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REVIEW

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# Titanium dioxide particles from the diet: involvement in the genesis of inflammatory bowel diseases and colorectal cancer

Frédéric Barreau<sup>1,2\*</sup>, Céline Tisseyre<sup>3</sup>, Sandrine Ménard<sup>1,2</sup>, Audrey Ferrand<sup>1,2</sup> and Marie Carriere<sup>3\*</sup>

## Abstract

The gastrointestinal tract is a complex interface between the external environment and the immune system. Its ability to control uptake across the mucosa and to protect the body from damage of harmful substances from the lumen is defined as the intestinal barrier function (IBF). The IBF involves four elements: the intestinal microbiota, the mucus layer, the epithelium and the immune system. Its dysfunction is linked with human diseases including inflammatory, metabolic, infectious, autoimmune and neurologic disorders. Most of these diseases are complex and involve genetic, psychological and environmental factors. Over the past 10 years, many genetic polymorphisms predisposing to inflammatory bowel disease (IBD) have been identified. Yet, it is now clear that they are insufficient to explain the onset of these chronic diseases. Although it has been evidenced that some environmental factors such as cigarette smoking or carbohydrate intake are associated with IBD, other environmental factors also present potential health risks such as ingestion of food additives introduced in the human diet, including those composed of mineral particles, by altering the four elements of the intestinal barrier function. The aim of this review is to provide a critical opinion on the potential of TiO<sub>2</sub> particles, especially when used as a food additive, to alter the four elements of the intestinal barrier function, and consequently to evaluate if this additive would likely play a role in the development and/or exacerbation of IBD.

**Keywords:** TiO<sub>2</sub>, Food additive, Toxicity, Gastrointestinal tract, Ingestion, Intestine, Inflammation, Cancer

## Background

The gastrointestinal tract is a complex interface between the external environment, internal tissues and the immune system, establishing a dynamic barrier that enables the absorption of dietary nutrients and the exclusion of potentially hazardous substances, including pathogenic bacteria and toxic chemical agents, from the intestinal lumen. The ability to control their uptake across the mucosa and to protect from these harmful substances present within the lumen is defined as the intestinal

barrier function (IBF). It includes several lines of defence, the first one being the lumen itself, where commensal bacteria contribute to the protection against pathogens (i.e. harmful biological agents) by producing anti-microbial substances and by competing with them for access to nutrients, thereby limiting their colonisation [1]. The second line of defence is the mucus layer, which first constitutes a tight physical protection of the epithelium against all harmful substances that may transit in the gut lumen, and second is rich in secreted IgA and antimicrobial peptides (AMP), thereby preventing the access of bacteria to the epithelium [2]. The third barrier is the monolayer of intestinal epithelial cells that further refrains toxic molecules and enteric pathogens from reaching internal tissues [3]. This constitutes the

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last line of defence before the mucosal immune system, which constitutes the ultimate barrier against pathogens [1]. Many human diseases including inflammatory, metabolic, infectious, neurologic, and cardiovascular disorders are linked to deficiency of the IBF [4].

Crohn's disease (CD) and ulcerative colitis (UC), the two main subtypes of inflammatory bowel diseases (IBD), are chronic relapsing inflammatory disorders of the gastrointestinal tract. CD can be distinguished from UC in that the inflammation associated with CD is transmural and often discontinuous [5]. IBD may be complicated by occlusions and perforations of the intestine. Patients suffering from IBD have a high risk of developing colitis-associated colorectal cancer (CAC) and have high mortality from these diseases [6, 7]. Indeed, chronic inflammation in the digestive tract results in particular pathological fibrosis that will affect the crypts. More importantly, in the majority of patients who did not show any signs of IBD pathogenesis prior to CRC onset, tumor-associated inflammation is evident in clinical samples and has been shown to drive cancer development in the gut, suggesting a fundamental role for inflammation in both CAC and sporadic colorectal cancer (CRC) development [8]. Current estimations indicate that 6.8 million people live with IBD worldwide [9]. The highest prevalence of IBD is in Europe and North America, but these countries show stabilized or decreased incidence of these diseases while their incidence is increasing in Africa, Asia, Eastern Europe and South America [10]. IBD is currently thought to be linked to environmental factors associated with the occidental way of life, which are currently unknown. Although cigarette smoking [11] or carbohydrate intake [12] are associated with CD, other environmental factors such as ingestion of food additives may present potential health risks by altering the IBF and then favouring IBD [13, 14]. Among them, the food additive E171, composed of titanium dioxide particles, has been shown to contain up to 55% of nanosized particles (TiO<sub>2</sub>-NPs) and is among the most commonly used mineral particle-based food additive in consumer products [15–18]. As reported in the present review, TiO<sub>2</sub> has already been described to alter the IBF and to translocate in the internal body (for instance, see [19] where the absorption of TiO<sub>2</sub> is demonstrated and [20, 21] where TiO<sub>2</sub> is observed in the liver of rats exposed via oral administration). For this reason, this food additive is a good candidate as an environmental factor that could be implicated in IBD and CAC.

The aim of this review is to compile the published data that show how TiO<sub>2</sub> food additive may impact the four elements of the IBF, in order to shed light and to provide a critical opinion on the mechanisms by which it could be implicated in IBD and CAC. It is not intended to evaluate the risk associated with the

ingestion of this additive, which has been already documented [22]. This review is based on a literature survey up to January 2021, using the databases Pubmed, IsiWeb and Scopus, which were searched with the keywords TiO<sub>2</sub>, titanium dioxide, E171, intestine, gut, inflammation, immunity, inflammatory bowel disease, mucus, microbiota, toxicity. The included publications were chosen thanks to quality criteria including minimal description of the origin and physico-chemical characteristics of the used TiO<sub>2</sub> particles, good laboratory practices and correct description of the employed methods, particularly in the *in vivo* studies, conclusions matching the presented results and presence of appropriate controls.

## Description of the four compartments of the intestinal barrier function

### Commensal bacteria

The human gut contains around 10<sup>14</sup> bacteria and constitutes the largest microbial community associated with human body [23]. The gut microbiota acts as a metabolic organ, through breakdown of indigestible dietary carbohydrates and proteins, with generation of fermentation end-products, vitamins, and ion absorption [24, 25]. It contributes to the barrier effect by constituting an obstacle to pathogen invasion. The microbiota also enhances the resistance to pathogens by both direct and indirect mechanisms of action including production of antimicrobial substances such as bacteriocins, pH modification and competition for nutrients, immune or epithelial cell-mediated mechanisms [1]. For example, *B. thetaiotaomicron* consumes carbohydrates used by *C. rodentium*, which contributes to the competitive exclusion of the pathogen. *B. thetaiotaomicron* also enhances the production by epithelial cells of the peptidoglycan-binding C-type lectin regenerating islet-derived protein IIIγ (REGIIIγ), which is an antimicrobial peptide that kills Gram<sup>+</sup> bacteria [26].

### The mucus layer

The mucus layer overlying the epithelium promotes the elimination of gut content, providing the second line of defence against injury caused by ingested food, particles and microorganisms [27]. This layer is present all along the GIT, from the stomach to the rectum, and is thicker in the stomach and colon than in the small intestine [2]. It is classically divided into a dense sterile inner layer, which is firmly attached to the epithelium, and a removable outer layer that is colonized by anaerobic commensal bacteria [2]. In rodents, the mucus layer in the small intestine is not attached to the epithelium and only shows one layer [28]. The major components of the mucus layer are glycoproteins termed mucins, which are secreted by goblet cells and form a gel at the surface of epithelial cells [29]. In addition to mucins, goblet cells

also synthesize trefoil factors, resistin-like molecule  $\beta$ , and Fc- $\gamma$  binding protein [30]. Paneth cells also contribute to this barrier function by secreting substances contained in secretory granules, including antimicrobial substances such as lysozyme, phospholipase A2,  $\alpha$ -defensins and the REGIII $\gamma$  [31]. Finally enterocytes produce  $\beta$ -defensins and REGIII $\alpha$  [32, 33]. All these mucins and AMP compose the mucus wall and act synergistically to maintain the homeostasis of the mucus layer [29, 34].

### The epithelial monolayer

The epithelial monolayer is a semi-permeable barrier that allows the passage of water and nutrients from the gut lumen to the underlying tissues. Passage occurs via the paracellular or the transcellular route. The paracellular route, through tight junctions (TJs) that link adjacent cells, makes possible the strictly controlled passage of water, solutes and ions and is determined by the size of TJ pores with a maximum opening reaching 120 Å, i.e., 12 nm [35]. Four transmembrane families of proteins contribute to TJ formation, which are occludin, claudin, junctional adhesion molecule and tricellulin [35]. The intracellular domains of these transmembrane proteins interact with cytosolic scaffolding proteins, i.e. zonula occludens proteins, which in turn anchor the transmembrane proteins to the perijunctional actomyosin meshwork [35]. The circumferential contraction of the actomyosin ring is regulated by the myosin light chain kinase [35, 36]. Other mechanisms like endocytosis of TJ proteins, regulation of TJ genes expression and epithelial cell apoptosis regulate the paracellular route. If TJs are not disrupted, they strictly regulate the passage of molecules and therefore the passage of nanoparticles with size > 120 Å, i.e., 12 nm, would be hindered.

A vast literature shows that particles larger than the TJ pore size would rather cross the gut epithelial layer by using the transcellular route, i.e. by passing through gut cells by transcytosis (for instance, see [36]). Although epithelial and dendritic cells are able to capture antigens and microbes from the gut lumen, full transcellular transport is mainly ascribed to microfold (M)-cells, which are part of the follicle associated epithelium (FAE) overlying isolated lymphoid follicles (ILF) or Peyer's patches (PP) [37]. The transcellular route across M-cells and other cells exhibits two classical pathways depending on the size of the substance. Proteins and bacterial products can be taken up by endocytosis [38]. Larger entities, including bacteria, are captured via macropinocytosis or phagocytosis [38]. After crossing the cell, these particles are released on the other side of M-cells at the vicinity of immune cells that populate PPs. These two passage routes across the epithelium are closely controlled so that the intestinal homeostasis is maintained.

In case of epithelial barrier disruption, enhanced passage of substances, including particles and microorganisms could lead to chronic inflammation [35].

