Effect of nano- and microplastics on the human immune system and their influence on inflammatory bowel disease

Bachelor thesis by
Maximiliaan T. Remmelts
S2757079

Abstract

In this paper the effects of micro- and nanoplastics are researched, specifically their effect on the immune system, microbiome, and the incidence of inflammatory bowel disease. Consumption of plastic particles differs per person and can easily be reduced after awareness. Plastic particles elicit an inflammatory response with mainly IL-1α, IL-6, IL-8 and TNF-α. Furthermore, a reduction of mucin production and increase in intestinal epithelial cell permeability is observed. Thus, plastics can possibly cause the leaky gut syndrome. Plastic particles also cause dysbiosis by reducing commensals and increasing pathogenic bacteria. Therefore, it is expected that high micro- and nanoplastic consumption can trigger and aggravate inflammatory bowel disease.

Introduction

Nowadays plastics are everywhere, they are in the food we eat, the water we drink and the air we breathe[1]. Since 1970 the worldwide plastic production has increased by more than 1000%.[2] No wonder, since we use a lot of plastics in our everyday lives, wrappers for food, plastic bottles, straws, and even in clothing. However, plastics are one of the main pollutants in the earth's open water bodies.[2] For example, there is an estimated 268.950 tons of plastic in the surface waters of the global ocean.[2] Larger plastic parts are an immediate threat to sea life since sea creatures mistake it for food, which can result in false satiety or even death through suffocation[3]. Larger plastics parts can degrade into smaller pieces such as micro- and nanoplastics[4] and these smaller plastic particles can contaminate food, water and air[1]. Smaller particles such as micro- and nanoplastic particles also pose a threat to sea life, albeit a more subtle threat.

In fish microplastics have been found distributed throughout the body. In a study conducted by Mattsson et al., nanoplastics were proven to be able to penetrate through the blood brain barrier of the crucian carp. Even more, in this study morphological changes in the brain of the fish were found, such as less water in the brain and the gyri in the cerebral lobes were larger in the fish that had been fed nanoplastics. The fish fed nanoplastics displayed a different behavior compared to the control group, this change in behavior became more evident after a longer period, suggesting that the accumulation of nanoplastics might play a pivotal role.[4] Whereas studies on fish, mice and cell-lines often do not last longer than 30 days, therefore long-term accumulation is often not included in these studies. Most studies have been conducted on non-human test subjects; we should take into consideration that micro- and nanoplastics might affect humans in the same way. However, most of us do not drink seawater, so how do humans consume plastic particles?

A review by Cox et al. shows that microplastics are also found in products destined for human consumption. According to Cox et al. this is due to consumption of microplastics by animals, contamination by plastic packaging, contamination during the production process and through contamination due to dust, containing microplastics, settling during consumption. The article by Cox et al. is an extensive literary review about the number of microplastics found in consumptional items and gives an estimation of microplastics consumed by humans. It is estimated that Americans consume between 39000 and 52000 microplastic particles per year with fluctuation depending on age and sex. These numbers are based on analyses of 15% of the daily intake of consumption goods with amounts based on the recommended daily caloric intake. Cox et al. did not correct for the other 85% of consumption goods. Therefore, it is to be expected that this estimate of microplastic particle intake is an underestimate. Furthermore, it is expected that the number of microplastics detected will increase due to the increasing possibility to detect smaller particles. For this reason, nanoplastics were not taken in account in Cox et al. its review. The amount of microplastic consumption also depends on whether tap- or bottled water is consumed, giving an extra yearly microplastic intake of 4000 particles or 90000 particles, respectively. Interestingly the majority of microplastics found were microplastic fibers and microplastic fragments were second most. Humans are also exposed to microplastics by inhalation, it remains unclear whether inhaled microplastics are ingested. However, the clearing of microplastics from the lungs through mucus will often result in ingestion and if clearing from the lungs fail the microplastics will remain trapped in the lungs. When microplastic exposure through inhalation is taken into account, adults are exposed to approximately an extra 48000 – 62000 microplastics annually.[1]

For the effect of microplastics to be significant usually a high dose of microplastics is used in a study; a dose of which researchers often mention that it is very unlikely that humans ever consume such a high dose. For babies this is, unfortunately, not always the case. A study by Li et al., focuses on the use of infant feeding bottles, specifically on those made of polypropylene; about 68,8% of the global market share. Following the formula preparation guidelines of the World Health Organization it is estimated that there is a release of microplastic

in infant feeding bottles between 1.310.000 and 16.200.000 particles per liter. Moreover, in this study a 0.8µm pore size filter was used, meaning that nanoplastics were not measured. However, further analysis by Li et al., showed that there were trillions of nanoplastics per liter with a mean diameter of roughly 100nm.[5]

