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New frontiers in nanotoxicology: Gut microbiota/microbiome-mediated effects of engineered nanomaterials

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ABSTRACT

It has been recently recognized that the gut microbiota, the community of organisms living within the gastrointestinal tract is an integral part of the human body, and that its genoma (the microbiome) interacts with the genes expressed by the cells of the host organism. Several important physiological functions require the cooperation of microbiota/microbiome, whose alterations play an important role in several human diseases. On this basis, it is probable that microbiota/microbiome may in part be involved in many biological effects of engineered nanomaterials (ENMs). There are still few reports on the possible toxicological effects of ENMs on microbiota/microbiome, and on their possible clinical consequences. Available data suggest that several ENMs, including carbon nanotubes (CNTs), titanium dioxide, cerium dioxide, zinc oxide, nanosilica and nanosilver may affect the microbiota and that clinical disorders such as colitis, obesity and immunological dysfunctions might follow. On the other hand, other ENMs such as iron nanoparticles may show advantages over traditional iron-based supplemental treatment because they do not interfere with the microbiota/microbiome, and some ENM-based therapeutic interventions might be employed for treating intestinal infections, while sparing the microbiota.
The final section of the review is focused on the possible future developments of the research in this field: new in vitro and in vivo models, possible biomarkers and new pathophysiological pathways are proposed and discussed, as well as the possibility that metabolic changes following ENMs/microbiota interactions might be exploited as a fingerprint of ENM exposure.
The potential toxicological relevance of physico-chemical modifications of ENMs induced by the microbiota is also highlighted.

1. Introduction

One of the most appealing perspectives in nanotoxicology is represented by the evaluation of the possible impact of nanoparticles with the enormous amount of micro-organisms living within the human body (the microbiota) and with their genomes (microbiome). In fact the genes of indigenous micro-organisms exceed the number of other human genes by at least two orders of magnitude. Knowledge about their role in physiologic and pathologic processes has enormously increased in recent years and it is now clear that micro-organisms are not simple potentially dangerous guests, but are part of the human body itself: indeed, humans may be seen as supraorganisms, composed also by microbial cells (Lederberg, 2000). Other important recent acquisitions regard the extent, composition and role of microbiome: thanks to next generation sequencing technology, genetic material belonging to micro-organisms has been detected in practically all human tissues and body fluids, including the blood (Nikkari et al., 2001), and it is has been demonstrated that not only bacteria, but also fungi and viruses are part of the microbiota. As far as the role of microbiome is concerned, it should be considered that our genome is actually an amalgama of human genes and the genes of our microbial "selves": In fact, the genomes and the proteomes of microbes in the human body frequently interact with those expressed by human cells, a functional overlapping labelled as interactome. Given this background, it is not surprising that microbiome takes part in crucial physiological processes such as immune response, the development and function of vital organs such as the brain, and the production of key elements such as vitamin B12 and folic acid (Bennett et al., 2015). On the other hand, changes in microbiome have been found to be associated with important disease states such as obesity, diabetes, rheumatoid arthritis, and inflammatory bowel disease (Marchesi et al., 2015).

On this basis, it is clear that knowledge of the interactions between engineered nanoparticles and microbiome may shed light on the pathophysiology of many reported biological activities of engineered nanomaterials (ENMs), and may also allow to discover new unrecognized biological effects. Furthermore, this new information might contribute to clarify crucial debated areas of nanotoxicology such as the identification of specific biomarkers (e.g. typical changes in microbiome

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composition and/or in microbial metabolite production) and the implementation of safe and effective post-exposure treatments (e.g. the administration of probiotics might attenuate or eliminate the impact of ENMs on pathological processes mediated by microbiome, with a similar mechanism as that observed when co-administered with oral antibiotics, i.e. by counter-balancing the negative effects on "beneficial" gut commensals).

As stated above, colonies of micro-organisms live almost everywhere in the human body, however substantial information is available only for species inhabiting the gastro-intestinal tract (a comprehensive molecular survey of the gut microbiota has been available since 2005 — Eckburg et al., 2005) and the gut microbiota far exceeds in size the other microbial communities present in our body. Therefore, the review will focus on this system. ENMs having a high chance of being introduced through the oral route are silver and copper, for their antimicrobial activity and consequent use in food packaging (Echegoyen and Nerin, 2013; Cushen et al., 2014), titanium dioxide, for its use as food colorant (Weir et al., 2012), and amorphous silica, used as an additive for clearing alcoholic beverages or as an anticaking agent Dekkers et al. (2013).