### The intestinal immune system

This section gives a brief overview of the intestinal immune system, which is highly complex. Some excellent reviews describe its components and functioning, the authors suggest the reader to refer to the reviews cited in the text for more details. Two main sites of immune response have been described, i.e., the inductive site (immune response initiation – antigen encountering) and the effector site. Gut-associated lymphoid tissue (GALT), including PPs and ILFs, represent the inductive compartment for B and T cells. After encountering antigens in the GALT, B and T cells leave the effector site, transit through the lymph, enter the circulation and join the effector site of the intestine, that is, the mucosal epithelia and the lamina propria. Intestinal lymphocytes are continuously exposed to food and microbial antigens via antigen presenting cells (APC) including dendritic cells (DCs). DCs present in GALT and lamina propria have a high tolerogenic potential and a break of this tolerance triggers inflammation leading to IBD or cancer (for review, see [39–41]). These lymphocytes help to maintain the integrity of the intestinal barrier and immune homeostasis. Owing to their proximity to luminal antigens, they have a dual function: regulatory function (i.e., maintaining tolerance toward food antigens and commensal microbiota) and effector function (i.e., prevention of pathogenic invasion) (for review, see [42]). Innate lymphoid cells (ILCs) are also present in the lamina propria, those lymphocytes do not express the type of diversified antigen receptors expressed on T cells and B cells. ILCs are tissue-resident cells participating in tissue homeostasis (for review, see [43]). Intraepithelial lymphocytes (IELs) are also an important contributor to intestinal homeostasis. They reside within the intestinal epithelial layer and are involved in intestinal repair and defence against infections [44]. In IBD, alterations of these four elements constituting the IBF have been described.

### Absorption, distribution and elimination of TiO<sub>2</sub> particles upon ingestion and associated toxicity

Upon request of the European Food Safety Authority (EFSA), a group of experts reviewed the nanomaterials that are currently used in agriculture, feed and food [45]. The food industry uses nanomaterials either in packaging or for nano-encapsulation of bioactive compounds such as vitamins. In addition, synthetic amorphous silica, titanium dioxide, silver and iron oxide are used as food additives for their anti-caking property or as food colorant [45]. Titanium dioxide food additive (E171) is a

powder composed of particles with an average primary grain size higher than 100 nm. It also contains a significant amount of nanosized particles, which may arise from the production process. Depending on the analysed powder, this nanosized fraction is reported to be up to 36% [17], 35% [18], 55% [15] or 10–15% [16]. Thus, E171 is not a nanomaterial with respect to the current European recommendation for a definition of nanomaterials [46]. Its use in food products was authorized in 1969 by the JECFA (Joint FAE/WHO Expert Committee on Food Additives) with an acceptable daily intake “not specified” (report: NMRS 46/TRS 445-JECFA 13/12). This authorization relies on a literature survey that proved that E171 was non-toxic and not absorbed in the gastro-intestinal tract (GIT). In March 2021, the EFSA panel on Food Additives and Flavourings re-evaluated the safety of E171 when used as food additive, based on recent literature including a study dedicated to the analysis of E171 reproductive and developmental toxicity. The Panel concluded on potential immunotoxicity and inflammation with E171, as well as potential neurotoxicity and potential induction of aberrant crypt foci in the intestine. Moreover, a concern for genotoxicity could not be ruled out. Consequently, the Panel concluded that “E171 can no longer be considered as safe when used as a food additive” [47].

Since it is used as a white food colorant, the largest quantity of  $\text{TiO}_2$  is found in candies, sweets and pastries, with 0.01–1 mg  $\text{TiO}_2$  per unit [17]. In sugar-coated chewing gums, the nanoparticulate fraction of  $\text{TiO}_2$  reaches 27.7–43.7 and 95% of  $\text{TiO}_2$ -NPs are swallowed upon chewing [48]. Based on consumer intake data, human exposure has been estimated at 1–2 mg and 0.2–0.7 mg  $\text{TiO}_2$ /kg/day for US children under 10 and other US consumers, respectively [17]. More recent estimation concludes that daily intake of  $\text{TiO}_2$  for Dutch population is rather 0.66–0.70 mg/kg/day for children under 7, 0.16–0.18 mg/kg/day for consumers between 7 and 70, and 0.05–0.07 for adults over 70, with lifelong intake of  $\text{TiO}_2$  estimated at 0.19 mg/kg/day [49]. Lastly, the EFSA panel on food additives and nutrient sources estimates that daily intake of  $\text{TiO}_2$  reaches 0.4 mg/kg bw per day for infants and the elderly and 10.4 mg/kg bw per day for children, for the maximum level exposure assessment scenario, at the mean, which is the highest estimated values reported in their publication [50]. Based on the latter estimated values, and taking into consideration the existing guidance for conversion of doses between animals and humans such as the Nair and Jacob practice guide or the FDA’s guidelines, it can be considered that a realistic *in vivo* exposure concentration would be up to 50–60 mg/kg b.w./day [51]. *In vitro*, exposing 2D-cells to 25  $\mu\text{g}/\text{cm}^2$  (of cell culture surface) would represent ~10,000-fold the daily ingested dose by a human adult

[52], therefore a realistic exposure concentration would be as low as 2.5  $\text{ng}/\text{cm}^2$ , i.e., ~5  $\text{ng}/\text{mL}$  in classical 2D cell culture conditions. In such *in vitro* mechanistic studies, unrealistically high concentrations are often used in order to determine the effect and no-effect level of NMs [53]. Although it is recommended to use realistic concentrations and long-term exposure *in vitro*, such high concentrations can be considered as worst case scenarios and are acceptable as long as they are interpreted with caution and as they do not exceed the concentration at which NMs agglomerate or interfere with the readout of the test, i.e., generally 1–100  $\mu\text{g}/\text{mL}$  [53].

Due to continuous exposure via ingestion,  $\text{TiO}_2$  might be distributed in intestinal tissues and in internal organs if absorbed through the gastrointestinal tract. Indeed, early reports describe the presence of so-called “pigmented cells” in human PPs. These cells contain some dark granules, which were analysed as being composed of Ti, Si and Al [54]. The authors propose that these pigments derive from the ingestion of synthetic food additive (Ti from  $\text{TiO}_2$ ), aluminosilicates (Al, Si) and mixed environmental silicates (Si). In addition to this local accumulation in immune cells, a recent study on human volunteers shows significant intestinal absorption of Ti after exposure via ingestion of two capsules containing 100 mg of food-grade  $\text{TiO}_2$  (which would represent 1–1.5 mg/kg b.w.) [19]. Ti is detected in the bloodstream of the volunteers as soon as 2 h after ingestion, and reflectance microscopy shows that Ti is in the physical form of a particle, not as ions that would have been released from the particles [19]. Moreover, the kinetics of appearance of Ti in the bloodstream suggests two routes of intestinal absorption, first in the proximal part of the small intestine, then in the distal part of the ileum, possibly through PPs [19]. In contrast, Jones et al. report insignificant absorption of  $\text{TiO}_2$  in human volunteers orally exposed to a single dose of 5 mg/kg b.w. of  $\text{TiO}_2$  micro and nanoparticles (15 nm, 100 nm and < 5  $\mu\text{m}$ ) dispersed in water [55]. However, in the Jones study the baseline level of Ti in the bloodstream is high, which impairs the detection of small amounts of Ti that would have been absorbed [55]. Finally, in 2017, Ruiz et al. show from a cohort study that the titanium level in the blood of human patients having active UC disease is higher than that of healthy donors [56].

Several *in vivo* studies in rodents report  $\text{TiO}_2$  absorption and toxicity when administered per os, although other studies report the opposite. When focusing on studies performed with E171 or  $\text{TiO}_2$  particles with physico-chemical properties that are close to those of E171, the literature is scarce. In 2015, three studies published in the same article clearly demonstrate absence of toxicity of  $\text{TiO}_2$  with physico-chemical characteristics close to those of food-grade  $\text{TiO}_2$ . These three studies

are performed according to the Organisation for Economic Co-operation and Development (OECD) guidelines for i) 90 day subchronic toxicity, ii) 28 days repeated dose toxicity and iii) acute oral toxicity [57]. In addition, negligible absorption, lack of distribution and an overall excretion in the feces is observed in rats exposed to E171 incorporated in food pellets at 30 mg/kg b.w./day for 7 days in a study conducted following the OECD TG417 [58]. Oral gavage of E171 at 10, 100 or 1000 mg/kg b.w./day for 90 days (OECD TG408) leads to accumulation of Ti only in the colon [59]. Conversely, some non-OECD guideline studies show the absorption and distribution of E171 in the liver of rats exposed for 7 days to E171 at 10 mg/kg b.w./day by intragastric gavage [20] and in mouse exposed by dripping some E171 into the mouth of the mouse at 5 mg/kg b.w./day, 3 days per week for 3 weeks [21].

In addition to this literature, some studies performed with TiO<sub>2</sub> particles with properties differing from those of E171 report either no or significant absorption of TiO<sub>2</sub> and in some of them some toxicity. Absorption of TiO<sub>2</sub>-NPs is reported for 25 or 80 nm NPs when administered to mice at a single dose of 5 g/kg b.w., which is an unrealistically high dose, with signs of toxicity in the liver, kidneys and brain as well as altered serum biochemical parameters [60]. The result of this study should be interpreted with caution due to the excessively high dose administered to the animals. Similarly, oral exposure of mice with 16 nm rutile or 20 nm anatase TiO<sub>2</sub>-NPs at the dose of 100 mg/kg/day for 28 days, which can also be considered as a high dose as compared to estimated human exposure, causes Ti distribution in the spleen, lung, and kidney without causing any significant effects on organ histology [61]. Jani et al. report in 1994 that 500 nm TiO<sub>2</sub>-NPs are absorbed in rats and accumulate in GALT, lungs and peritoneal tissues after oral administration at 12.5 mg TiO<sub>2</sub>/kg b.w. per day for 10 days. No Ti is detected in the liver and spleen [62]. More recently, a systematic study performed with five benchmark TiO<sub>2</sub> particles from the JRC repository, differing in their physico-chemical characteristics, shows that none of these particles are significantly absorbed by rats after 5 consecutive days of oral exposure at the realistic concentration of 2.3 mg/animal per day (i.e., 7–12 mg/kg b.w. depending on the weight of the animal). No distribution to the liver, spleen and mesenteric lymph nodes is observed, except in a small number of animals [63]. This study also shows that when exposed by intravenous injection, TiO<sub>2</sub> distributes mainly to the liver, spleen and lung. No TiO<sub>2</sub> is detected in the urine and feces during the 90 days post-exposure, proving that its elimination is negligible [63]. This suggests that even if TiO<sub>2</sub> absorption through the GI tract is mild, lifelong cumulative absorption combined to low elimination would result in