Similarly, a study performed by Hernandez et al. on tea bags showed that humans can consume high doses of microplastics. The teabags used were pyramid shaped and made of nylon and polyethylene terephthalate and during the brewing of a single cup of tea approximately 2.3 million microplastics and 14.7 billion nanoplastics were released. This adds up to about 16µg of micro- and nanoplastic consumed in a single cup of tea. Moreover, humans can have multiple cups of tea increasing the consumption of microplastics and tea drinking is a habit that can lasts years or a lifetime.[6]

Whether it is due to consuming sea salt, or drinking a cup of tea[6], exposure to plastic particles is an everyday occurrence to our guts[1]. The bacteria that live in out gut are also exposed to ingested plastic particles. Possibly, plastic particles affect the microbiome and cause dysbiosis, an imbalanced microbiome. Dysbiosis can cause a change in the immune response and overall health of human beings. Moreover, there is a strong link between dysbiosis and inflammatory bowel disease[7].

Because microplastics are relatively new in the human "diet" there is still a lot unknown about the effects microplastics have on the human body. The immune system is tasked with the detection and neutralization of viruses, bacteria, pathogens and other foreign particles that do not belong in the body.[8] Plastic particles are also foreign to our bodies and to the immune system. Therefore, it is expected that plastic particles elicit an immune response. Inflammatory bowel disease is a disease that is characterized by chronic inflammation of the gastrointestinal tract. Furthermore, the incidence of inflammatory bowel disease is increasing globally[7]. If plastic particles elicit an immune response, plastic particles can possibly trigger and aggravate inflammation, thereby affecting inflammatory bowel disease severity.

In this paper the focus will be on the effect microplastics have on the gut its microbiome, the immune system, and the incidence of inflammatory bowel disease. To uncover how plastics affect the human gut we first must investigate the effects of plastic on the microbiome. Thereafter, the effect of plastic particles on the immune system and finally we will be looking into how inflammatory bowel disease is affected by the changes in the microbiome and immune system after exposure to plastic particles.

Microplastics directly affect the gut microbiome

The human intestines are inhabited by more than 10^{14} bacteria of over 1000 species.[8] Over 80% of microbiome consists of bacteria from 2 phyla: the *Firmicutes* and the *Bacteroides*.[8] Most of these bacteria are in a mutually beneficial relationship with their host. Therefore, it is important to let commensal bacteria live and flourish, however it is equally important to not let pathogenic bacteria get a foothold in the lumen and overgrow the commensal bacteria. For defense the gut epithelium relies on; a layer of mucus so that there is no direct contact with the bacteria, anti-microbial peptides, secretory IgA and tight connections in the epithelium so that bacteria cannot slip through the cracks.[9] Since the microbiome is the first to be exposed to plastic particles does it affect the bacteria in the microbiome?

Because our microbiome is important to our health, the effects that microplastics have on bacteria are also important. Li et al., investigated the effects of polyethylene microplastics on mice. The mice were fed with either 6µg, 60µg or 600µg of microplastic.[10] To confirm whether microplastics affect bacteria changes in abundance and flora diversity were taken into account.[10] It shows that the group of mice fed 600µg had a significantly increased bacterial abundance and a significantly increased flora diversity.[10] The other two groups also showed an increase in both aspects however not significantly.[10] The abundance of bacteria from the phyla Bacteroides was significantly

decreased in 60µg and 600µg.[10] In the group exposed to 6µg a decrease of *Bacteroides* abundance was observed albeit not significantly.[10] A study by Hiippala et al. shows that six members of the *Bacteroides* phyla have anti-inflammatory characteristics as well as *P. distasonis*. These bacteria are able to alleviate the reaction to *E. coli* lipopolysaccharide from HT-29 cells.[11] Furthermore, the aforementioned bacteria from the *Bacteroides* phyla are able to strengthen the intestinal epithelial.[11] It must be noted that Li et al. did not specify which strains of *Bacteroides* were affected by the microplastic treatment. Thus, it remains unclear whether microplastics harm the specific anti-inflammatory strains of Bacteroides.

