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Inorganic nanoparticles for oral drug delivery: opportunities, barriers, and future perspectives

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Oral delivery is the preferred route of drug administration due to patient compliance and convenience. Despite this, nanomedicines have so far primarily been developed for the parenteral route. Inorganic nanoparticles hold great promise as theranostics for oral drug delivery. This is gaining importance especially for the local treatment of gastrointestinal (GI) diseases. However, successful oral delivery of inorganic nanoparticles is challenged by complex physiological conditions in the GI tract. We discuss the main GI barriers and their impact on nanoparticle biotransformation and toxicity. An improved understanding of the complex interplay of inorganic nanoparticles with the dynamic GI environment can facilitate the development of efficient oral nanomedicines.

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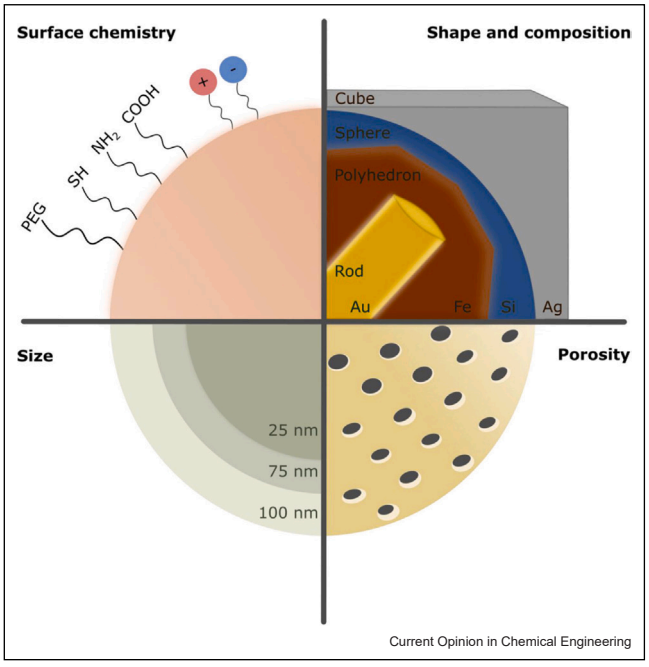
Introduction

Nanomedicine has been an active area of research since the discovery of liposomes in the 1960s [1]. Nanotechnology in medicine and pharmaceutical research has aided great discoveries in the treatment, imaging, and diagnosis of various conditions, especially in oncology [2,3]. Today, more than 30 types of nanoparticles have been approved in the clinic. The Food and Drug Administration (FDA) approval of lipid nanoparticle-based carriers for the mRNA vaccines against COVID-19 has fueled even greater interest in nanomedicine [4]. In fact, parenterally administered lipid nanoparticles make up the largest fraction of clinically approved nanoparticles, followed by inorganic nanoparticles as the second largest group. The latter are primarily iron oxide-based nanoparticles used in the systemic treatment of iron-deficient anemia and kidney disease [5•].

Compared with systemically administered nanoparticles, orally administered ones have been much less studied, despite the oral route being the most common (approx. 60% of approved drug products) and showing best patient compliance. Nevertheless, orally administered inorganic nanoparticles are well-established as food additives, for example, in the form of SiO_2 (E551), a common and generally-regarded-as-safe food additive [6]. Furthermore, the successful clinical translation of systemically administered nanomedicines, offer promising pharmaceutical and biomedical applications of inorganic nanoparticles in the gastrointestinal tract (GIT). Orally administered inorganic nanoparticles can be used i) as carriers for systemic drug absorption, ii) for local treatment of gastrointestinal (GI) diseases such as inflammatory bowel disease (IBD) and colorectal cancer (CRC), and iii) for imaging applications. Thus, the function of orally administered nanoparticles does not solely rely on their translocation across the intestinal epithelium. However, oral administration of inorganic nanoparticles is challenging due to multiple barriers presented by the GIT. These include its high enzymatic activity, variations in pH, the intestinal mucosal barrier, and absorption across the intestinal epithelium. The functionality and fate of inorganic nanoparticles in the GIT depend on their physicochemical properties such as composition, size, shape, porosity, and surface chemistry (Figure 1).