potential accumulation of TiO<sub>2</sub> in the tissues. Noteworthy, data from this study have been considered as the most accurate toxicokinetics data, and have been used for risk assessment of TiO<sub>2</sub> oral exposure [22]. In contrast, while no TiO<sub>2</sub>-NP is detected in the liver, kidneys, spleen and brain of rats exposed by repeated gavage to 520, 1041 or 2083 mg/kg b.w./day for 13 weeks (OECD TG409) of 26 nm-diameter TiO<sub>2</sub>-NPs, TiO<sub>2</sub> is significantly eliminated in the faeces [64]. The doses administered to rats in the latter study are unrealistic and higher than the dose in the study by Geraets et al. [63], which can explain the stronger elimination. Regarding TiO<sub>2</sub> toxicity, while no significant distribution of TiO<sub>2</sub> to the blood, liver, kidney and spleen is reported by Wang et al. in rats exposed to 10–200 mg/kg b.w. of 75 nm TiO<sub>2</sub>-NPs for 30 days, significant damage is observed both in young and adult animals [65]. In young rats, this repeated oral administration induces liver oedema and heart damage as well as cell activation in the stomach, but only at doses > 50 mg/kg b.w., which are higher than the estimated human exposure [65]. In adult rats, it causes damage to the liver, kidneys and compromises intestinal permeability, also at the highest doses, showing age-dependent impact of TiO<sub>2</sub> [65]. Conversely, no systemic toxicity of P25 TiO<sub>2</sub>-NPs (21 nm in diameter, mix anatase/rutile) is observed in rats exposed for 28 or 90 days (OECD TG407 and 408) at 250, 500 or 1000 mg/kg b.w./day by gavage [66]. Finally, Ruiz et al. show dose-dependent distribution of TiO<sub>2</sub> in the spleen of mice having dextran sodium sulfate (DSS)-induced colitis, when orally exposed to 30–50 nm rutile TiO<sub>2</sub>-NPs repeatedly for 7 days, at 50 or 500 mg/kg b.w./day [56]. This shows that the colitis caused by DSS facilitates absorption of TiO<sub>2</sub> nanoparticles, which was expected as colitis perturbs intestinal permeability. Finally, a series of articles from the Fashui Hong team reports impact of TiO<sub>2</sub> nanoparticles on diverse organs after oral administration, but these articles are not considered in this review because of flaws in the presentation and statistical analysis of the data, as has already been pointed out on a series of articles from the same team related to the pulmonary impact of similar TiO<sub>2</sub> nanoparticles [67]. In these articles the reported standard errors are exactly 5% of the reported mean, rather than the actual standard errors.

Overall, it can be considered that TiO<sub>2</sub> absorption is mild and its elimination in the faeces is slow, in realistic exposure conditions. Taking these conclusions into consideration, risk assessment for oral exposure to TiO<sub>2</sub> concludes that a potential risk exists for liver, ovaries and testes [22]. Such an evaluation, including TiO<sub>2</sub>-NPs kinetics, indicates a possible effect on liver, spleen, ovaries and testes [22].

### Impact of TiO<sub>2</sub> on the intestinal barrier function

Early reports suggest that ingestion of food additives composed of mineral particles such as TiO<sub>2</sub> may participate in the aetiology and pathogenesis of IBD [13]. Current studies suggest that TiO<sub>2</sub> particles alter all four elements of the IBF. Therefore, they may promote the development and/or the aggravation of IBD and the associated CRC.

### Alteration of the intestinal microbiota by TiO<sub>2</sub> particles

The antibacterial properties of metals such as silver and zinc are known for centuries and this has been exploited in modern medicine for infection control. For example, silver nitrate was one of the first substances suggested for the management of dental cavities [68]. In addition, metal-containing nanomaterials arise in several chemical forms, including solid nanoparticles of metal or metal oxides like Ag or TiO<sub>2</sub>-NPs as well as composite materials with layers of distinct metals. Some of these nanoparticles are used as antibacterial agents [69] and there is now interest to use them for controlling bacterial infections [70]. The precise mechanisms for bacterial toxicity of these NPs is still being elucidated, but the current paradigms include free metal ion toxicity arising from the dissolution of metals from the surface of the NPs (e.g., Ag<sup>+</sup> from Ag NPs) [71] or oxidative stress via the generation of reactive oxygen species (ROS) on the surface of some NPs.

It is unlikely that TiO<sub>2</sub> nanoparticles, which are considered to be poorly soluble [72], can release sufficient quantities of Ti ion to confer them antibacterial properties. TiO<sub>2</sub> is known as an antimicrobial agent because of its photocatalytic properties, which lead to the release of ROS when TiO<sub>2</sub> is irradiated with UV light [73]. This property is currently used in many applications such as water treatment [74], but cannot explain the potential dysbiosis caused by TiO<sub>2</sub> since no UV light reaches the GIT. Still, it is also demonstrated that TiO<sub>2</sub> can generate some free radicals in the dark [75]. Such free radicals can then synergistically act by attacking polyunsaturated phospholipids in bacteria and damage their DNA [76, 77], which eventually may cause bacterial cell death. In the context of the GIT, such damage to bacteria could alter the intestinal microbiota.

Generally, the impact of TiO<sub>2</sub> on the microbiota is reported to be mild (Table 1). Considering the E171 food additive, Radziwill-Bienkowska et al. show mild impact of E171 on a panel of Gram<sup>+</sup>/Gram<sup>-</sup> bacterial strains chosen to be representative of gut microbiota [78]. E171 is internalized in 7% of *E. coli* cells, with minor consequences on bacterial viability [78]. According to Dufroi et al., E171 does not affect bacterial gas production and only mildly impact bacterial fatty acid profiles in a defined human gut bacterial community composed of 33

different strains (microbial ecosystem therapeutic 1, MET-1) [79]. DNA profiles and phylogenetic distributions confirm limited impact on the bacterial community, with a modest decrease of *Bacteroides ovatus* in favour to *Clostridium cocleatum* [79]. In a human colon model reactor, after 5 days of exposure at 36 mg/L (corresponding to oral exposure to ~0.3–0.7 mg/kg), both E171 and 21 nm TiO<sub>2</sub>-NPs hinder the natural shift in microbial composition that is observed in the control condition, favouring Firmicutes phylum over Proteobacteria [80]. The authors' hypothesis is that this natural shift is probably due to the shift from a vegetarian diet (host) to a diet with high levels of animal proteins and fats (colon medium) [80]. After exposure to 21 nm TiO<sub>2</sub>-NPs, the amount of Firmicutes and Bacteroidetes increases, with Firmicutes being approximately as abundant as Proteobacteria [80]. Particles with diameter 122 nm, supposed to represent food grade TiO<sub>2</sub> particles, trigger a slight decrease of Proteobacteria as well as a minor increase of Firmicutes and Bacteroidetes, but overall do not affect the initial ratio of these phyla. TiO<sub>2</sub>-NPs also decrease colonic pH, with 122 nm particles having a higher impact than 21 nm NPs [80]. The authors hypothesize that it could be due to a direct interaction between TiO<sub>2</sub>-NPs and bacteria, and/or binding of microbial nutrients at the particle surface [80]. More recently, a comprehensive study reports the impact of E171 or TiO<sub>2</sub>-NPs (33 nm in diameter) on the microbiota and the colon inflammatory status in non-obese and obese mice [81]. Exposure for 8 weeks to 0.1 wt% TiO<sub>2</sub> incorporated into the feed leads to microbiota dysbiosis, characterized by increased abundance of Firmicutes and decreased abundance of Bacteroidetes phyla, i.e. the same trend as observed in IBD patients, together with reduced abundance of *Bifidobacterium* and *Lactobacillus* genera [81]. This dysbiosis concurs with decreased cecal levels of short-chain fatty acids (SCFAs), which has also been reported to be associated with IBD. Histological observation shows typical signs of inflammation in the colon, both in directly exposed animals and in control animals that have received fecal transplant from TiO<sub>2</sub>-exposed mice, suggesting direct impact of the microbiota on the intestinal inflammatory status [81]. Overall, these effects are more intense in obese mice compared to non-obese mice, and E171 shows mild impact while TiO<sub>2</sub>-NPs exert the strongest effect [81].

Other studies also report mild impact on the microbiota of TiO<sub>2</sub> nanoparticles with physico-chemical properties that differ from E171. Chen et al. find no disturbance of gut microbiota in mice exposed by oral gavage to 19 nm TiO<sub>2</sub>-NPs, at 2.5 mg/kg/day for 7 days [82]. In faeces of both control and TiO<sub>2</sub>-NP exposed mice, the Bacteroidetes phylum is preponderant,