A study by Liu et al. shows that in Chinese mitten crabs polystyrene particles of 5µm can induce a change in microbiome composition and abundance.[12] For example, the relative abundance of both *Firmicutes* and *Bacteroides* was decreased and that of *Fusobacterium* and *Proteobacteria* was increased.[12] Similarly, Lu et al., found a reduction in abundance of both *Firmicutes* and *Bacteroides* and an increase in *Bifidobacterium* in mice that were administered 1000µg/L polystyrene.[3] Interestingly, these studies show that bacteria that are part of the most abundant phyla in humans are affected by plastic particles.

In the study by Li et al. an increased abundance of *Staphylococcus* was shown in mice after exposure to polyethylene.[10] This increase was significant for all treated groups.[10] It is thought that *Staphylococcus* can directly aggravate inflammation due to its ability to generate super anti-gens. An increase in pathogenic bacteria is also observed in zebrafish after polystyrene exposure.[13] This increased abundance was significant when treated with 100nm and 200µm particles.[13] Both treated groups showed increased abundance of different phyla.[13] However, zebrafish exposed to 5µm polystyrene did not show significant changes in pathogenic bacteria abundance, even though *Staphylococcus* abundance was increased 1.77-fold.[13] Thus an increase in pathogenic bacteria abundance is observed after microplastic exposure.

The first measure of defense against these pathogenic bacteria is the layer of mucus on the intestinal epithelium.[14] Microplastics do not only facilitate growth of these pathogenic bacteria, but it has been observed to also decreases mucus secretion. Lu et al. observed that mice treated with 500nm and 500µm polystyrene particles show a significant reduction of mucus secretion in the gastrointestinal tract.[3] Furthermore, it is mentioned that the decrease in secreted mucus indicates that the barrier function might be impaired.[3] Contrary, an increase of mucus secretion and genes related to mucus secretion was also found in the 100nm group by Gu et al.[13] Furthermore, downregulation of genes related to secretory IgA production by B-cells was found.[3]

In conclusion, microplastics not only affect commensal bacteria it also causes an increase of pathogenic bacteria abundance. Therefore, it is expected that microplastics directly cause dysbiosis[3,10,12]. It is likely that plastics reduce the secretion of mucus and a reduction in mucus secretion can compromise the first measure of defense. Because the microbiome is affected by microplastics it is probable, that the immune system is affected by not only plastic particles but also to the changes in the microbiome and the reduction in mucus secretion.

Microplastics and their effect on the immune system

An immunological response is an intricate multi-faceted process. It starts with the uptake and destruction of a pathogen by innate anti-gen presenting immune cells. The antigen presenting cell in turn presents the anti-gen acquired from the destroyed pathogen to a cell from the adaptive immune system which is able to fully or partly recognize the antigen. T-cells and B-cells are lymphocytes that can recognize antigens. T-cells will differentiate into an effector T-cell and start orchestrating an immunological response. B-cells that recognize the anti-gen start producing antigen specific anti-bodies. Although, this oversimplified immunological response is somewhat applicable to the gut, the gut also has its own manner of generating an immune response.

Generating an immunological response

In the gut most of the immunological responses are generated in the so-called gut associated lymphoid tissue. Peyer's patches are part of the gut associated lymphoid tissue and are found in the ileum. Furthermore, Peyer's patches, as depicted in figure 1, are important in the generation of an immunological response. Microfold cells continuously sample the contents of the lumen and transport the samples into the Peyer's patch. The sample is then taken up by dendritic cells and the dendritic cells present the antigens derived from the sample to naïve T-cells and B-cells in the Peyer's patch. The naïve T-cells that recognize these antigens then become effector T-cells; effector T-cells are considered to be one of the main orchestrators of the immune response. After being activated effector T cells reenter the bloodstream and travel to mucosal tissues to fulfill their effector function.[8]

