitinger, G., Zimmer, A., Roblegg, E.: Development of an advanced intestinal in vitro triple culture permeability model to study transport of nanoparticles. Mol. Pharm. **11**, 808–818 (2014). https://doi.org/10.1021/mp400507g
187. Hilgers, A.R., Conradi, R.A., Burton, P.S.: Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. Pharm. Res. **7**, 902–910 (1990). https://doi.org/10.1023/A:1015937605100
188. Beloqui, A., des Lieux, A., Préat, V.: Mechanisms of transport of polymeric and lipidic nanoparticles across the intestinal barrier. Adv. Drug Deliv. Rev. **106, Part B**, 242–255 (2016). https://doi.org/10.1016/j.addr.2016.04.014
189. He, B., Lin, P., Jia, Z., Du, W., Qu, W., Yuan, L., Dai, W., Zhang, H., Wang, X., Wang, J., Zhang, X., Zhang, Q.: The transport mechanisms of polymer nanoparticles in Caco-2 epithelial cells. Biomaterials **34**, 6082–6098 (2013). https://doi.org/10.1016/j.biomaterials.2013.04.053
190. Russel-Jones, G.J., Arthur, L., Walker, H.: Vitamin B12-mediated transport of nanoparticles across Caco-2 cells. Int. J. Pharm. **179**, 247–255 (1999). https://doi.org/10.1016/S0378-5173(98)00394-9
191. Sheng, J., Han, L., Qin, J., Ru, G., Li, R., Wu, L., Cui, D., Yang, P., He, Y., Wang, J.: N-Trimethyl chitosan chloride-coated PLGA nanoparticles overcoming multiple barriers to oral insulin absorption. ACS Appl. Mater. Interfaces **7**, 15430–15441 (2015). https://doi.org/10.1021/acsami.5b03555
192. Luo, Y., Teng, Z., Li, Y., Wang, Q.: Solid lipid nanoparticles for oral drug delivery: Chitosan coating improves stability, controlled delivery, mucoadhesion and cellular uptake. Carbohydr. Polym. **122**, 221–229 (2015). https://doi.org/10.1016/j.carbpol.2014.12.084
193. Araújo, F., Shrestha, N., Shahbazi, M.-A., Fonte, P., Mäkilä, E.M., Salonen, J.J., Hirvonen, J.T., Granja, P.L., Santos, H.A., Sarmento, B.: The impact of nanoparticles on the mucosal translocation and transport of GLP-1 across the intestinal epithelium. Biomaterials **35**, 9199–9207 (2014). https://doi.org/10.1016/j.biomaterials.2014.07.026
194. Lichtenstein, D., Ebmeyer, J., Meyer, T., Behr, A.-C., Kästner, C., Böhmert, L., Juling, S., Nieman, B., Fahrenson, C., Selve, S., Thünemann, A.F., Meijer, J., Estrela-Lopis, I., Braeuning, A., Lampen, A.: It takes more than a coating to get nanoparticles through the intestinal barrier in vitro. Eur. J. Pharm. Biopharm. **118**, 21–29 (2017). https://doi.org/10.1016/j.ejpb.2016.12.004
195. Giannasca, K.T., Giannasca, P.J., Neutra, M.R.: Adherence of *Salmonella* typhimurium to Caco-2 cells: identification of a glycoconjugate receptor. Infect. Immun. **64**, 135–145 (1996)

196. Jahn, K.A., Biazik, J.M., Braet, F.: GM1 Expression in Caco-2 cells: characterisation of a fundamental passage-dependent transformation of a cell line. J. Pharmaceut. Sci. **100**, 3751–3762 (2011). https://doi.org/10.1002/jps.22418
197. Behrens, I., Vila Pena, A.I., Alonso, M.J., 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. **19**, 1185–1193 (2002). https://doi.org/10.1023/A:10198543
198. Ke, Z., Guo, H., Zhu, X., Jin, Y., Huang, Y.: Efficient peroral delivery of insulin via vitamin B_{12} modified trimethyl chitosan nanoparticles. J. Pharm. Pharm. Sci. **18**, 155–170 (2015)
199. Yoshida, T., Yoshioka, Y., Takahashi, H., Misato, K., Mori, T., Hirai, T., Nagano, K., Abe, Y., Mukai, Y., Kamada, H., Tsunoda, S., Nabeshi, H., Yoshikawa, T., Higashisaka, K., Tsutsumi, Y.: Intestinal absorption and biological effects of orally administered amorphous silica particles. Nanoscale Res. Lett. **9**, 532–538 (2014). https://doi.org/10.1186/1556-276X-9-532
200. Wiwattanapatapee, R., Carreño-Gomez, B., Malik, N., Duncan, R.: Anionic PAMAM dendrimers rapidly cross adult rat intestine in vitro: a potential oral delivery system? Pharm. Res. **17**, 991–998 (2000). https://doi.org/10.1023/A:1007587523543