**Table 1** Impact of TiO<sub>2</sub> particles on the intestinal microbiota<sup>a</sup>

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
in vitro					
Waller et al., 2016 [80]	Model human colon reactor: bacteria isolated from a fecal sample from a human donor, grown in aerobic condition	5 days, three times a days. TiO <sub>2</sub> diluted in a fluid simulating digested food entering the large intestine. Exposure under flow, in the dark	P25 (Evonik, 21 nm, anatase/rutile) and commercially available TiO <sub>2</sub> , similar to food grade TiO <sub>2</sub> (122 nm)	Sonication for 30 min in water	Decrease of bacteria number. Inhibition of the bacterial shift observed in control condition (Proteobacteria to Firmicutes phyla). Decreased colonic pH. Mild effect on microbial stability (microbial community hydrophobicity, electrophoretic mobility). Food-grade TiO <sub>2</sub> particles show greater effect.
Duderoi et al., 2017 [79]	MET-1 bacterial community (33 bacterial strains), grown in anaerobic condition	100–250 ppm. Incubation in the dark under agitation.	Two different E171 and NM105 TiO <sub>2</sub> -NP (21 nm, anatase/rutile)	Suspension in distilled water, no sonication	No impact on gas production by bacteria. Minor effect on fatty acids profiles. Limited effect on bacterial communities: decrease of <i>Bacteroides ovatus</i> , increase of <i>Clostridium cocleatum</i> .
Radziwill-Bienkowska et al., 2018 [78]	Eight Gram-positive/ Gram-negative bacterial strains	32–320 µg/mL, 15 min to 24 h, in the dark, under agitation or not, depending on the strain.	E171 and NM105 TiO <sub>2</sub> -NP (22 nm, anatase/rutile)	Probe sonication in 0.05% water/BSA, 27 min	Adsorption of TiO <sub>2</sub> on the surface of bacteria, with accumulation in 7% of <i>E. coli</i> , as measured by nano-SIMS. Alteration of growth profiles and moderate reduction of cell cultivability. Morphological damage on a small number of bacteria.
in vivo					
Chen et al., 2017 [82]	Mice, oral gavage	2,5 mg/kg b.w./day, once a day for 7 days	TiO <sub>2</sub> -NPs, anatase, 17 nm (TEM)	Suspension in water, bath sonication for 15 min	No change of gut microbiota composition.
Li et al., 2018 [61]	Mice, oral gavage	100 mg/kg b.w./day, once a day for 28 days	TiO <sub>2</sub> -NPs, anatase (20 nm, DLS) and rutile (16 nm, DLS)	Suspension in distilled water, no sonication	No decrease of gut microbiota diversity, but its structure is shifted: Proteobacteria increased by rutile TiO <sub>2</sub> , <i>Prevotella</i> decreased by anatase and rutile TiO <sub>2</sub> , <i>Rhodococcus</i> increased by rutile TiO <sub>2</sub> , <i>Bacteroides</i> increased by anatase TiO <sub>2</sub> .
Pinget et al., 2019 [133]	Mice, drinking water	2, 10, 50 mg TiO <sub>2</sub> /kg b.w./day for 21 days	E171	Suspension in drinking water	Limited impact on bacterial diversity, richness, evenness or Faith's diversity in fecal samples in the colon. Impact at the genus level in the colon, depending on the dose. No impact in the small intestine. Significant modulation of commensal bacterial activity at 50 mg/kg b.w./day. Increased biofilm formation of <i>E. coli</i> , <i>E. faecalis</i> and commensal bacteria exposed to 2, 10 and 50 µg/mL for 24 h or 72 h, in vitro.
Cao et al., 2020 [81]	Mice, exposure in food pellets	0.1 wt% in food pellets for 8 weeks	E171 or TiO <sub>2</sub> -NPs, anatase, 33 nm (TEM)	Mixed with food, either low-fat or high-fat diet	Microbiota dysbiosis: increased abundance of Firmicutes and decreased abundance of Bacteroidetes; decreased abundance of <i>Bifidobacterium</i> and <i>Lactobacillus</i> genera, concurrent with decreased level of short-chain fatty acids. Associated with colon inflammation. More intense in obese mice and in mice treated with TiO <sub>2</sub> -NPs



**Table 1** Impact of TiO<sub>2</sub> particles on the intestinal microbiota<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
					compared to E171. Microbiota transplant experiment suggests that inflammation is directly linked with dysbiosis.

<sup>a</sup>Abbreviations: BSA bovine serum albumin, DLS dynamic light scattering, SIMS secondary ion mass spectroscopy, TEM transmission electron microscopy

followed by Firmicutes then Proteobacteria and exposure to TiO<sub>2</sub>-NPs does not affect their ratio [82]. Moreover, anatase or rutile TiO<sub>2</sub>-NPs, orally administrated to mice for 28 days at 100 mg/kg b.w./day, do not alter gut microbiota diversity but modifies its composition [61]. Rutile NPs have a more pronounced impact than anatase NPs [61]. The most altered phylum is Proteobacteria, which is significantly increased by rutile but not by anatase. At the genus level, the abundance of *Prevotella* is decreased by both TiO<sub>2</sub>-NPs, while the abundance of *Rhodococcus* is enriched by rutile NPs, and the abundance of *Bacteroides* is increased by anatase NPs [61]. Finally, food grade TiO<sub>2</sub> minimally affects the composition of the microbiota in mice exposed in the drinking water, but it alters bacterial metabolite release and enhances biofilm formation from commensal bacteria, in vitro [83].

Overall, although some articles show that TiO<sub>2</sub> may alter gut microbiota, other articles report the opposite. Therefore, this impact of TiO<sub>2</sub> on gut microbiota is still a matter of debate. In vivo, human microbiome has been characterised, and its alleged composition varies among authors. However, Firmicutes and Bacteroidetes phyla are always shown to be predominant with a similar abundance, with Proteobacteria and Actinobacteria being far less present but still more abundant than all remaining phyla [84–86]. Dysbiosis has been linked to some intestinal pathologies, the most severe one being *Clostridium difficile* infection, but still alteration of the microbiota concurs with intestinal disorders such as IBD, irritable bowel disease and CRC [87]. For this reason any disturbance of the intestinal microbiota by TiO<sub>2</sub> food additive could contribute to the aggravation of the symptoms of IBD and CRC in predisposed individuals.

#### Impact on the epithelial monolayer

Studies assessing the impact of TiO<sub>2</sub> on intestinal epithelial cells are summarized in Table 2. In vivo, exposure of mice to E171 at 50 mg/kg b.w./day for 4 weeks reduces the length of colonic crypts but not the total length of the colon [83]. Tight junction protein 1 (zonula occludens type 1, ZO-1) expression in the colon is not affected when mice are exposed to 10 or 50 mg/kg b.w./day of E171 for 4 weeks, which suggests no alteration of intestinal permeability [83]. Decreased crypt length is also observed in mice exposed for 16 weeks to 5 mg/kg

b.w./day of E171, provided in the drinking water when the mice are fed with either a regular fat diet or a high-fat diet [88]. Conversely, Talamini et al. do not observe any structural and histological modifications in the intestine of mice exposed by dripping ~ 2 mg/kg b.w./day of E171 in the mouth of the mice, 3 days per week for 3 weeks [21]. The mode of administration of E171 could explain such apparently discrepant results.

Although in vivo experiments are considered to be more informative than in vitro experiments on established cell lines, which are often derived from cancers, in vitro experiments might be useful to decrypt the mode of action of toxic substances such as nanomaterials. In vitro, E171 causes disruption of microvilli organization in Caco-2<sub>BBE1</sub> cells exposed to concentrations of E171 as low as 100 ng/cm<sup>2</sup> (i.e. 350 ng/mL) [89]. This disruption could be due to sedimentation of particles on the microvilli, which appears limp and fewer in number in exposed cells. When exposing Caco-2<sub>BBE1</sub> cells to E171 using an inverted configuration, in which particles cannot sediment on top of the cell layer, disruption of microvilli is also observed [89]. Therefore, exposure to TiO<sub>2</sub> affects the morphology of intestinal epithelium. Regarding the impact on intestinal epithelium of non-food-grade TiO<sub>2</sub> nanoparticles, impact on intestinal villi and intestinal permeability is also reported. Li et al. show that daily gavage of mice for 28 days (100 mg/kg/day) with 16 nm rutile NPs but not with 21 nm anatase NPs results in longer intestinal villi and irregular arrangement of villus epithelial cells [61]. The ratio of villi height/crypt depth is modified in mice exposed for 1 or 3 months to anatase NPs or rutile microparticles via their feed (1% w./w.) and the level of ZO-1 and occludin is increased in the ileum after 1 month of exposure [90]. In vivo and ex vivo, agglomerates of 12 nm anatase TiO<sub>2</sub>-NPs alter the paracellular permeability of the ileum and colon epithelia of mice after a single oral gavage at 12.5 mg/kg b.w. [52]. Gene expression of ZO-1 and -2, claudin 2 and 3 and occludin are down-regulated in the ileum, which is attributed by the authors to tight junction remodelling that may occur due to epithelial integrity alteration [52]. In the same study, in vitro exposure of a monoculture of Caco-2 cells (regular epithelium) and a co-culture of Caco-2 and HT29-MTX cells (mucus-secreting regular epithelium) to 50 µg/mL of these TiO<sub>2</sub>-NPs does not alter the

**Table 2** Impact of TiO<sub>2</sub> particles on intestinal epithelium<sup>a</sup>

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Faust et al., 2014 [89]	Caco-2 <sub>BBE1</sub> cells, differentiated	Exposure using microgravity bioreactor ("floating" epithelia) for 24 h	E171 (anatase from XRD, 122 nm from TEM, "E171") and E171 extracted from gum (141 nm, TEM, "gum E171")	Probe sonication > 2 min, then dilution in exposure medium, then probe sonication > 2 min	Disruption of the brush border independently of particle sedimentation (in a microgravity reactor, or in inverted epithelium configuration): fewer microvilli, limp, less erected, at 350 ng/mL and 3.5 µg/mL of gum E171 or E171.
Brun et al., 2014 [52]	In vitro: differentiated Caco-2 and co-culture of Caco-2/HT29-MTX; ex vivo: Ussing chamber; in vivo: mice, oral gavage	In vitro: 50 µg/mL for 6 h, 24 h or 48 h; ex vivo: 50 µg/mL for 2 h; in vivo: 12.5 mg/kg b.w., sacrifice after 6 h	anatase (12 nm, TEM)	Probe sonication 30 min in water	In vitro: no impact on TEER value, paracellular and transcellular transport properties, activity of the P-gp. Upregulation of mRNA levels of proteins involved in tight and adherens junctions. In vivo and ex vivo: increased paracellular permeability after exposure to TiO <sub>2</sub> -NPs, downregulation of mRNA expression of junction proteins (TJP1, TJP2, OCLN) in the ileum of mice exposed in vivo.
Dorier et al., 2015 [91]	Caco-2 cells, differentiated	50 µg/mL for 6 h, 24 h or 48 h	Anatase (12 nm, TEM) and rutile (20 nm, TEM)		Early and transient modulation of mRNA expression of ABC transporter genes, late modulation of mRNA expression of some SLC transporter genes. At 48 h significant increase of ABC transporter protein level (significant decrease at 4 h).
Guo et al., 2017 [92]	Co-culture of Caco-2/HT29-MTX cells	10 <sup>6</sup> , 10 <sup>8</sup> and 10 <sup>10</sup> particles/cm <sup>2</sup> (low, medium, high concentration), for 4 h (acute) or 5 days (chronic)	TiO <sub>2</sub> -NPs, 20–40 nm from TEM	Suspension in water, mixing, no sonication	Chronic exposure decreased TEER value at the three doses (not acute), enlargement of the gaps between cells, as shown via OCLN protein immunostaining. Decreased Fe, Zn and fatty acid transport transport. mRNA expression of nutrient transporters affected.
Li et al., 2018 [61]	Mice (10 mice per group), oral gavage	100 mg/kg b.w./day, once a day for 28 days	Anatase (20 nm, DLS) and rutile (16 nm, DLS)	Suspension in distilled water, no sonication	Longer intestinal villi in the colon (mild effect) and disturbed arrangement of villus epithelial cells when exposed to rutile but not anatase.
Jensen et al., 2019 [134]	Rats, oral gavage once a week during 10 weeks	50 µg/kg b.w./week (low dose) and 500 µg/kg b.w./week (high dose)	E171 (anatase, sizes: 135 nm, 305 nm and 900 nm from TEM)	Indirect cup-type sonication (high energy) in 2% FBS, 16 min	Decreased mRNA expression of TJP1 in the colon mucosa at the high dose.
Dorier et al., 2019 [95]	Differentiated Caco-2 and co-culture of Caco-2/HT29-MTX	Repeated exposure (twice a week for 21 days); 10 and 50 µg/mL	E171 (anatase, 119 nm), A12 (anatase, 12 nm), NM105 (anatase/rutile, 21 nm)	Indirect cup-type sonication (high energy) in water, 30 min	No perturbation of the in vitro epithelial barrier development over the 21 days of exposure: TEER, mRNA expression of junction proteins and markers of microvilli differentiation unaffected. Decreased (E171) or decreased (NM105) levels of ABC transporters when repeated exposure.
Pinget et al., 2019 [133]	Mice, drinking water	2, 10, 50 mg TiO <sub>2</sub> /kg b.w./day for 21 days	E171	Suspension in drinking water	No impact on Tjp1 expression, suggesting no impact on intestinal permeability. Expression of Defb3, encoding beta-defensin 3, increased; granzyme B, cathelin-related antimicrobial