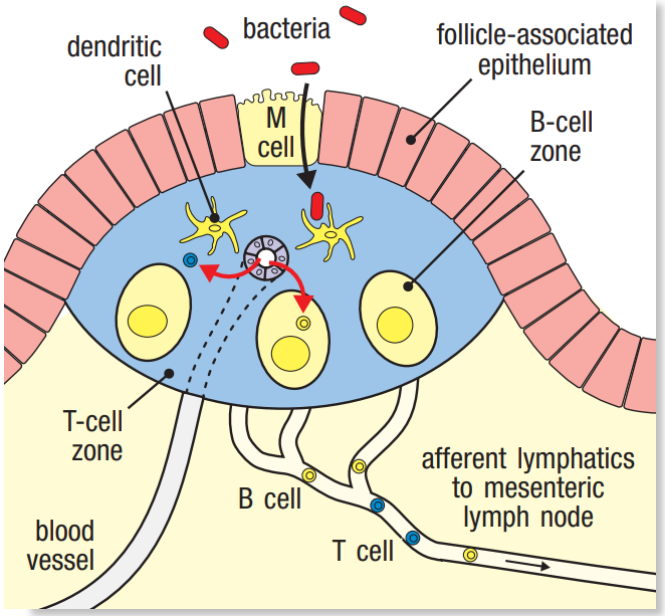

Figure 1. Peyer's patch. Courtesy of Janeway's 9th Edition

T-cell polarization

Effector T-cells consist of various polarization the T helper 1 cell (T_h1), T helper 2 cell (T_h2), T helper 17 cell (T_h17), and induced regulatory T-cell (T_{reg}). Generally speaking, T_h1 and T_h17 cells are pro-inflammatory and Treg cells are anti-inflammatory. The polarization of naïve T-cells largely depends on the co-stimulatory signals the naïve T-cells receive. These co-stimulatory signals largely consist of cytokines in the microenvironment of the naïve T-cell.[8]

A naïve T-cell that receives a lot of pro-inflammatory cytokines has a bigger change of becoming a pro-inflammatory T-cell.[8] Cytokines IL-6 and TGFβ promote the differentiation of naïve T-cells into T_h17 cells.[8] Whereas a naïve T-cell that is activated in a microenvironment that has low levels of pro-inflammatory cytokines has a bigger change of becoming a regulatory T-cell.[8] In the absence of IL-6 and other pro-inflammatory cytokines and in the presence of all-trans retinoic acid and TGFβ naïve T-cells differentiate into regulatory T-cells.[8] Furthermore, the $CD4^+$ T-cell phenotype can adapt to their local environment, T-cell plasticity. Induced Treg cells can transition into: T_h17 under influence of IL-1 and IL-6, and into T_h1 with IL-12.[8] T_h17 can transition into a T_h1 phenotype under influence of IL-12.[8] A balance between the T-cell subsets is of importance, an imbalance can be linked to autoimmune and inflammatory diseases.[15]

Cytokines are not only important for the polarization of naïve T-cells, but cytokines are important signaling molecules for all cells. Cytokines are not solely produced by lymphocytes; some cytokines can be produced by all cells that are in distress. However, most cytokines are mainly produced by certain lymphocytes. T-cells are the main orchestrators of a correct immune response and to this end they produce large quantities of cytokines. Of particular interest are the following cytokines;

- Tumor necrosis factor alpha is a pro-inflammatory cytokine that can be soluble or membrane bound, it has a wide array of pro-inflammatory effects on cells.[16] And it plays an important role in the activation of effector T-cells and macrophages.[16]
- IL-1 is pro-inflammatory cytokine that is important in the activation of T-cells and macrophages.[8]
- IL-6 can enhance pro-inflammatory functioning of anti-gen presenting cells and T-cells.[16] Furthermore, IL-6 can stimulate pro-inflammatory cytokine secretion in T-cells.[16]
- IL-8, also known as CXCL8, is powerful chemoattractant for neutrophils.[8]

Plastic particles affect the immune response

Are micro- and nanoplastics able to induce an immunological reaction in humans when ingested? Due to lack of studies in humans, there is no definite answer for the time being. However, studies in animals and human- and animal cell-lines give some insights as to how plastic particles may affect our immune system.

In bloodclams a significant reduction in pro-inflammatory expression levels of TRAF6, IKKα and NF-κB was observed after exposure to polystyrene particles of 500nm and 30µm.[17] These results suggest that plastic particles reduce the pro-inflammatory immune response in bivalves. Interestingly, in other species a pro-inflammatory immune response is often observed.