Inorganic nanoparticles are most often explored for their theranostic potential, that is, combined diagnosis and treatment. This level of functionality is difficult to achieve with lipid-based or polymeric nanoparticles. The most commonly used inorganic nanoparticles for biomedical applications include pure metals (especially plasmonic nanoparticles such as Au and Ag), metal oxides (e.g. solid or mesoporous SiO_2, γ-Fe_2O_3/Fe_3O_4), semiconductor materials (quantum dots), and calcium phosphates. Gold nanoparticles are extensively studied due to their high biocompatibility, ease of synthesis, and bioconjugation, as well as tunable optical and thermal properties. The latter properties make gold

Figure 1



Physicochemical properties that influence the biological fate and behavior of inorganic nanoparticles in the GIT. Sizes, shapes, and compositions are depicted as examples.

nanoparticles of particular interest for photothermal therapy (PTT), in which near-infrared (NIR) light is used to trigger localized hyperthermia. Iron oxide nanoparticles have also been widely studied for hyperthermia, magnetically targeted drug-delivery applications, and as contrast agents in magnetic resonance imaging. Recently, hybrid materials have been developed that combine the benefits of several nanoparticle classes [7]. For the interested reader, there are detailed reviews covering the synthesis, physicochemical properties, and biomedical applications of the most commonly used inorganic nanomaterials: gold [8], silver [9], iron oxide [10,11], titanium dioxide [12], and silica dioxide [13,14].

Here, we review recent advances in orally administered inorganic nanoparticles for disease treatment and diagnosis. First, we highlight the barriers encountered by nanoparticles in the GIT and how these impact their dissolution, aggregation, and biodistribution. We focus specifically on the interplay between the GI environment and the nanoparticles themselves. The interested reader is referred to recently published reviews on drug solubility [15], dissolution [16], and ultimately absorption [17] from the GIT. We emphasize the importance of developing *in vitro* methods that more closely mimic the dynamic physiological conditions encountered by nanoparticles in the GIT. A special focus is placed on the emerging use of oral inorganic nanoparticles as theranostics for GI diseases. Finally, we address toxicity issues related to nanoparticles and their impact on the successful clinical translation of oral inorganic nanomedicines.

Gastrointestinal barriers encountered by orally administered inorganic nanoparticles

The GIT is well-adapted for its physiological tasks of processing food and absorbing nutrients while at the same time protecting the body from harmful substances and microorganisms. These opposing functions of uptake and exclusion are the main barriers for orally administered inorganic nanoparticles. The physiological environment in the GIT varies between different GI regions, prandial states (i.e. fasted versus fed), and health conditions (healthy or diseased). These parameters all influence the complex dynamics of GI transport and oral drug absorption [17]. For an inorganic nanoparticle-based drug-delivery system to exert a systemic therapeutic effect, the nanoparticle and/or its cargo must cross the mucus lining of the intestinal epithelium and permeate the epithelial cells. Here, it is important to consider the release of the cargo from the nanoparticle. Both the properties of the drug (e.g. logP and pK_a) and the nanoparticle (e.g. size and material) influence when, where, and at which rate the drug is released from the carrier. Examples include immediate drug release from the nanoparticle upon contact with the GI fluid or controlled release at the target site after systemic absorption of the nanoparticle. Surface coatings/modifications (such as pH-responsive coatings) can modulate drug release [18,19]. Below, we outline parameters governing GI transport and cellular uptake of inorganic nanoparticles and their impact on oral drug delivery.

Dynamics of nanoparticle dissolution and aggregation in gastrointestinal fluids

GI fluids are aqueous solutions or suspensions comprising exogenous (e.g. food) and endogenous constituents. The fluids vary in pH, ionic strength, and composition (e.g. lipids, bile salts, and proteins). In turn, the fluid properties affect the dynamic behavior of inorganic nanoparticles in the GIT with respect to nanoparticle degradation (due to pH changes or enzymes [20]), dissolution, aggregation, and surface properties (Figure 2). Nanoparticle transport through the GIT is governed by nanoparticle diffusion and motility-driven transport.

Table 1 provides an overview of recent studies on the dynamic aggregation and dissolution behavior of inorganic nanoparticles in simulated GI fluids. Many of these *in vitro* studies focus on a specific GI location (e.g. in the small intestine) and the resulting effects on

the hydrodynamic particle diameter or particle concentration. To improve the correlation of *in vitro* and *in vivo* data, dynamic *in vitro* assays have been developed to account for the processes occurring upon transport through different GI regions (i.e. cascade set-ups). Further, cascade setups have been compared with the dissolution behavior of various metal oxide nanoparticles in individual fluids (mimics of fasted-state saliva, and gastric and intestinal fluids) [21]. Cascade transport through various biological compartments may significantly affect the toxicological profile of certain nanomaterials [22••].