201. Lautenschläger, I., Dombrowski, H., Frerichs, I., Kuchenbecker, S.C., Bade, S., Schultz, H., Zabel, P., Scholz, J., Weiler, N., Uhlig, S.: A model of the isolated perfused rat small intestine. Am. J. Physiol. **298**, G304–G313 (2010). https://doi.org/10.1152/ajpgi.00313.2009
202. Sinnecker, H., Krause, T., Koelling, S., Lautenschläger, I., Frey, A.: The gut wall provides an effective barrier against nanoparticle uptake. Beilstein J. Nanotechnol. **5**, 2092–2101 (2014). https://doi.org/10.3762/bjnano.5.218
203. Bergin, I.L., Witzmann, F.A.: Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int. J. Biomed. Nanosci. Nanotechnol. **3**, 163–210 (2013). https://doi.org/10.1504/ijbnn.2013.054515
204. Delie, F.: Evaluation of nano- and microparticle uptake by the gastrointestinal tract. Adv. Drug Deliv. Rev. **34**, 221–233 (1998). https://doi.org/10.1016/S0169-409X(98)00041-6
205. Bölke, T., Krapf, L., Orzekowsky-Schroeder, R., Vossmeyer, T., Dimitrijevic, J., Weller, H., Schüth, A., Klinger, A., Hüttmann, G., Gebert, A.: Data-adaptive image-denoising for detecting and quantifying nanoparticle entry in mucosal tissues through intravital 2-photon microscopy. Beilstein J. Nanotechnol. **5**, 2016–2025 (2014). https://doi.org/10.3762/bjnano.5.210
206. Lee, C.-M., Lee, T.K., Kim, D.-I., Kim, Y.-R., Kim, M.-K., Jeong, H.-J., Sohn, M.-H., Lim, S.T.: Optical imaging of absorption and distribution of RITC-SiO2 nanoparticles after oral administration. Int. J. Nanomed. **9**(Suppl 2), 243–250 (2014). https://doi.org/10.2147/ijn.s57938
207. Howe, S.E., Lickteig, D.J., Plunkett, K.N., Ryerse, J.S., Konjufca, V.: The uptake of soluble and particulate antigens by epithelial cells in the mouse small intestine. PLoS ONE **9**, e86656 (2014). https://doi.org/10.1371/journal.pone.0086656
208. Loeschner, K., Hadrup, N., Qvortrup, K., Larsen, A., Gao, X., Vogel, U., Mortensen, A., Lam, H.R., Larsen, E.H.: Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. Part. Fibre Toxicol. **8**, 18 (2011). https://doi.org/10.1186/1743-8977-8-18
209. Jani, P., Halbert, G.W., Langridge, J., Florence, A.T.: Nanoparticle uptake by the rat gastrointestinal mucosa: quantitation and particle size dependency. J. Pharm. Pharmacol. **42**, 821–826 (1990). https://doi.org/10.1111/j.2042-7158.1990.tb07033.x
210. Geraets, L., Oomen, A.G., Krystek, P., Jaobsen, N.R., Wallin, H., Laurentie, M., Verharen, H.W., Brandon, E.F.A., de Jong, W.H.: Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. Part. Fibre Toxicol. **11**, 30 (2014). https://doi.org/10.1186/1743-8977-11-30
211. Janer, G., Mas del Molino, E., Fernández-Rosas, E., Fernández, A., Vázquez-Campos, S.: Cell uptake and oral absorption of titanium dioxide nanoparticles. Toxicol. Lett. **228**, 103–110 (2014). https://doi.org/10.1016/j.toxlet.2014.04.014

212. Jovanovic, B.: Critical review of public health regulations of titanium dioxide, a human food additive. Integr. Environ. Assess. Manag. **11**, 10–20 (2015). https://doi.org/10.1002/ieam.1571
213. Böckmann, J., Lahl, H., Eckert, T., Unterhalt, B.: Titan-Blutspiegel vor und nach Belastungsversuchen mit Titandioxid. Pharmazie **55**, 140–143 (2000)
214. Jones, K., Morton, J., Smith, I., Jurkschat, K., Harding, A.-H., Evans, G.: Human in vivo and in vitro studies on gastrointestinal absorption of titanium dioxide nanoparticles. Toxicol. Lett. **233**, 95–101 (2015). https://doi.org/10.1016/j.toxlet.2014.12.005
215. Pele, L.C., Thoree, V., Bruggraber, S.F.A., Koller, D., Thompson, R.P.H., Lomer, M.C., Powell, J.J.: Pharmaceutical/food grade titanium dioxide particles are absorbed into the bloodstream of human volunteers. Part. Fibre Toxicol. **12**, 26 (2015). https://doi.org/10.1186/s12989-015-0101-9
216. Rompelberg, C., Heringa, M.B., van Donkersgoed, G., Drijvers, J., Roos, A., Westenbrink, S., Peters, R., van Bemmel, G., Brand, W., Oomen, A.G.: Oral intake of added titanium dioxide and its nanofraction from food products, food supplements and toothpaste by the Dutch population. Nanotoxicology **10**, 1404–1414 (2016). https://doi.org/10.1080/17435390.2016.1222457
217. Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goertz, N.: Titanium dioxide nanoparticles in food and personal care products. Environ. Sci. Technol. **46**, 2242–2250 (2012). https://doi.org/10.1021/es204168d
218. Jani, P.U., McCarthy, D.E., Florence, A.: Titanium dioxide (rutile) particle uptake from the rat GI tract and translocation to systemic organs after oral administration. Int. J. Pharm. **105**, 157–168 (1994). https://doi.org/10.1016/0378-5173(94)90461-8
219. Hummel, T.Z., Kindermann, A., Stokkers, P.C.F., Benninga, M.A., ten Kate, F.J.W.: Exogenous pigment in Peyer's patches of children suspected of having IBD. J. Pediatr. Gastroenterol. Nutr. **58**, 477–480 (2014). https://doi.org/10.1097/MPG.0000000000000221
220. Shepherd, N.A., Crocker, P.R., Smith, A.P., Levison, D.A.: Exogenous pigment in Peyer's patches. Human Pathol. **18**, 50–54 (1987). https://doi.org/10.1016/S0046-8177(87)80193-4
221. Feliu, N., Docter, D., Heine, M., Del Pino, P., Ashraf, S., Kolosnjaj-Tabi, J., Macchiarini, P., Nielsen, P., Alloyeau, D., Gazeau, F., Stauber, R.H., Parak, W.J.: In vivo degeneration and the fate of inorganic nanoparticles. Chem. Soc. Rev. **45**, 2440–2457 (2016). https://doi.org/10.1039/C5CS00699F
222. Carambia, A., Freund, B., Schwinge, D., Bruns, O.T., Salmen, S.C., Ittrich, H., Reimer, R., Heine, M., Huber, S., Waurisch, C., Eychmüller, A., Wraith, D.C., Korn, T., Nielsen, P., Weller, H., Schramm, C., Lüth, S., Lohse, A.W., Heeren, J., Herkel, J.: Nanoparticle-based autoantigen delivery to Treg-inducing liver sinusoidal endothelial cells enables control of autoimmunity in mice. J. Hepatol. **62**, 1349–1356 (2015). https://doi.org/10.1016/j.jhep.2015.01.006
223. Jung, C.S.L., Heine, M., Freund, B., Reimer, R., Koziolek, E.J., Kaul, M.G., Kording, F., Schumacher, U., Weller, H., Nielsen, P., Adam, G., Heeren, J., Ittrich, H.: Quantitative activity measurements of brown adipose tissue at 7 T magnetic resonance imaging after application of triglyceride-rich lipoprotein 59Fe-superparamagnetic iron oxide nanoparticle: intravenous versus intraperitoneal approach. Invest. Radiol. **51**, 194–202 (2016). https://doi.org/10.1097/RLI.0000000000000235
224. Wang, Y., Zhao, Y., Cui, Y., Zhao, Q., Zhang, Q., Musetti, S., Kinghorn, K.A., Wang, S.: Overcoming multiple gastrointestinal barriers by bilayer modified hollow mesoporous silica nanocarriers. Acta Biomater. **65**, 405–416 (2018). https://doi.org/10.1016/j.actbio.2017.10.025
225. Bartelt, A., Bruns, O.T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M.G., Tromsdorf, U.I., Weller, H., Waurisch, C., Eychmüller, A., Gordts, P.L.S.M., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., Heeren, J.: Brown adipose tissue activity controls triglyceride clearance. Nat. Med. **17**, 200–205 (2011). https://doi.org/10.1038/nm.2297
226. Freund, B., Tromsdorf, U.I., Bruns, O.T., Heine, M., Giemsa, A., Bartelt, A., Salmen, S.C., Raabe, N., Heeren, J., Ittrich, H., Reimer, R., Hohenberg, H., Schumacher, U., Weller,

H., Nielsen, P.: A simple and widely applicable method to 59Fe-radiolabel monodisperse superparamagnetic iron oxide nanoparticles for in vivo quantification studies. ACS Nano **6**, 7318–7325 (2012). https://doi.org/10.1021/nn3024267

227. Kreyling, W.G., Hirn, S., Möller, W., Schleh, C., Wenk, A., Celik, G., Lipka, J., Schäffler, M., Haberl, N., Johnston, B.D., Sperling, R., Schmid, G., Simon, U., Parak, W.J., Semmler-Behnke, M.: Air-blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size. ACS Nano **8**, 222–233 (2014). https://doi.org/10.1021/nn403256v
228. Schleh, C., Semmler-Behnke, M., Lipka, J., Wenk, A., Hirn, S., Schäffler, M., Schmid, G., Simon, U., Kreyling, W.G.: Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration. Nanotoxicology **6**, 36–46 (2012). https://doi.org/10.3109/17435390.2011.552811