A 28-day exposure study by Huang et al., using polystyrene microplastics of 32-40µm, shows that there is a pro-inflammatory immune response to microplastics in the gut of guppies.[18] The protein levels of TNF-α, IFN-γ, TLR4 and IL-6 were significantly increased in both the 100µg/L and 1000µg/L microplastics treatment.[18] For IFN-γ, TLR4 and IL-6 there was a dose dependent increase in protein release.[18] For TNF-α there was no dose dependent increase in TNF-α levels.[18] Similarly, zebrafish exposed to 100nm, 5µm and 200µm polystyrene particles did not show an increase in TNF-α levels.[13]

Li et al. used a murine model with 10-150µm polyethylene particles.[10] Three groups of mice were fed 6µg, 60µg or 600µg of microplastics per day.[10] In all groups there was a 2-fold increase in IL-1α.[10] In the 6ug group a 3.2-fold increase of IL-6 and a halving of IL-2 was observed.[10] IL-2 is a regulatory cytokine that can help induce naïve T-cell to differentiate into regulatory T-cells.[8]

A study by Lehner et al. shows that microplastics in the size of 50-500µm caused no significant release of pro-inflammatory cytokines; TNF-α, IL-8 and IL-1β. However, a continuous dose dependent increase of IL-8 was observed after exposure but never reached significant levels. A 3D intestinal barrier model was used for this study. In this study Caco-2, HT29-MTX and a mixture of these cell lines was used with the addition of human blood monocyte derived macrophages as well as human dendritic cells. The particles used in this study mainly resembled materials used in tires such as polyurethanes, both the cross-linked and thermoplastic version, polyamide, and polypropylene.[19] Particles from tires are often found in the air and according to Cox et al. accounts for roughly 30% of the annual intake of microplastics[1]. Thus, it is interesting that these plastic particles elicit no significant pro-inflammatory immune response in the gut.

Different plastics elicit a different effect from the immune system. A study by Han et al. shows the effect that microplastics have on human peripheral blood mononuclear cells. A lower dose of smaller polyvinylchloride particles, 25-75µm, resulted in higher IL-6 and TNF-α secretion than higher doses of bigger, 75-200µm, particles. However, these results were only significant for TNF-α levels. Acrylonitril-butadieen-styreen particles elicited a dose dependent increase of IL-6 levels in which smaller particles had more effect. A low dose of small acrylonitril-butadieen-styreen particles increased TNF-α levels, whereas a higher dose of small particles suppressed TNF-α. TNF-α levels decreased with a higher dosage of bigger acrylonitril-butadieen-styreen particles but it did not suppress TNF-α secretion.[20]

Most studies show an increase in inflammatory immune response after microplastic exposure. Even though a consensus on the effects of microplastics is not yet reached, most studies indicate that microplastics induce a pro-inflammatory immune response.[10,18–20] Since smaller plastic particles elicit a higher level of cytokine secretion, as shown in the study by Han et al., is size a possible factor in the generation of the immune response?

Size does matter; nanoplastics

Nanoplastics, plastic particles smaller than 1um, affect the immune response in a different way than microplastics. According to a study conducted by Forte et al. the size of plastic particles is important in regard to the immune response it elicits. In this study polystyrene nanoplastics from 44nm (NP44) and 100nm (NP100) are tested on gastric adenocarcinoma cells.[21] The NP100 did not induce significant changes in pro- or anti-inflammatory gene expressions. However, NP44 induced a 7-fold increase in mRNA expression of both IL-6 and IL-8.[21] Brown et al. reported that in the lungs of a rat 64nm polystyrene nanoplastics had a

significantly greater neutrophil influx compared to 202- and 535nm nanoplastics.[22] Furthermore, A549 cells treated with the 64nm polystyrene had a significant increased IL-8 mRNA expression and increased amount of reactive oxygen species. Whereas, on both accounts, 202- and 535nm did not.[22] Even though gastric adenocarcinoma cells and A549 cells are cells that are not present in the guts, it is interesting that human cells responded so fiercely to nanoplastics of 44nm.

Most studies focus on the effects of virgin plastic particles. However, the human in vivo digestive process can alter the properties of plastic particles.[23] Liu et al., found that a corona had formed on the surface of nanoplastics, after the nanoplastics were exposed to an in vitro digestive process. Further analysis showed that a 100nm spherical polystyrene nanoplastic was transformed to a 440nm nanoplastic due to the effects of the in vitro digestive process.[23] This increase in size can be explained by the corona, consisting of digestive enzymes and complement factors, that had formed around the particles.[23] The corona reduces electrostatic repulsion among the plastic particles thereby facilitating the agglomeration of particles.[23] Moreover, Liu et al. show that digested 100nm (440nm) nanoplastics induce a significant increase in both IL-8 and CCL2 levels in a Caco-2 model.[23] Interestingly this upregulation was not observed in virgin 100nm polystyrene nanoplastics.[23] Showing the importance of further study so that all aspects of plastics are known and are taken into account. To conclude, smaller plastic particles, especially nanoplastics, elicit a stronger pro-inflammatory immune response compared to bigger plastic particles.[20–23]