The impact of varying physiological conditions in the GIT due to disease state, for example, during an acute inflammation, when investigating inorganic nanoparticle fate *in vivo*, is often overlooked. Physiological changes can include impaired integrity of the intestinal wall and altered motility and flow conditions (e.g. due to diarrhea). Leonard et al. demonstrated that larger (~2 μm) particles adhere more strongly than smaller (~80 nm) ones to inflamed Caco-2 cells during flow conditions [23]. Thus, cell studies under flow could more closely mimic GI motility and the resulting nanoparticle–cell interactions than static experiments.

Enzyme corona formation in gastrointestinal fluids

GI-fluid components such as pancreatic enzymes can interact with the surface of inorganic nanoparticles to form an enzyme corona. This considerably increases the hydrodynamic diameter of the particles, alters their surface properties, and increases particle recognition and elimination by the innate immune system. This is a well-studied phenomenon for parenterally administered nanoparticles for which the systemic transport and biodistribution of nanoparticles is strongly influenced by the formation of a serum-protein corona [24]. In contrast, corona formation in GI fluids still remains understudied. Although one recent study has explored the influence of particle properties in this context [25••], more studies are needed to understand the dynamic interaction of digestive enzymes with inorganic nanoparticles and their impact on GI toxicity and cellular uptake. The formation of enzyme corona can be minimized, but not abolished by coating the nanoparticle surface with neutrally charged molecules or polymers such as polyvinyl alcohol and polyethylene glycol (PEG) [26].

Transport of inorganic nanoparticles through the intestinal mucus layer

The GIT is covered by a protective mucus layer consisting of hydrogel-forming mucins, which are large, glycosylated proteins secreted by goblet cells [27]. The morphology and thickness of the mucus layer varies throughout the GIT. The mucus itself is a barrier and the constant mucus turnover, combined with the GI-fluid flow, efficiently removes particles [28]. Taken together, thise creates problems for nanoparticle-mediated systemic drug delivery (Figure 2). The hydrogel mesh sterically excludes particles > 200 nm [29]. Furthermore, mucus can interact with nanoparticles and their cargo via electrostatic, hydrophobic, and hydrogen-bonding interactions and thereby hinder transport toward the epithelium. In particular, electrostatic interactions between the overall negatively charged mucus and cationic delivery systems can retain nanoparticles in the mucus layer.

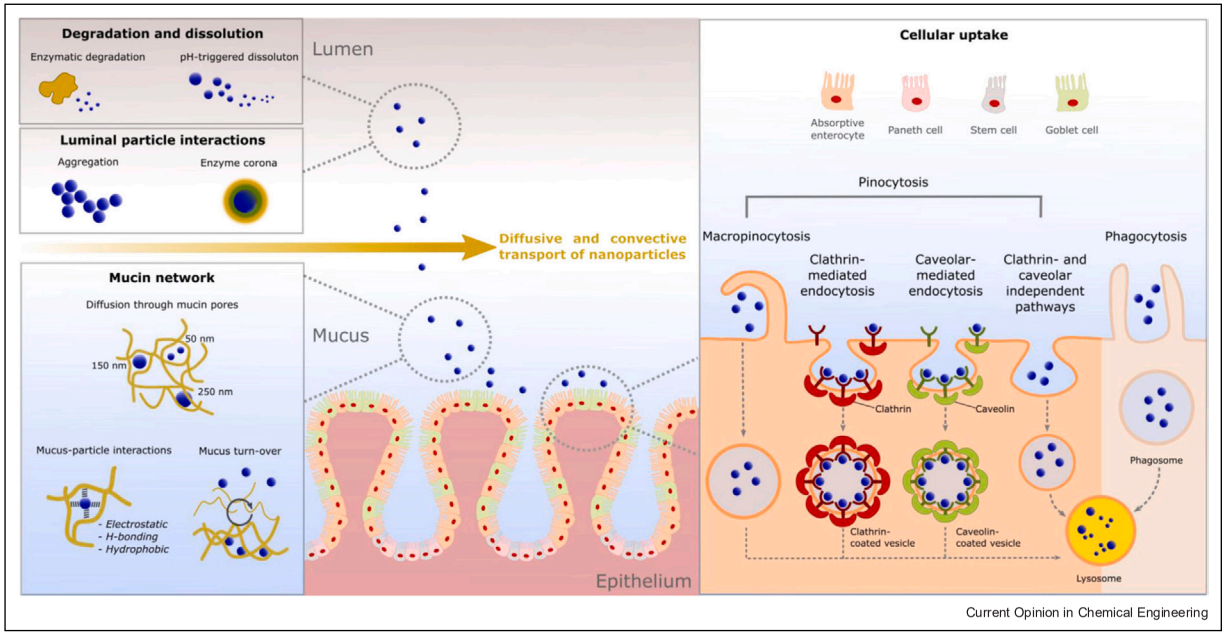
Thus, cationic and hydrophobic nanoparticles interact more strongly with mucin than anionic and hydrophilic ones, thus rendering nanoparticles either mucoadhesive or mucopenetrating, respectively [30]. Bhattacharjee et al. observed that the transport of SiO_2 nanoparticles through porcine jejunal mucus depends on particle size, surface charge, and coating [31]. Specifically, anionic and methyl-PEGylated SiO_2 nanoparticles were transported more readily than their cationic and noncoated counterparts. Additionally, Au nanoparticles can interact with mucin through the formation of gold–sulfur or disulfide bonds (for thiol-stabilized Au) [32]. Nanoparticle shape can also influence transport through mucus. Using mesoporous silica and calcium phosphate nanoparticles, Yu et al. showed that nanorods display better mucus-transport properties than nanospheres of the same chemistry [29]. Finally, nanoparticles can impact the mucus layer itself. For example, Bredeck et al. reported an altered expression of mucin genes upon exposure to CeO_2 and Ag nanoparticles, which may lead to an elevated susceptibility toward intestinal inflammation and infection [33].