**Table 2** Impact of TiO<sub>2</sub> particles on intestinal epithelium<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Talamini et al., 2019 [21]	Mice, TiO <sub>2</sub> dripped into the mouse's mouth	~ 2 mg/kg b.w./day 3 days/week for 3 weeks	E171	Suspension in water, no sonication, dripped into the mouth	peptide, regenerating islet-derived protein 3 gamma and p-lysozyme not significantly modulated. Reduction of colonic crypt length at 50 mg/kg b.w./day, no impact on colon length.
Medina-Reyes et al., 2020 [88]	Mice with either high fat diet (HFD) or regular diet (RD)	5 mg/kg b.w. for 16 weeks	E171	Suspension in drinking water	No overt structural or morphological alteration in the stomach and intestine, no disruption of crypt structure, no atypical cell proliferation. Decreased colon crypt length, as compared to the respective control (HFD or RD).
Zhang et al., 2021 [90]	Mice	1% w/w TiO <sub>2</sub> for 1, 3 or 6 months		TiO <sub>2</sub> incorporated in food pellets	Increased villi height/crypt depth ratios at 1 and 3 months; increased expression of ZO-1 and occludin at 1 month; spare microvilli in small intestine at 6 months. No change in intestinal permeability.

<sup>a</sup>Abbreviations: ABC ATP-binding cassette, FBS foetal bovine serum, OCLN occludin, SLC solute liquid carrier, TEER transepithelial resistance, TEM transmission electron microscopy, TJP tight junction protein, XRD X-ray diffraction

**Table 3** Impact of TiO<sub>2</sub> particles on intestinal mucus<sup>a</sup>

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Urrutia-Ortega et al., 2016 [96]	Oral gavage, mice. Wild-type or model of CAC via AOM/DSS induction.	5 mg/kg body weight/5 days/10 weeks	E171	Sonication for 30 min in water	Reduced number of goblet cells in the distal colon, in both groups (WT and CAC), higher decrease in the CAC model.
Talbot et al., 2018 [94]	HT29-MTX and in vivo rat acute and sub-chronic oral exposure	In vitro: 250 µg/mL; in vivo acute (7 days, daily gavage): 10 mg/kg bw/day; in vivo sub-chronic (60 days, drinking water): 10 and 0,1 mg/kg bw/day	E171 and P25 (anatase/rutile, 21 nm)	Probe sonication in 0,05% water/BSA 27 min	Penetration of TiO <sub>2</sub> into the mucoid area of HT29-MTX cells (patchy structures of mucus). When E171 and P25 were administered in vivo, cecal short-chain fatty acid profiles and gut mucin O-glycosylation patterns remained unchanged
Dorier et al., 2019 [95]	Caco-2/HT29-MTX co-culture	Acute (6-48 h; 50 µg/mL) or repeated twice a week for 3 weeks; 10 and 50 µg/mL	E171, anatase 12 nm (A12), anatase/rutile, 21 nm (P25)	Indirect cup-type sonication (high energy) 30 min in water	No impact on cell differentiation. Inflammatory profile (chronic and acute), increased mucus secretion (chronic). Epithelial integrity unaltered, content of ABC family of xenobiotic efflux pumps modified (either increased or decreased depending on the particle: decreased with E171, increased with P25).
Pinget et al., 2019 [133]	Mice, drinking water	2, 10, 50 mg TiO <sub>2</sub> /kg b.w./day for 21 days	E171	Suspension in drinking water	Decreased <i>Muc2</i> gene expression in the colon at 10 and mg/kg b.w./day
Medina-Reyes et al., 2020 [88]	Mice with either high fat diet (HFD) or regular diet (RD)	5 mg/kg b.w. for 16 weeks	E171	Suspension in drinking water	Hyperplasia and hypertrophy of goblet cells; increased <i>Muc2</i> , <i>Muc5AC</i> , <i>Muc1</i> and <i>Muc13</i> mRNA expression.

<sup>a</sup>Abbreviations: ABC ATP-binding cassette, AOM azoxymethane, BSA bovine serum albumin, CAC model of colitis-associated cancer, DSS dextran sulfate sodium, WT wild-type

paracellular permeability of the epithelia, nor transport through P-glycoprotein [52]. Moreover, acute exposure of Caco-2 cells to 50 µg/mL of anatase (12 nm) or rutile (20 nm) TiO<sub>2</sub>-NPs induces early upregulation of a battery of efflux pumps and nutrient transporters, suggesting that it might affect the efflux of toxic substances and nutrient absorption [91]. Moreover, another in vitro study demonstrates that, in addition to altering tight junctions, acute exposure for 4 h and repeated daily exposure for 5 days of Caco-2/HT29-MTX to 30 nm TiO<sub>2</sub>-NPs reduces absorptive cell microvilli, which are involved in nutrient absorption. This concurs with alteration of nutrient transport (specifically Fe and Zn) as well as fatty acid uptake [92]. The concentration used in this study are 10<sup>6</sup>, 10<sup>8</sup> and 10<sup>10</sup> particles/cm<sup>2</sup> (acute exposure), or three times these concentrations (chronic exposure).

Overall, all these data highlight the fact that the protective and absorptive functions of intestinal epithelial cell can be impaired by TiO<sub>2</sub> exposure.

#### Impact of TiO<sub>2</sub> on the mucus layer

Concerning the impact of TiO<sub>2</sub> on the mucus and AMP secretions from epithelial cells including goblet cells, Paneth cells and enterocytes, the literature is scarce (Table 3). HT29-MTX cells, developed by Lesuffleur et al. [93], are an ideal model to study the interaction of NPs with the intestinal mucus, in vitro. The mucus is organized as islands on top of these cells, and these islands of mucus can trap E171 [94]. In vitro, repeated exposure of Caco-2/HT29-MTX cells for 21 days to 10, 50 or 100 µg/mL of E171 or 21 nm anatase/rutile TiO<sub>2</sub>-NPs causes mucus secretion to increase, while mucin gene expression remains unchanged [95]. In vivo, Medina-Reyes et al. show hyperplasia and hypertrophy of goblet cells and increased mucin gene expression in mice exposed for 16 weeks to 5 mg/kg b.w. of E171 in the drinking water and fed with either regular diet or high fat diet [88]. Inversely, intragastric administration of E171 to mice, both wild type and with chemically induced colitis-associated cancer, at 5 mg TiO<sub>2</sub>/kg b.w./day, 5 days a week during 10 weeks, decreases the number of goblet cells in the distal colon [96]. This would rather suggest that TiO<sub>2</sub> reduces the capacity of mucus secretion in exposed animals. Moreover, Talbot et al. show no modification of cecal short-chain fatty acid (SCFA) profiles, which play a role in mucin synthesis and mucus characteristics, and of gut mucin O-glycosylation patterns in rats orally exposed to E171 or 21 nm anatase/rutile TiO<sub>2</sub>-NPs, which suggests that these particles show no significant effect on mucus quantity and quality [94]. In the latter study, rats have been exposed to 0.1 mg E171/kg b.w./day for 60 days or 10 mg E171/kg b.w./day for 7 days. While the concentration of E171 to which

animals are exposed within the three latter studies are comparable and realistic, the exposure time varies from 7 days to 16 weeks. Their results point to the time-dependence of E171 impact on mucus secretion. They suggest that mucus secretion would not be affected at short exposure times (7 or 60 days) [94], it would then be reduced after 10 weeks of exposure [96] and finally increased after 16 weeks of exposure [88]. Still, such an interpretation should be treated with caution, as exposure patterns and E171 preparation procedure vary from one study to another. It is accepted that the procedure of TiO<sub>2</sub> dispersion plays a pivotal role on its agglomeration state and biocorona; for instance TiO<sub>2</sub>-NPs agglomerate due to high ionic strength and when submitted to simulated digestion [97, 98], while E171 strongly adsorb some mucins on its surface, which leads to reduced agglomeration [99]. Thus, exposing animals via intragastric gavage or via drinking water would result in different biocorona on the particles and different agglomeration states, which could explain these apparently discrepant results.

To put into perspective these recent studies, an abundant literature demonstrates the impact of TiO<sub>2</sub>-NPs on the mucus secretion from the respiratory system and on the development of the pulmonary diseases like asthma, chronic obstructive pulmonary disease or cystic fibrosis. Analysis of this literature is reported as [Supplementary materials](#) and suggests the implication of calcium signaling in the induction of mucus secretion triggered by TiO<sub>2</sub> [100].