In conclusion, after exposure to plastic particles an increase in the secretion of pro-inflammatory cytokines is observed.[10,13,18–20,12,21,22,24–26] This increased proinflammatory response can directly influence the generation of pro-inflammatory cells and reduce the generation of anti-inflammatory cells. Thereby accelerating the inflammation and removing cells that can slow down inflammation. Can these changes in the immune response affect patients and at-risk patients of inflammatory bowel disease?

Inflammatory Bowel Disease and microplastics

Inflammatory bowel disease is a chronic inflammation of the digestive tract. There are two major subtypes of inflammatory bowel disease, ulcerative colitis and Crohn's disease. Ulcerative colitis is characterized by inflammation limited to the colon whereas inflammation with Crohn's disease can be found in the entirety of the digestive tract. However, the inflammation seen in Crohn's disease is often localized in the ileum.[27] Genetic mutations are the underlying cause for this response, in identical twins there is a 50% chance that both are affected by Crohn's disease and for ulcerative colitis it is a 10% chance.[28] Thus, it stands to reason that not only genes play a role in the incidence of inflammatory bowel disease but, environmental factors also play a substantial part in triggering the immune response. Studies have shown that among these triggers are: a westernized diet, low levels of vitamin D, and the use of antibiotics during childhood.[29] The key question is do microplastics contribute to the manifestation of chronic inflammation and are they a possible trigger for inflammatory bowel disease? To find out whether microplastics affect the progress of inflammatory bowel disease, we must understand cytokines and their interaction with inflammatory bowel disease.

IL-6 is a cytokine of which increased secretion levels are measured after cells are exposed to plastic particles.[10,18,20–22] Even more, patients with inflammatory bowel disease show an increased production of IL-6 by macrophages and $CD4^+$ T-cells.[16] In mice the use of an IL-6 receptor antagonist was successful in suppressing chronic intestinal inflammation.[16] An increase in IL-6 levels is observed after exposure to microplastics[10,18,20,22] and even more a 7-fold increase was seen after exposure to nanoplastics[21]. Possibly, microplastics can promote naïve T-cells to differentiate into a pro-inflammatory phenotype thereby reducing the percentage of induced Treg cells. Under

influence of microplastics the microenvironment of Tregs becomes pro-inflammatory and possibly cause Treg phenotype to shift towards T_h17. Shifting the polarization of naïve T-cells towards an inflammatory phenotype is like adding extra fuel to an inflammation. Whereas repolarizing Treg cells towards an inflammatory phenotype is like removing the existing brakes. However, Li et al. showed that polyethylene microplastics caused a decrease in the percentage of both T_h17 and Treg cells in the $CD4^+$ population, but the balance between both did not differ.[10]

An increase in TNF-α levels is observed after cells are exposed to plastic particles.[18,20] TNF-α plays a role in the progressing of inflammatory bowel disease, for example, neutralization of membrane bound TNF using anti-bodies is a proven treatment for inflammatory bowel disease.[16] Moreover, TNF-α can increase paracellular permeability in intestinal epithelial cells, and the increased permeability can be reversed by using an anti-TNF agent.[30] Therefore, TNF-α is linked to a decreased intestinal integrity. Exposure to microplastics increased levels of TNF-α and other cytokines, will these increased cytokines affect macrophages?