Nanoparticle uptake by the intestinal epithelium for systemic drug delivery

The intestinal epithelium consists of a tight layer of cells with different functions, for example, absorptive enterocytes and mucus-producing goblet cells (Figure 2). The transport of inorganic nanoparticles is generally very low across these cell layers for systemic absorption to occur [24,25••,34–36]. Inorganic nanoparticles can cross the intestinal epithelium via endocytotic pathways, classified as pinocytosis and phagocytosis. The main pinocytotic pathways are macropinocytosis, clathrin-mediated endocytosis, caveolar-mediated endocytosis, and clathrin- and caveolar-independent pathways. Phagocytosis is a receptor-mediated process carried out mainly by specialized cells such as macrophages and neutrophils to remove invading microorganisms and exogenous material.

Some studies also suggest that the paracellular route can be an absorption pathway, although the available space (< 10 Å) there is limited [37]. Two recent studies demonstrated that anionic SiO_2 nanoparticles can enhance intestinal paracellular permeability via tight-junction relaxation. This enabled oral delivery of a

Figure 2



Orally administered nanoparticles and the physiological obstacles they face in the GIT. *Lumen*: upon contact with GI fluids, nanoparticles may lose their function due to enzymatic degradation or pH-triggered dissolution. Nanoparticle properties may be significantly affected due to aggregation events and protein corona formation. *Mucus*: particles are entrapped in the mucin network due to the size-dependent diffusion through the mucosal pores and/or mucus–particle interactions. The constant renewal of mucus contributes to the removal of particles from the mucosal layer. *Cellular uptake*: nanoparticles are taken up by absorptive enterocytes through various pinocytotic pathways, such as macropinocytosis, clathrin-mediated endocytosis, caveolar-mediated endocytosis, or clathrin- and caveolar-independent pathways. Eventually, the particles might degrade in the lysosome. Efficient removal of pathogens by specialized cells, such as macrophages or neutrophils, is performed via phagocytosis.

Table 1

Aggregation and dissolution behavior of inorganic nanoparticles in simulated GI fluids.