#### Impact on the intestinal immune system

A paradigm of TiO<sub>2</sub> toxicity, especially via inhalation, is that it causes inflammation [101]. Several studies have also reported that TiO<sub>2</sub> would induce an inflammatory profile in the intestine when ingested (Table 4). In rats orally exposed for 7 days to 10 mg/kg b.w./day of E171, increased DC (CD103 + MHCII<sup>+</sup>) population and decreased helper T cells (Th) (CD4 + CD25<sup>+</sup>) and regulatory T cells (Treg) (CD4 + CD25 + Foxp3<sup>+</sup>) populations were observed in PPs [20]. This observation of immune cells population shift is completed by functional in vitro experiments through TcR stimulation of isolated cells from PP and spleen. Interestingly, E171 decreases CD3/CD28-induced IFN $\gamma$  at the intestinal level but increases its secretion at the systemic level. Higher IL-17 secretion is also observed in the spleen of rats exposed to E171 without modification at the intestinal level [20]. Moreover, 100 days of oral exposure to E171 at 10 mg/kg b.w./day lead to decrease of Th and Treg populations in PP [20]. This inflammatory signature could lead to IBD in susceptible individuals. In contrast, Blevins and al. do not report any modification of DCs, nor Th, nor Treg in PPs after 7 or 100 days of exposure to E171 at 400 mg/

**Table 4** TiO<sub>2</sub> and intestinal pathologies<sup>a</sup>

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
<i>Inflammatory bowel disease</i>					
Powell et al., 1996 [54]	Human GALT from donors with CD, UC, and colon carcinoma	n/a	n/a	n/a	Presence of particles in phagolysosomes of macrophages from GALT ("pigmented" cells). Anatase TiO <sub>2</sub> (100–200 nm), flakes of aluminosilicates with some Fe on the surface (< 100–400 nm), mixed silicates without aluminium (100–700 nm).
Powell et al., 2000 [123]	Human intestinal biopsies from donors with CD, UC and healthy donors, EL4.NOB-1 murine lymphoma lymphoblasts, CTLL murine T cells	5 µg/mL TiO <sub>2</sub> or 5 µg/mL TiO <sub>2</sub> , together with 1 ng/mL LPS and 4 mM CaCl <sub>2</sub> or 1 ng/mL LPS	Anatase TiO <sub>2</sub> , 0.2 µm	Suspension in culture medium, or suspension together with LPS and CaCl <sub>2</sub> (ion bridge) followed by ultrasonication	Increased secretion of IL-1 in cells and organ cultures stimulated with the mixture of TiO <sub>2</sub> and LPS, not with TiO <sub>2</sub> or LPS alone.
Nogueira et al., 2012 [104]	Mice	Oral gavage, 100 mg/kg bw/day; 10 days	TiO <sub>2</sub> -NPs (66 nm, home-made) and MP-TiO <sub>2</sub> (260 nm, Kronos 1171, food-grade)	Suspension in distilled water, sonicated (no indication of the method) immediately before administration	Increased levels of CD4+ T cells in duodenum, jejunum and ileum of animals exposed to TiO <sub>2</sub> . Increased levels of IL-12, IL-4, IL-23, TNF-α, IFN-γ and TGF-β (pro-inflammatory), suggesting a Th1-mediated inflammatory response. Hypertrophy and hyperplasia of the mucosal epithelium in the duodenum, jejunum and ileum. Effects of NPs and MPs are not significantly different.
Ammendolia et al., 2014 [126]	<i>Listeria monocytogenes</i> (LM2 and LM9) biofilms and Caco-2 cells	0.8, 8 and 80 µg/mL TiO <sub>2</sub> for 24 h and 48 h	commercial TiO <sub>2</sub> -NPs, anatase, 139 nm mean diameter	Probe sonication in bacterial medium for 45 min	Exposure of bacteria to TiO <sub>2</sub> -NPs, then exposure of Caco-2 cells to TiO <sub>2</sub> -exposed bacteria. No impact on bacterial cell survival. Increased biofilm production for LM2 with 0.8, 8 and 80 µg/mL TiO <sub>2</sub> for 24 h and for LM9 with 80 µg/mL TiO <sub>2</sub> for 24 or 48 h. Modification of the biofilm structure. Increased interaction of LM2 with Caco-2 cells when exposed to TiO <sub>2</sub> , reduced intracellular accumulation of LM2 and LM9, reduced multiplication of LM2 and LM9 in Caco-2 cells when exposed to TiO <sub>2</sub> .
Hummel et al., 2014 [124]	Biopsies from children suspected to having IBD	n/a	n/a	n/a	Amount of pigment (defined in Powell et al., 2000 as agglomerates of mineral particles) in Peyer's patches. The amount of pigment increased with the age of patients. Significantly less pigment in patients supposed to having CD as compared to

**Table 4** TiO<sub>2</sub> and intestinal pathologies<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Urrutia-Ortega et al., 2016 [96]	Mice, or mice with chemically-induced CAC (12.5 mg/kg body weight/ ip AOM and 2% DSS)	Oral gavage, 5 mg/kg b.w./day, 5 days a week, 10 weeks	E171	Suspension in water, sonication for 30 min at 60 Hz. 300 nm in diameter (DLS)	healthy donors and patients suspected to having UC. Transient inflammation observed in the CAC group, more intense in the CAC + E171 group. Decreased interleukin expression (IL-2, TNF- $\alpha$ , INF- $\gamma$ , IL-10) in the CAC + E171 group, compared to the E171 group. Migration of TNF- $\alpha$ from the cytoplasm to the nucleus of colorectal epithelial cells in E171 and CAC groups.
Ruiz et al., 2016 [56]	Mice, wild-type or deficient in (NLRP3), with chemically-induced colitis (DSS). In vitro: Caco-2, HT29 and other epithelial cells, THP-1 treated or not with siRNA for caspase-1, ASC and NLRP3. Bone marrow-derived macrophages (from mice). Human patients with IBD having active disease	In vivo: oral gavage, 50 or 500 mg TiO <sub>2</sub> /day/kg b.w. for 7 days (WT) or 500 mg TiO <sub>2</sub> /day/kg b.w. for 7 days (Nlrp3 <sup>-/-</sup> mice). In vitro: exposure to 5, 20 or 100 $\mu$ g/mL of TiO <sub>2</sub> for 24 h.	In vivo: 30–50 nm, rutile. In vitro: TiO <sub>2</sub> rutile (30–50 nm) and anatase with size ca 0.36 $\mu$ m (food-grade)	Suspension in drinking water (in vivo); suspension in ultrapure water and sonication for 5 min (in vitro)	In mice: no sign of colitis in WT mice exposed to TiO <sub>2</sub> with no pre-existing colitis. In mice with chemically-induced colitis: colitis symptoms are worsened in WT mice, not in Nlrp3 <sup>-/-</sup> mice. In vitro: both TiO <sub>2</sub> particles triggers NLRP3-ASC-caspase-1 assembly, caspase-1 cleavage and the release of NLRP3-associated IL-1 $\beta$ and IL-18. TiO <sub>2</sub> induces intracellular ROS accumulation. In human volunteers with IBD: Higher TiO <sub>2</sub> level in the bloodstream compared to healthy patients.
Bettini et al., 2017 [20]	Rats or rats with chemically-induced carcinogenesis (DMH)	Oral gavage 10 mg/kg of b.w./day, 7 days; or exposure via the drinking water at 200 $\mu$ g or 10 mg/kg of b.w./day for 100 days	E171 and NM105 (21 nm, anatase/rutile TiO <sub>2</sub> -NP)	Probe sonication for 16 min in water/BSA 0.05%	Disturbance in intestinal and systemic immune homeostasis, colon microinflammation after 100 days of treatment.
Chen et al., 2017 [82]	Mice	Oral gavage, 2.5 mg/kg b.w./day, once a day for 7 days	TiO <sub>2</sub> -NPs, anatase (not food-grade)	suspension in water, sonication in a water bath at 12–15 °C for 15 min	No evidence of inflammation (only a few inflammatory cells observed), no pathological changes by H&E staining. Increase of IL-1 $\beta$ in the colon only (measurement of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ in the colon and small intestine).
Hong et al., 2017 [135]	Mice	Oral gavage, 1.25, 2.5, and 5 mg/kg b.w./day, once a day for 9 months	TiO <sub>2</sub> -NPs, anatase (not food-grade), 5–6 nm	Suspension in 0.5% w/v HPMC, ultrasonication for 45 min and mechanic vibration for 10 min	Inflammatory cell infiltration in the stomach. Increased protein expression of NF- $\kappa$ B, TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, COX-2, and PGE2 in the stomach, decreased protein expression of I $\kappa$ B.
Dorier et al., 2019 [95]	Caco-2/HT29-MTX co-culture	Acute (6–48 h; 50 $\mu$ g/mL) or repeated twice a week for 3 weeks; 10 and 50 $\mu$ g/mL)	E171, A12 (inhouse synthesis, anatase, 12 nm), P25	Suspension in distilled water, sonication indirect cup-type sonicator in continuous	No impact on cell differentiation. Inflammatory profile (chronic and acute),

**Table 4** TiO<sub>2</sub> and intestinal pathologies<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
			(anatase/rutile, 21 nm)	sonication mode for 30 min at 52.8 W (80% amplitude)	increased mucus secretion (chronic). Epithelial integrity unaltered, content of ABC family of xenobiotic efflux pumps modified (either increased or decreased depending on the particle: decreased with E171, increased with P25).
Pinget et al., 2019 [83]	mice, drinking water	2, 10, 50 mg TiO <sub>2</sub> /kg b.w./day for 21 days	E171	Suspension in drinking water	Proliferation of macrophages in the colonic myeloid compartment of mice exposed at 10 or 50 mg/kg b.w./day, no impact on the population of neutrophils, dendritic cells, total monocytes. Upregulation of IL-6, TNF- $\alpha$ and IL-10 in the colon.
Blevins et al., 2019 [102]	Rats, exposure to TiO <sub>2</sub> included in food pellets	40, 400 or 5000 pm in food pellets, 7 or 100 days	E171	n/a	No effect on immune parameters: percentage of dendritic, CD4+ T or Tregs in Peyer's patches, in their periphery. No effect on cytokine release in the colon, jejunum and plasma.
<i>cancer</i>					
Urrutia-Ortega et al., 2016 [96]	Mice, or mice with chemically-induced CAC (12.5 mg/kg body weight/ ip AOM and 2% DSS)	Oral gavage 5 mg/kg bw/ 5 days a week/10 weeks	E171	1 mg/ml in water, water and sonication for 30 min at 60 Hz	E171 in non-chemically-induced animals: no tumor formation but in the distal colon: slight vascularization, increase of crypts size and number, hyperplastic epithelium with slight dysplastic changes. CAC: purulent material and blood discharge; abnormal behavior; well differentiated adenocarcinomas in the distal region of the colon. E171 in CAC: exacerbated blood and purulent discharge and higher inflammation with rectal prolapse (vs CTL CAC animals) + abnormal behavior. Enhanced the tumor formation in distal colon; mildly differentiated adenocarcinoma. Increased fluorescence of markers of tumor progression. Decrease IL2, IL10, TNF $\alpha$ , INF $\gamma$ . Kidney weight reduced. Both groups: decrease of goblet cells in the distal colon, higher in CAC + E171.