Macrophages can express different phenotypes, best known are the M1 and M2 polarization. Simply put, M1 macrophages are pro-inflammatory and M2 macrophages are regulatory and repair damaged tissue.[31] In vitro monocytes differentiate into M1 macrophages using lipopolysaccharides, TNF-α and IFN-γ, in combination of each other or alone.[31] Furthermore, it is thought that polarization is not set-in stone but macrophagic phenotype is dependent on cues in their environment. However, in vitro, M2 macrophages can shift their regulatory phenotype towards that of a M1 macrophage.[31] Potentially, this could mean that a repolarization of macrophages to an inflammatory phenotype happens in the gastrointestinal tract due to microplastics, thereby strengthening ongoing inflammation and hampering repair and regeneration. Ling et al. observed a metabolic shift towards glycolysis after microplastic uptake by macrophages.[24] This could be due to the depolarizing properties that microplastics exert on mitochondria[32] or because M1 macrophages rely more on glycolysis for their metabolism[33]. Furthermore, human macrophages expressed more CD80 and CD86 after microplastic engulfment, supporting a more pro-inflammatory phenotype.[24] Hwang et al. observed a shift in macrophage polarization towards a M1 phenotype under influence of polypropylene microplastics.[34] Contrary, a study by Gu et al. showed a significant decrease in M1 macrophages in Zebrafish exposed to polystyrene particles with sizes 100nm, 5µm and 200µm.[13] Lissner et al. show that M1 macrophages significantly reduce the transepithelial resistance in Caco-2 cells.[35] It also shows that an anti-TNF-α factor is able to mitigate the weaking effect that lipopolysaccharide has on the transepithelial resistance.[35]

Intestinal epithelial integrity and permeability

An impaired intestinal barrier is one of the observed pathologies in inflammatory bowel disease patients.[9] Whenever the intestinal barrier is compromised, contents in the gut can cross the intestinal epithelial cells and reach the lamina propria.[9] Lumen content like pathogens, toxins and bacterial constituents come in direct contact with immune cells and the immune system reacts with a strong inflammation after contact.[9] There are indications that genetic alterations in mucin genes are associated with a predisposition to develop inflammatory bowel disease.[27] The intestinal epithelial cells are covered with a layer of mucus to form a barrier against pathogens but still leaves sufficient capacity for the uptake of nutrients.[9] If the mucin layer is reduced or absent pathogens can come into direct contact with intestinal epithelial cells and can enter the cells.[9] Lu et al. observed a reduction in mucus production after microplastic exposure.[3] In zebrafish microplastics caused a significant decrease in goblet cells[36], which are important for the mucin secretion[36]. Gu et al. observed an increase in mucus production, increased expression of mucin genes, and mucous hypersecretion in the intestinal mucosa of zebrafish after polystyrene exposure.[13] Hypersecretion can lead to depletion of stored mucin thereby overtime reducing the mucus layer.[27] Hypersecretion can be induced by pathogens[27] and potentially by microplastics. Muc1 and Muc2 are genes that code for the production mucins, Klf4 is important for the differentiation of goblet cells

in the gastrointestinal tract of mice[37]. Polystyrene particles also significantly decreased gene transcription of Muc1 and Klf4.[3] Muc2 was decreased, albeit not significant.[3] The decrease of Klf4 is a possible explanation for the observed decrease in goblet cells in polystyrene particle treated cells[36].

Even if the mucus layer is compromised it does not mean that the gut is permeable. Intestinal epithelial cells by themselves form a physical barrier, that functions both ways; it keeps pathogenics out but at the same time keeps important things from leaking into the lumen. The intestinal epithelial cells are able to maintain the barrier function through tight connections with their neighboring cells.[9] These connections consist mainly of tight junctions.[9] ZO-1 and occludin are both proteins that are directly involved in maintaining tight junctions.[38] In a study by Liu et al. in a Caco-2 model shows that 20µg/ml of 100nm polystyrene nanoplastics can downregulate the expression of both ZO-1 and occludin significantly.[23] Interestingly a lower dose of 1µg/ml had no significant impact on the expression of these proteins[23]. The polystyrene microplastics of 5µm also showed no impact on the expression levels of ZO-1 and occludin[23]; showing the importance of the size of plastic particles. Furthermore, genes correlated with epithelial integrity were also downregulated in the gastrointestinal tract of zebrafish[36], which could mean that microplastics increase gut permeability. In addition, epithelial detachment was observed in the intestine after exposure to polyethylene and polystyrene.[36] One of the theories about the manifestation of inflammatory bowel disease is that the leaky gut evokes a severe inflammation which in turn becomes a chronic inflammation.[9] Thus, the manifestation of the leaky gut is thought to come before the development of inflammatory bowel disease. Since these early results indicate that microplastics can compromise mucin secretion and possibly decrease intestinal epithelial cell integrity, therefore, it is possible that plastic particles increase the chance on developing a leaky gut and thus increase the change of developing inflammatory bowel disease.