Nanomaterial	Size (nm) and shape[a]	Surface modification	GI model[b]	GI media[c]	Dissolution and aggregation behavior	Ref.
Ag	16 ± 4 (TEM)	–	Static	• Artificial saliva • SGF (pH 1.2, 6.8) ± pepsin • SIF (pH 6.8) ± pancreatin	• Aggregation in saliva and SGF without pepsin • Very strong aggregation in SIF with pancreatin • Protein matrix around the nanoparticle in SGF with pepsin	[87]
	20, 110 (supplier)	Citrate, PVP	Static	• SGF (pH 2, 3.5, 4.5, 5)	• Aggregation in SGF increased with decreasing pH • Aggregation initiated via surface dissolution. Surface precipitation of Ag^+ as AgCl • Size and capping agent-dependent aggregation (citrate 20 > PVP 20 > citrate 110 > PVP 110)	[88]
	40, 70 (supplier)	Citrate	Static	• SGF	• High and size-dependent dissolution in SGF (40 > 70) • Aggregation in SGF due to formation of AgCl	[89]
	50 (DLS)	Citrate, lipoic acid	Cascade	• Artificial saliva (pH 6.8) • SGF (pH 5) • SIF (pH 6.5) • All fluids with proteins and enzymes	• Particulate Ag was reduced by 86–92% for lipoic acid-coated and 48–79% for citrate-coated nanoparticles after *in vitro* digestion	[4-1•]
Au	40, 80 (supplier)	–	Static	• SGF (pH 2, 3.5, 4.5, 5)	• Size-dependent aggregation in SGF (40 > 80) • No dissolution in SGF	[89]
	5, 50, 100 (TEM)	PVP	Static	• FaSSGF (pH 1.2) • FaSSIF (pH 6.5), FeSSIF (pH 5) • From biorelevant.com	• Aggregation in FaSSGF • No aggregation in FaSSIF and FeSSIF	[36]
CeO_2	30–50 (SEM)	–	Static	• SGF (pH 2, 3.5, 4.5, 5)	• Concentration- and temperature-dependent aggregation in SGF	[89]
Fe_2O_3	80–120 rods (TEM), 70 acicular-shaped NPs (TEM)	–	Static, cascade	• Artificial saliva (pH 6.5) • SGF (pH 1.4) • SIF (pH 8.1) • All fluids with proteins and enzymes	• Low dissolution in all stages of *in vitro* digestion with highest dissolution in SGF • Strong aggregation in SIF • Dissolution: rods < acicular-shaped nanoparticles • Aggregation: rods > acicular-shaped nanoparticles	[21]
	Nanoparticles differing in crystallinity and shape, ~100 (TEM)	–	Cascade	• Artificial saliva (pH 6.4) • SGF (pH 2) • SIF (pH 7.5) • All fluids with proteins and enzymes	• Low dissolution during all stages of *in vitro* digestion • Highest dissolution in SGF	[43]
Fe_3O_4	11 (TEM)	–	Cascade	• Artificial saliva (pH 6.4) • SGF (pH 2) • SIF (pH 7.5) • All fluids with proteins and enzymes	• Low dissolution during all stages of *in vitro* digestion • Highest dissolution in SGF	[43]
SiO_2	10–20 (TEM)	–	Static, cascade	• Artificial saliva (pH 6.5) • SGF (pH 1.4) • SIF (pH 8.1) • All fluids with proteins and enzymes	• Static: 2.6% and 34.5% dissolution in saliva and SGF, respectively. No aggregation in saliva. Strong aggregation in SGF. Complete dissolution in SIF. • Cascade: Dissolution after transfer from saliva to SGF. Precipitation after transfer from SGF to SIF.	[21]

Table 1 (*continued*)

Nanomaterial	Size (nm) and shape[a]	Surface modification	GI model[b]	GI media[c]	Dissolution and aggregation behavior	Ref.
TiO_2	100–150 (TEM)	–	Static, cascade	• Artificial saliva (pH 6.5) • SGF (pH 1.4) • SIF (pH 8.1) • All fluids with proteins and enzymes	• Static: No aggregation in saliva, slight aggregation in SGF, aggregation in SIF. No dissolution in any media. • Cascade: No dissolution. Aggregation after transfer from saliva to SGF, unchanged after transfer to SIF.	[21]
ZnO	100–150 (TEM)	–	Static, cascade	• Artificial saliva (pH 6.5) • SGF (pH 1.4) • SIF (pH 8.1) • All fluids with proteins and enzymes	• Static: Low dissolution in saliva and SIF. Complete dissolution in SGF. • Cascade: Complete dissolution after transfer from saliva to SGF. Remained dissolved after transfer from SGF to SIF.	[21]
	< 100 and 80–200 (supplier)	–	Static	• SGF (pH 2, 3.5, 4.5, 5)	• High dissolution in SGF • Smaller nanoparticles (< 100 nm) dissolved faster than bigger ones (80–200 nm)	[89]
	15–70 (uncoated), 50–200 (silane) (TEM)	Uncoated and silane-coated	Cascade	• Artificial saliva (pH 6.4) • SGF (pH 2.2) • SIF (pH 7.5) • All fluids with proteins and enzymes	• Low dissolution in saliva, complete dissolution after transfer to SGF. • *De novo* formation of $ZnPO_4$ nanoparticles after transfer from SGF to SIF (14–34% remained dissolved). • No difference between bare and silane-coated nanoparticles.	[90]

Abbreviations: DLS, dynamic light scattering, FaSSGF, fasted-state simulated gastric fluid, FaSSIF, fasted-state simulated intestinal fluid, FeSSGF, fed-state simulated gastric fluid, FeSSIF, fed-state simulated intestinal fluid, PVP, polyvinylpyrrolidone, SGF, simulated gastric fluid, SIF, simulated intestinal fluid, TEM, transmission electron microscopy.