**Table 4** TiO<sub>2</sub> and intestinal pathologies<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Bettini et al., 2017 [20]	Rats or rats with chemically-induced carcinogenesis (DMH)	Oral gavage 10 mg/kg of BW/day, 7 days; or exposure via the drinking water at 200 µg or 10 mg/kg of BW/day for 100 days	E171	Probe sonication for 16 min in water/BSA 0.05%	Increased number of aberrant crypt foci per colon after 100 days of treatment with E171 at 10 mg/kg b.w./day, proving initiation of preneoplastic lesions. In DMH-induced rats, increased number of aberrant crypts per colon and of large aberrant crypt foci per colon after exposure for 100 days at 10 mg E171/kg b.w./day, proving promotion of carcinogenesis.
Proquin et al., 2017 [129]	Mice	Oral gavage, 5 mg/kg b.w./day, 2, 7, 14 and 21 days (5 times per week)	E171	1 mg/ml in water, water and sonication for 30 min at 60 Hz	Analysis of the colon. Structure of crypts altered, hyperplastic epithelium. No effect on intestinal cell proliferation. Transcriptome analysis with pathways affected: olfactory/GPCR receptor family, cancer, oxidative stress, cell cycle, immune response, metabolism of proteins.
Proquin et al., 2018 [130]	Mice with chemically-induced CAC (12.5 mg/kg body weight/ip AOM and 2% DSS)	Oral gavage, 5 mg/kg b.w./day, 2, 7, 14 and 21 days (5 times per week)	E171	1 mg/ml in water, water and sonication for 30 min at 60 Hz	Analysis of the colon. Transcriptome analysis with pathways affected: olfactory/GPCR receptor family, cancer, metabolism of xenobiotics, immune system, oxidative stress.
Setyawati et al., 2018 [132]	SW480 cells, in vitro	100, 250 and 500 µM, 4 days	TiO <sub>2</sub> -NP, 21 nm (P25 Evonik)	Sonication (no detail) in 0.5% FBS DMEM	Epithelial to mesenchymal (EMT) transition: phenotypic change (fibroblastic elongated morphology, lost of cell-cell junctions, diffuse actin staining), modulation of levels of EMT marker proteins: alpha-SMA, VIM, E-cadherin. Cell mobility and invasive behavior increased. Decreased cell proliferation. Activation of the TGF-beta (not via Smad3, rather via MAPK) pathway, concomitant with ROS level elevation. Activation of the Wnt pathway.
Blevins et al., 2019 [102]	Rats, exposure to TiO <sub>2</sub> included in food pellets	40, 400 or 5000 pm in food pellets, 7 or 100 days	E171	n/a	No effect on histopathological observation: aberrant crypt foci, goblet cell number, colonic gland length.
Talamini et al., 2019 [21]	Mice, TiO <sub>2</sub> dripped into the mouse's mouth	~ 2 mg/kg b.w./day 3 days/week for 3 weeks	E171	Suspension in water, no sonication, dripped into the mouth	No atypical cell proliferation in the intestine.



**Table 4** TiO<sub>2</sub> and intestinal pathologies<sup>a</sup> (Continued)

Study	Test system	Exposure conditions: dose range and exposure time	Material	Dispersion procedure	Main conclusions
Medina-Reyes et al., 2020 [88]	Mice with either high fat diet (HFD) or regular diet (RD)	5 mg/kg b.w. for 16 weeks	E171	Suspension in drinking water	Increased number of adenomas in the colon. No difference in the volume of adenomas. No abnormal cell proliferation (as probed via the PCNA and Ki67 proliferation markers mRNA expression)

<sup>a</sup>Abbreviations: AOM azoxymethane, ABC ATP-binding cassette, BSA bovine serum albumin, CAC colitis-associated cancer, CD Crohn's disease, CTL control, DLS dynamic light scattering, DMH 1,2-dimethylhydrazine, DSS dextran sulfate sodium, GALT gut-associated lymphoid tissue, H&E hematoxylin and eosin, HPMC hydroxypropyl methyl cellulose, IBD inflammatory bowel disease, LPS lipopolysaccharide, PMA phorbol 12-myristate 13-acetate, UC ulcerative colitis

kg of bw/day, when E171 is incorporated in the feed [102]. However, after 100 days of E171 oral exposure, IL-17 concentration is higher in the colon of exposed animals [102]. So far, the consequences of oral exposure to TiO<sub>2</sub> on intestinal ILC, IEL or B cells populations are unknown. Accumulation of TiO<sub>2</sub>-NPs inside the intestinal cells, especially in PP, might lead to damaging outcomes such as inflammation and has been postulated as being potentially involved in the pathogenesis of IBD [13, 62, 103]. In 2012, Nogueira et al. evidenced that TiO<sub>2</sub>-NPs induce a pro-inflammatory response in the small intestine by increasing inflammatory cytokine production and T CD4<sup>+</sup> cell proliferation [104]. TiO<sub>2</sub> induces recruitment of CD4<sup>+</sup> T cells in the ileum, as shown via histological analyses, associated with higher secretion of IL-12, IFN $\gamma$ , TNF $\alpha$ , IL-4, IL-23 and TGF- $\beta$  [104], which suggests that TiO<sub>2</sub> commits immune response toward Th1. Moreover, hypertrophy and hyperplasia of the small intestinal mucosa are also observed in mice exposed to TiO<sub>2</sub>-NPs. This induction of inflammation in the small intestine is probably linked to the presence of more M-cells in this area (PP and ILF), which are known to be the main pathway of nanoparticle uptake across the gastrointestinal tract [105, 106]. Although there remains some ambiguity surrounding the specific immunomodulatory and cytotoxic effects resulting from in vivo TiO<sub>2</sub>-NPs exposure, it seems clear that TiO<sub>2</sub> has the potential to cause immune cell and tissue damage and dysfunction.

To our knowledge, only these three recent articles report in vivo consequences of TiO<sub>2</sub> exposure –only two of them having been conducted with food-grade TiO<sub>2</sub>– on the intestinal immune system. The difficulty to characterize intestinal immune response could explain this lack of data. Therefore, to complete this section, we include some data related to the consequences of oral exposure to TiO<sub>2</sub>-NPs with characteristics differing from those of E171 on the systemic immune system, i.e., in the spleen, liver, PBMC and microglia. In vivo, administration of TiO<sub>2</sub>-NPs has been demonstrated to have multiple immunomodulatory effects, characterized by

TiO<sub>2</sub>-NPs distribution in peripheral lymphoid organs, alterations of immune cell number, viability and function. Although they do not recapitulate the complexity of the local intestinal immune system, simple in vitro or ex vivo models using systemic immune cells could inform on the mechanisms of TiO<sub>2</sub>-driven immune cell dysfunctions. TiO<sub>2</sub>-NPs (5–6 nm) administered by intragastric gavage at 2.5, 5 or 10 mg/kg b.w./day for 90 days cause macrophage infiltration in the spleen [107]. Moreover, mice gavage with 2.5, 5 or 10 mg/kg b.w./day of the same 5–6 nm anatase TiO<sub>2</sub>-NPs for six consecutive months causes distribution of TiO<sub>2</sub>-NPs in the liver, associated with liver dysfunction, infiltration of inflammatory cells and altered mRNA levels of Th-1 and Th-2 type cytokines, i.e., IL-4, IL-5 and IL-12, as well as their targets IFN- $\gamma$ , GATA3, GATA4, T-bet, STAT3, STAT6, eotaxin, MCP-1, and MIP-2 [108]. In vitro, treatment with TiO<sub>2</sub>-NPs of the peripheral tissue equivalent (PTE) or the modular immune in vitro construct (MIMIC) system, which is an immunological construct comprising blood vein endothelial cells (HUVECs) and peripheral blood mononuclear cells (PBMCs), also shows some inflammatory response. Indeed, treatment with TiO<sub>2</sub>-NPs leads to a dose-dependent elevation of inflammatory cytokines including IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, IFN- $\gamma$  and TNF- $\alpha$ , as well as upregulated expression of the maturation marker CD83 and the co-stimulatory molecule CD86 on dendritic cells [109]. Notably, 48 h of exposure to TiO<sub>2</sub>-NPs also results in a 10–20-fold increase of ROS levels in PBMCs [109]. Treatment of RAW 264.7 macrophages with very low concentrations of TiO<sub>2</sub>-NPs, such as those that might be found in consumer products, induces NF- $\kappa$ B activation, which results in upregulation of pro-inflammatory gene expression [110]. Similarly, TiO<sub>2</sub>-NPs alter the expression of adhesion molecules and the chemokine receptor type 4 (CXCR4) in PBMCs and trigger an increase in T cell proliferation in response to PHA stimulation at concentrations that are not cytotoxic to the immune cells [111]. The direct exposure of microglia (which is also a tissue specific macrophage-like cell) to TiO<sub>2</sub>-NPs elevates the iNOS

expression associated to an increase of NO release from the microglia [112]. The expression levels of MCP-1, MIP-1 $\alpha$ , TNF $\alpha$ , IL-1 $\beta$  and IL-6 are also upregulated, as is the activation of NF- $\kappa$ B [112]. Further demonstrating the direct impact of TiO<sub>2</sub>-NPs on immune cells, TiO<sub>2</sub>-NPs induce the expression of NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome by dendritic cells and macrophages [113, 114]. TiO<sub>2</sub>-NPs activate NLRP3 inflammasome in macrophages via a mechanism that is dependent upon the release of ATP. Namely, the activation of phospholipase C- $\beta$  has been shown to be necessary for TiO<sub>2</sub>-NP induced NLRP3 activation and for the resulting IL-1 $\beta$  production. IL-1 $\beta$  production in response to TiO<sub>2</sub>-NPs is augmented by ATP and ADP hydrolysis, and suppressed by adenosine degradation or selective A2<sub>A</sub> or A2<sub>B</sub> receptor inhibition [115].