Microbiome & dysbiosis

In patients with inflammatory bowel disease, the intestinal microbiome is changed.[39] Furthermore, these changes in the microbiome play an important part in the pathogenesis of inflammatory bowel disease.[39] The butyrate producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* are reduced.[40] Intestinal epithelial cells use butyrate in their metabolism[41] and lower levels of butyrate can cause a decrease in mucus secretion.[42] Furthermore, a decrease in *Firmicutes* and *Bifidobacterium* and increases in *Escherichia Coli*, *Fusobacterium* and *Proteus* is observed in patient with inflammatory bowel disease.[40] Even more, an impaired intestinal barrier has been found after infection by enteropathogenic *E. coli* before the manifestation of inflammatory bowel disease.[9] Exposure to plastic particles also causes changes in microbiome composition in animals, for example a decreased abundance of both the commensals *Bacteroides*[3,10,12] and *Firmicutes*.[4,27] However, Lu et al., also observed an increase in the commensal *Bifidobacterium,* a phylum that can also decrease inflammation[43]. Moreover, an increase in pathogenic bacteria is also seen after microplastic exposure, namely that of *Staphylococcus*.[10,13]

A balanced microbiome is of importance because, for instance, there are bacteria that can influence the immune system. In mice, the commensal *Clostridia* bacteria is able to suppress the retinal dehydrogenase Rdh7 thereby reducing conversion of vitamin A into all-trans retinoic acid.[44] All-trans retinoic acid is important in the differentiation of naïve T-cells into induced regulatory T-cells.[8] Whereas a shortage of all-trans retinoic acid results in an increase in T_h17 cells, thereby increasing immune response towards pathogens.[8]

A balanced microbiome is important to our health. Unfortunately, exposure to plastic particles is likely to cause dysbiosis.[3,10,12] Even more, an increase in pathogenic bacteria combined with an impaired intestinal barrier might prove to be a bad combination for the pathogenesis of inflammatory bowel disease.

Concluding remarks

Even though there is no scientific consensus as to what different studies show, based on these studies it is safe to say that microplastics affect

the immune system, intestinal epithelial cells and microbiome. Furthermore, the consumption of microplastics can reach high levels.[5,6] By drinking 3 cups of tea from specific brands, micro- and nanoplastic intake is around 48µg.[6] Thus, inflammatory bowel disease patients might benefit from avoiding products that cause high micro- and nanoplastic consumption. Please keep in mind that most of these in vitro studies used virgin plastic particles. Liu et al. showed that nanoplastics elicit a stronger immune response when nanoplastics underwent an in vitro digestive process.[23] However, it should be noted that possibly digested particles mitigate the effects seen on tight junctions, mucin production and bacteria, because the formed corona possibly alters the intrinsic properties of plastic particles. Based on the results presented in this paper we can conclude the following:

- Plastic particles elicit a pro-inflammatory immune response.[10,13,18–2012,21,22,24–26]
- Size does matter, nanoplastics elicit a stronger pro-inflammatory immune response then microplastics.[21–23]
- Possibly, plastic particles cause a shift in polarization of naïve T-cells and monocytes towards a pro-inflammatory phenotype.
- Possibly, plastic particles cause repolarization of Treg and M2 macrophages towards a pro-inflammatory phenotype.
- Possible, plastic particles reduce mucin production[3,13,36]
- Possibly, plastic particles decrease intestinal integrity by reducing tight junctions.
- Possibly, plastic particles can cause the leaky gut syndrome.
- Plastic particles cause dysbiosis by reducing commensals[3,10,12] and increasing pathogenic bacteria[10,13].

Even though, plastic particle doses used in studies fluctuate, with some using low dosage and some high dosage, we expect that plastic particles are a trigger for the development of inflammatory bowel disease. Cox et al. showed that microplastic intake is likely not high enough to cause this. However, Hernandez et al. showed that drinking tea from specific brands can dramatically increase plastic particle intake[6] and so did Li et al. with plastic infant drinking bottles[5]. Furthermore, we expect that high plastic particle consumption severely aggravates inflammation in inflammatory bowel disease. A lot more research is needed into this particular subject, since little is known about the effects that different plastics of different sizes have on humans. However, based on these preliminary results governing bodies should reassess whether micro- and nanoplastics pose a threat to public safety. Especially the use of plastics in infant feeding bottles and teabags should be reassessed. Micro- and nanoplastics likely play a role in inflammatory bowel disease and micro- and nanoplastics might provide an explanation for the globally rising incidence of inflammatory bowel disease.

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