[a] Spherical shape if not stated otherwise. Method used to determine size is given in parentheses.

[b] Static GI model = incubation in a single GI fluid. Cascade GI model = nanoparticles are incubated in a series of GI fluids, which mimic their *in vivo* transfer from mouth to intestine.

[c] The complexity of the GI media varies greatly between studies. For the detailed media composition, the reader is referred to the references given in the table.

coadministered peptide drug [35•,38]. For oral peptide delivery, nanoparticulate carriers have another advantage: they can enhance stability by protecting the peptide from the harsh GI environment, thereby positively affecting oral bioavailability [39,40].

Endocytosis of inorganic nanoparticles, including Ag [41•], Au [36,42], Fe_xO_y [43], SiO_2 [44–46], and TiO_2 [47], has been studied in multiple ways. These include *in vitro* cell models with Caco-2 cells as proxies for the intestinal epithelium [23,24,35•,42–46], *ex vivo* with intestinal tissues, and *in vivo* after oral administration [35•,45,47]. In general, cellular uptake is influenced by nanoparticle size [35•,42,43,45], shape [45], and surface coating [24,35•,46]. In contrast to endocytosis, exocytosis mechanisms are rarely studied, although a recent study by Lichtenstein et al. highlighted the influence of particle composition on transcytosis mechanisms [48].

The correlation of the dynamic GI environment encountered by orally administered nanoparticles and the resulting impact on their cellular uptake is often overlooked in the literature. Preclinical studies — with pristine nanoparticles under experimental conditions that do not reflect the GI environment — may under- or overestimate nanoparticle uptake *in vivo*. For example, Jiang et al. showed that the type of assay medium can influence nanoparticle uptake in *in vitro*-simulated gastric and intestinal fluids [36]. Abdelkhaliq et al. demonstrated that the uptake of *in vitro*-digested Ag nanoparticles is lower than that of their pristine counterparts [41•]. These examples underline the importance of considering the *in vivo* GI environment when designing and interpreting *in vitro* studies.

Orally administered inorganic nanoparticles for the treatment and diagnosis of gastrointestinal diseases

The incidence and prevalence of GI diseases are increasing worldwide, placing a huge economic burden on society in terms of medical costs [49]. Many GI disorders, if inadequately treated, can lead to serious complications. Thus, there is a great need to develop more efficient and patient-compliant diagnosis and treatment options for GI diseases. Inorganic nanoparticles hold great promise here as theranostics (Table 2). Furthermore, they can be locally delivered to the diseased GIT by oral administration, circumventing the need to cross the intestinal epithelium for systemic exposure.

Colorectal cancer

CRC is the third most common type of cancer and cause of death by cancer worldwide [50]. Au nanoparticle-mediated PTT combined with chemotherapy has been successfully applied for treatment of CRC and the method is under clinical trial investigation for prostate cancer (NCT02680535) and glioblastoma (NCT03020017). Especially promising are therapies that combine chemotherapeutic drugs with mild hyperthermia induced by NIR photoactivation of Au nanoparticles [51]. Theranostic metal-based nanoparticles can also offer spatiotemporal control of the nanosystem. Au nanoparticles can be tagged with a radionuclide before positron emission tomography/computed tomography (CT), to guide precise delivery of the nanoparticles to the CRC tumor [52]. Iron oxide can act as a T2 contrast agent to visualize nanoparticle accumulation in the cancerous tissue by magnetic resonance imaging [53]. Furthermore, the iron oxide enables precise localization of the nanoparticles at the target site using an external magnet.

However, only a few studies have attempted the oral administration of theranostic nanomaterials for CRC treatment. Recently, an oral hybrid system was developed using solid lipid nanoparticles loaded with doxorubicin and iron oxide for local hyperthermia therapy of CRC [54•]. The system was designed to evade absorption in the small intestine, while enhancing cellular uptake by the tumorous tissue through targeting of folate receptors. Thus, this oral delivery system enables colonic delivery of the nanostructure, by using coating layers that prevent biorecognition by absorption transporters and enzymatic degradation.