Although multiple studies indicate that TiO<sub>2</sub>-NPs induce immune cell activation and the subsequent release of inflammatory factors, TiO<sub>2</sub>-NP exposure also possesses the potential to cause immune cell death. For example, exposure of dendritic cells to TiO<sub>2</sub>-NPs induces a dose-dependent decrease of cell viability, with concentrations of TiO<sub>2</sub> nanoparticles in the range of 10–100  $\mu$ M eliciting an approximate 20% decline in viability [109]. Human dermal fibroblasts capable of exhibiting inflammatory responses also show a time and dose-dependent decrease in cell viability upon exposure to TiO<sub>2</sub> nanoparticles, however, an increase in ROS levels and expression of several pro-inflammatory genes including the TLRs, genes of the MAP kinase cascade and IL-1 $\beta$  is observed. Notably, several mediators of the NF- $\kappa$ B pathway are activated: ERK1/2 expression is upregulated and p38 is phosphorylated. Interestingly, NF- $\kappa$ B knockdown fibroblasts are resistant to TiO<sub>2</sub> nanoparticle-induced cell death, suggesting that the activation of the NF- $\kappa$ B pathway plays a role in TiO<sub>2</sub> nanoparticle-induced immune cell death [116]. In conclusion, although they are not strictly derived from experiments involving the intestinal immune system and the E171 food additive, these data underscore the ability of TiO<sub>2</sub>-NPs to modulate multiple aspects of immune function.

### **Potential implications of TiO<sub>2</sub> particles in chronic intestinal pathologies**

Despite much research on this topic, the pathophysiology of IBD and associated CRC are not well understood. The IBD pathogenesis model is based on the dysregulation of the normally controlled immune response to commensal bacteria, which could be precipitated by infection or by defects in the mucosal barrier. This involves infiltration of immune cells producing chemokines and cytokines, which in turn exacerbate the

dysfunctional immune response and activate either Th1, Th2 or Th17 cells in the gut mucosa of IBD patients [117]. More precisely, the pathogenesis of IBD occurs via three distinct stages: (i) penetration of luminal contents into underlying tissues which may be facilitated by environmental factors like infection [38], toxic particles or inherent defects in mucosal barrier linked with genetic mutations [118, 119], (ii) impaired clearance of luminal material from the bowel wall [120, 121] and (iii) a compensatory acquired immune response which leads to a chronic inflammatory response and gives rise to characteristic IBD lesions and further on CRC [122].

### **Implication of TiO<sub>2</sub> particles in inflammatory bowel diseases**

Various specific and non-specific environmental factors have been associated with the induction and/or exacerbation of disease activity in patients with CD and UC [54, 123]. Among them, some papers have suspected that TiO<sub>2</sub> from food may have a detrimental role in the pathogenesis of the IBD (Table 4). The first study to evidence a link between particles and alterations of the immune cell of the GALT is the article of Powell et al., published in 1996 [54]. It shows the presence of microparticles, including TiO<sub>2</sub> particles, in the phagolysosomes of macrophages populating the GALT and suggests that the pathogenicity of these microparticles should be further investigated. Indeed, in susceptible individuals, the same intracellular distribution of these three types of particles can cause chronic latent granulomatous inflammation which is one cardinal feature of CD [54]. The same group of researchers has also evidenced that ultrafine particles of TiO<sub>2</sub> strongly enhance the secretion of inflammatory cytokines by peripheral blood mononuclear cells and intestinal macrophages from IBD patients [123]. This result demonstrates that dietary particles are not immunologically inert and may be important adjuncts in overcoming normal gut cell hypo-responsiveness to endogenous luminal molecules [123]. As already discussed in the previous sections of this review, the same type of microparticles is observed in children having IBD or not; 42% of the patients show these pigmented cells, with significant difference between healthy children, children with UC and children with CD [124]. Patients with CD show lower number of pigment cells as compared with non-IBD patients and patients with UC [124]. A hypothesis to explain this observation is that these pigmented cells are involved in the inflammatory process in CD patients, and consequently they migrate from their initial site of activation to active sites of the inflammatory response [124]. Therefore, these microparticles may play a role in the inflammatory process of CD [124].

Since these discoveries, some researches have focused on the impacts of these fine and ultrafine particles on IBD development and/or exacerbation. As described in the previous sections of this manuscript, TiO<sub>2</sub> may impact the gut microbiota. In addition to the perturbations described in this previous sections, recent studies evidence that TiO<sub>2</sub>-NPs enhance the development of the infection and/or the immune cells response to LPS. Due to its non-degradability, TiO<sub>2</sub> can also interact with intestinal microorganisms or their products and induce or aggravate inflammatory processes. For example, bacterial lipopolysaccharide (LPS), abundant in the gut, avidly binds TiO<sub>2</sub>-NPs surface, facilitated by calcium-bridging cations and mucosal secretions. This complex induces release of inflammatory cytokines in primary human mononuclear phagocytes [125] or in intestinal explants [123]. Similarly, other studies evidence that 139 nm TiO<sub>2</sub>-NP exposure, at 80 µg/mL, influences both production and structural architecture of the biofilm synthesized by *Listeria monocytogenes* [126] and that food-grade TiO<sub>2</sub> promotes biofilm formation of *E. coli*, *E. faecalis* and mice commensal bacteria, in vitro [83]. Moreover, TiO<sub>2</sub>-NPs alter bacterial interaction with the intestinal cells [126]. Thus, increased biofilm production due to TiO<sub>2</sub> exposure may favour bacterial survival in the environment and its transmission to animal and human hosts. In addition to its role on the gut microbes, including bacteria (pathogenic or not) and viruses, as developed in the previous sections of this review, TiO<sub>2</sub>-NPs are also able to disrupt the integrity of three others components of the intestinal mucosa: epithelium, mucus layer and the immune system. TiO<sub>2</sub>-NPs also have a synergistic effect with some bacterial product to aggravate the inflammation [127]. As TiO<sub>2</sub>-NPs can aggravate intestinal inflammation, it is of interest to investigate their pathogenicity on models mimicking IBD patients, such as in a study by Ruiz et al., which shows that oral administration of TiO<sub>2</sub> NPs triggered the aggravation of acute colitis in a dextran sodium sulfate (DSS) mouse model of colitis through the activation of the NLRP3 inflammasome [56]. As already commented, these TiO<sub>2</sub> NPs accumulate in a dose-dependent manner in the spleen of these mice, highlighting the fact that ingested TiO<sub>2</sub> NPs can cross an altered intestinal barrier and reach the systemic circulation. Those results were corroborated by the fact that, after oral administration of TiO<sub>2</sub> NPs, patients with active ulcerative colitis presented increased levels of titanium in their systemic circulation [56].

#### Implication of TiO<sub>2</sub> particles in colorectal cancer

The International Agency for Research on Cancer (IARC), after comprehensively studying its hazardous potential, has rated TiO<sub>2</sub> as possibly carcinogenic for humans by inhalation (Group 2B) [128]. This

classification, in addition to the fact that TiO<sub>2</sub> can disrupt the intestinal barrier and induce intestinal inflammation, calls for studies investigating the impact of titanium on intestinal carcinogenesis. While TiO<sub>2</sub> does not appear as carcinogenic for healthy mice, it enhances the formation of intestinal tumours in mice suffering from induced colitis associated cancer (CAC) [96] (Table 4). This aggravated tumour formation is linked to dysplastic changes in the colonic epithelium, but also to a dramatic decrease in goblet cells (mucin-secreting cells) leading to intestinal barrier disruption [96]. Intestinal cancer formation could also be favoured by gene expression alterations caused by TiO<sub>2</sub>, and in this perspective transcriptomic studies show that food-grade TiO<sub>2</sub> affects the response to oxidative stress, the immune response, DNA repair and carcinogenesis [129, 130]. Tumorigenesis could also be linked to ROS production as well as to DNA damage triggered by the interaction of E171 with microtubules or oxidative DNA damage in cells acutely or repeatedly exposed to E171 or TiO<sub>2</sub>-NPs [15, 131]. In addition, TiO<sub>2</sub>-NPs have been reported to promote the epithelial to mesenchymal transition (EMT) process in colorectal cancer cells [132]. More precisely, TiO<sub>2</sub>-NPs activate the transforming growth factor-β (TGF-β)/mitogen-activated protein kinase (MAPK) and wingless (Wnt) pathways that drives the EMT process [132]. Moreover, the colon of rats exposed for 100 days to E171 presents pre-neoplastic lesions, this exposure condition favouring the development of aberrant crypt foci in a chemically induced carcinogenesis model [20]. Nevertheless, when provided to rats in a food matrix, E171 do not produce such effects [102], suggesting that the food matrix would be protective for the intestinal epithelium. Taken together, although some studies support the fact that exposure to TiO<sub>2</sub> could promote the development of CRC, additional studies must be conducted to validate or exclude a deleterious impact of these particles on colonic stem cell capacity to derive in carcinogenic cells.

#### Conclusions and perspectives

In conclusion, these data indicate that TiO<sub>2</sub> is able to alter the four compartments of IBF and to induce a low-grade intestinal inflammation associated or not with pre-neoplastic lesions. However, since multiple evidence has shown that the intestinal homeostasis in adults results directly from the perinatal establishment of the IBF, it is of major importance to investigate the impact of TiO<sub>2</sub> for the early phases of the intestinal development, i.e., during the embryogenic and lactating periods. Moreover, since IBD is a complex disease involving genetic, psychologic and environmental factors, it is important to check the impact of this material in models exhibiting IBD susceptibility like models mutated in the genes encoding

the *NOD2*, *ATG16L1*, *IRGM* or *IL-23* receptors. Finally, to demonstrate the likely involvement of these particles in the IBD genesis, it is also crucial to investigate whether exposure to these particles in combination with episodes of stress, which are described to be involved in the IBD, may induce an intestinal inflammation and linked colorectal cancer similar to those observed in IBD, in mice with genetic susceptibility to IBD.

#### Abbreviations

TiO<sub>2</sub>: Titanium dioxide; IBD: Inflammatory bowel disease; AMP: Antimicrobial peptide; CAC: Colitis-associated cancer; CD: Crohn's disease; CXCR4: Chemokine receptor type 4; EFSA: European food safety agency; FAE: Follicle-associated epithelium; GALT: Gut-associated lymphoid tissue; IBF: Intestinal barrier function; ILF: Isolated lymphoid follicle; JECFA: Joint FAE/WHO expert committee on food additives; MAPK: Mitogen-activated protein kinase; NLRP3: NOD-like receptor family, pyrin domain containing 3; OECD: Organisation for economic co-operation and development; PBMC: Peripheral blood mononuclear cell; PP: Peyer's patches; REGILly: Regenerating islet-derived protein 3; ROS: Reactive oxygen species; TGFβ: Transforming growth factor β; Th: T-helper; IARC: International agency for research on cancer; TJ: Tight junction; SAS: Synthetic amorphous silica; UC: Ulcerative colitis; Wnt: Wingless and Int-1

#### Supplementary Information

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#### Additional file 1.

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#### Authors' contributions

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#### Consent for publication

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#### Competing interests

The authors declare no competing interest.

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