Inflammatory bowel disease

IBD is a chronic inflammation in the intestine. Examples of IBD are Crohn's disease and ulcerative colitis. Diagnosis and treatment are challenging, partly because the disease has periods of active inflammation versus remission, but also due to the lack of accurate diagnostic procedures. Inorganic nanoparticles have been explored as imaging agents for diagnosis and because of their unique capability to mediate catalytic IBD treatment (Table 2). In a mouse model of colitis, Naha et al. administered dextran-coated CeO_2 nanoparticles orally as contrast agents for CT imaging. The particles successfully accumulated in the inflamed GIT locations [55•]. Zhao et al. capitalized on the enzymatic activity of CeO_2 to scavenge excessive reactive oxygen species (ROS) in active IBD [56]. Scavenging of ROS molecules could alleviate inflammation while also serving as a targeting site for IBD. Similarly, Zhao et al. used Prussian blue particles (ferric hexacyanoferrate(II)) with manganese as highly stable, inflammation-targeting ROS nanoscavengers [57,58]. This approach is particularly promising as Prussian blue is already a clinically established oral antidote for heavy-metal poisoning.

Gastrointestinal toxicity of inorganic nanoparticles

The oral administration of inorganic nanoparticles raises concerns regarding their potential toxic effects in the

Table 2

Orally administered inorganic nanoparticles for diagnosis and treatment of GI diseases.

Disease	Nanomaterial	Characteristics	Disease model	Outcomes	Application	Ref.
Colon cancer	Fe_2O_3	• Size: not stated • Coated with oleic acid and loaded into solid lipid nanoparticles together with doxorubicin	• Murine colorectal carcinoma CT26 cells • Surgery and colonic injection of colon adenocarcinoma (CT26) in BALB/C mice	• Delivery of lipid nanoparticles to cancerous tissue via oral delivery. • Tumor-growth inhibition achieved *in vitro* and *in vivo* by combining hyperthermia and chemotherapy.	Drug delivery	[5-4•]
IBD	CeO_2	• Size: 1.6 ± 0.2 nm (TEM) • Loaded on MMT sheets at a ratio of 1:9	• Murine macrophage-like RAW264.7 cells and human colonic epithelial HT-29 cells • Oral administration of DSS to C57BL/6 mice	• *In situ* assembly of nanoparticles onto drug carrier-enhanced therapy and delivery of orally administered particles. • Targeting of inflamed colon and reduction of inflammation through efficient scavenging of excessive ROS *in vitro* and *in vivo*.	Drug delivery	[56]
	CeO_2	• Size: 4.8 ± 1.2 nm (TEM) • Coated with dextran, resulting in total size of 17.5 ± 0.7 nm (TEM)	• Human fibroblast (BJ5ta), human colorectal adenocarcinoma (C2BBe1) among other cell lines • Oral administration of DSS to C57BL/6 mice	• Dextran-coated nanoparticles provide protection against oxidative damage *in vitro*. • Strong CT contrast and accumulation in inflamed areas of intestines *in vivo*.	Theranostic	[5-5•]
	Mn–Prussian blue	• Size: ~60 nm (TEM)	• Human colon adenocarcinoma DLD-1 cells and murine macrophage-like RAW264.7 cells • Oral administration of DSS to BALB/C mice	• Increased accumulation of negatively charged particles in inflamed mucosa *in vivo*. • Efficient catalytic activity leading to improved colitis *in vivo*.	Drug delivery	[58]
	Prussian blue	• Size: 60 nm (TEM) • Modified with PVP	• Oral administration of DSS to BALB/C mice	• Great scavenging capability of particles against hydroxyl radicals. • Significant reduction of colitis *in vivo* following intravenous administration of particles.	Drug delivery	[57]

GIT, especially with the recent EU ban of TiO_2 (E171) and re-evaluation of SiO_2 (E551) as food additives. The current FDA-approved amounts of TiO_2 and SiO_2 are 1% and 2% by weight of the food, respectively [59,60]. However, with the continuous influx of new reports and due to the lack of international regulations regarding approval of nanomedical products, these certified numbers are continuously updated and adjusted. The approval of nanomedical products is limited by regulatory aspects, where the toxicity of the nanomaterial is of major importance [61,62].

Nanotoxicity is commonly attributed to properties such as size, surface charge, chemical composition, shape, and the delivered dose [63–65]. These properties dictate the ability of nanomaterials to interact with biological components or (mechanically) damage tissues upon direct contact by penetrating cell membranes. Ultimately, the properties of the nanomaterials also govern their distribution and clearance from the body. The high surface-to-volume ratio of nanoparticles can potentiate reactivity of the material. Materials such as gold are inert in their bulk form, but can exhibit toxic effects at the nanoscale level. Although some nanoparticles, such as the CeO_2 mentioned above, act as ROS scavengers, other nano-sized metal oxides can generate ROS [66]. *In vivo* studies of GI nanotoxicity are still scarce. The high doses of particles used for *in vitro* studies do not necessarily reflect the *in vivo* local particle concentrations in the GIT. As a result, studies report contradictory results regarding the harmful effects of inorganic nanoparticles intended for oral administration.

Toxicity is strongly dependent on the nanoparticle size. For example, 10-nm SiO_2 nanoparticles, but not 30-nm ones, administered orally exacerbated intestinal inflammation in a colitis mouse model [67]. For Au nanoparticles (10, 30, and 60 nm), *in vivo* biodistribution and excretion was also size-dependent [68]. The smallest nanoparticles were most harmful, probably because of their greater penetration into the cell nucleus and consequently more DNA damage. Comparable results were obtained by Yao et al., where small Au nanoparticles accumulated more than the larger ones (15 nm > 50 nm > 100 nm) in Caco-2 cells [42].

The cytotoxicity of iron oxide nanoparticles is commonly considered minor or associated with the highest dose and/or longest exposure time for a given experiment [69]. Voss et al. investigated the correlation between cellular effects and physicochemical properties of iron oxide and reported no alterations in ROS production, apoptosis, or mitochondrial membrane potential in Caco-2 cells [43]. Similar results were reported by Moskvin et al., who tested iron oxide nanoparticles with various surface modifications on human CRC cell lines [70]. SiO_2 is generally considered a biocompatible material and therefore often used as a benign coating layer on inorganic nanoparticles such as iron oxide [71]. However, recent studies have shown that SiO_2 can exhibit *in vitro* cytotoxicity in macrophages and colon-cancer cells [72,73]. Chen et al. demonstrated that the oral ingestion of SiO_2 nanoparticles by mice induces microbial diversity in the intestine as well as significantly increased levels of pro-inflammatory cytokines, however, no overall toxicity was reported [74].

Finally, orally administered metal-based nanoparticles such as Au [75], Ag, or TiO_2 [76] can have a negative impact on gut microbiota. For the latter two, the microbial dysbiosis is attributed to the antimicrobial activity of these materials [71,75–82]. This has to be taken into account when treating GI diseases such as IBD that already present impaired gut microbiota. It has been reported that oral administration of TiO_2 to mice with acute colitis can worsen inflammation [81,83••,84]. However, other studies contradict any adverse effects of titanium- and silver based nanoparticles *in vivo* [85,86]. The experimental and analytical methods as well as different dose regimens in the various studies might explain these conflicting findings. The assessment of nanotoxicology is complex and the underlying cellular mechanisms are not yet fully understood.

Conclusion

Inorganic nanoparticles have a promising future as oral drug-delivery systems, especially because of their promise as theranostics. An emerging field of research in oral nanomedicine focuses on the use of theranostic nanoparticles for GI diseases. Here, the oral administration of nanoparticles can replace systemically administered medicines by exerting an optimized, local effect in the GIT. Inorganic nanoparticles can destroy tumorous tissue by thermal treatment (Au, iron oxides), scavenge inflammatory ROS (CeO_2), and simultaneously enable imaging or precise localization of the delivery systems *in vivo*. Such concepts could also be expanded to include other GI disorders such as celiac disease.

Recent advances in nanomaterial engineering enable close control of physicochemical particle properties such as size, composition, shape, and surface chemistry to optimize optical and thermal properties and enhance targeting or biocompatibility. This is essential to overcome the physiological barriers encountered by orally administered inorganic nanoparticles such as complex transport phenomena in GI fluids, the mucus layer, and crossing the intestinal epithelial cells. The impact of physicochemical particle properties on these processes is fairly well-understood, however, there is still a need to develop rigorous *in vitro* systems that more closely mimic the dynamic GI transit of inorganic nanoparticles. GI physiology — such as pH variations, flow, and

enzyme corona formation — affects, among other things, nanoparticle dissolution and aggregation. The GI physiology ultimately governs the interaction of the nanoparticles with the cell and thus the biological fate. Physiological differences can be further pronounced in patients suffering from GI diseases, impacting the safe and effective oral inorganic nanoparticle delivery in these patient groups. The complex interplay of inorganic nanoparticles with the GI environment, including the gut microbiota, is also evident by contradicting reports on GI nanotoxicity.

Future studies should consider the use of *in vitro* dose exposures that more closely reflect the local concentrations *in vivo*, as well as the dynamic physicochemical nanoparticle properties in the GIT. For example, it is imperative to test nanoparticles in biosimilar intestinal fluids and to take enzyme corona formation into account in toxicological assays. Such systematic investigations will enhance our understanding of the complex and dynamic behavior of inorganic nanoparticles in the GIT and pave their way for patient-compliant and safe oral use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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