Although exposure to ENM through the gastro-intestinal tract may be important for consumers (Pietroiusti, 2012), it is considered less relevant for workers, at least in comparison to the skin and the pulmonary routes. It should be considered, however, that a substantial percentage of inhaled nanoparticles are cleared by the muco-ciliary escalator cells into the oral cavity and thereafter into the gastrointestinal tract (Geiser and Kreyling, 2010), and that ENMs deposited in the skin may reach the gut lumen through hand-mouth contact.

Therefore, gut microbiome is probably involved even in the case of pulmonary and skin exposure. On the other hand, effects on the GI tract microbiome may spread to distant organ and organ systems (e.g. through the systemic effects of metabolites generated by the ENM/microbiome interaction and adsorbed through the intestinal epithelium).

2. Available literature

To identify the pertinent publications, we considered the database Scopus, Pubmed and ISI web of Knowledge using the following search terms "Nanoparticles (or nanomaterials) and: a) microbiome, b) microbiota, c) gut, d) bacteria, e) micro-organisms. In some cases, original articles and reviews were identified through the references of recently published papers or by using as search terms the names of authors internationally recognized as leading experts in the field.

There are still few experimental studies on the interaction between ENMs and gut microbiome/microbiota, however available data suggest that ENMs may cause adverse health effects mediated by microbiome. This may happen through the direct killing of gut micro-organism, or through alterations of their function. Clinically relevant outcomes such as colitis, obesity, and immunological changes may follow.

In an in vitro study (Chen et al., 2013) gut flora micro-organisms such as the *Lactobacillus acidophilus*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus fecalis* were exposed to several types of carbon nanotubes (CNTs) (single [S]- and multi-walled [MW], pristine and functionalized, short and long) at doses ranging from 20 to 100 μg/L: all CNTs showed a marked dose-dependent anti-bacterial activity (evaluated through the inhibition of bacterial growth) towards all the tested microbes. Some physico-chemical properties of CNTs such as rigidity, diameter and length, were associated with antibacterial activity. Furthermore, the shape of bacteria had also a role in their resistance, rod-shaped bacteria being more resistant than spherical ones to the injury. The anti-bacterial activity of SWCNTs was confirmed in a subsequent study (Zhu et al., 2014), who found that exposure of *S. aureus*, *Bacillus subtilis*, *E. coli*, and *Ochrobactrum* sp. for 24 h at a concentration range of 10–50 mg/L^{-1}, induced a dose dependent inactivation of the bacteria which was more marked for long SWCNTs in comparison to short SWCNTs, to MWCNTs and to short oxidated SWCNTs (the latter showing the mildest toxic effect).

The most susceptible strains were *E. coli* and *Ochrobactrum* sp. The loss of bacterial viability was paralleled by an increase in cytoplasmic membrane fluidity. Although both oxidative and non oxidative mechanisms may underlie the toxic effects of CNTs on cytoplasmic membrane (for an in depth review on this topic see Shvedova et al., 2012), available evidence seems to support oxidative stress as the pathogenetic pathway explaining their antibacterial effect (Rajavel et al., 2014). Interestingly, different bacterial species developed different strategies in order to counteract the membrane injury: *E. coli* and *Ochrobactrum* sp. increased the levels of saturated fatty acids and decreased the amount of unsaturated fatty acids, whereas *S. aureus* and *B. subtilis* increased branched chain fatty acids and decreased straight chain ones. Therefore, this report added the important notions that the damaging action of CNTs occurs through modifications of the bacterial membrane, and that exposed bacteria may develop different adaptative mechanisms, probably having a different adaptative efficiency. It can be speculated that the presence of CNTs may cause a selective pressure on some species of the gut microbiota, with the selective survival of species more able to adapt to their presence. This situation resembles that determined by some antibiotics given by mouth, which may cause gastrointestinal symptoms due to an imbalance produced in microbiota. In some instances, the outcome may be a severe colitis, for example when there is the suppression of commensal bacteria antagonizing the pathogen bacterium *Clostridium difficile* (Ciaran et al., 1994). The development of colitis associated to alterations in gut microbiota has been reported in mice exposed to ambient particulate matter (which contains a substantial portion of carbon-based nanoparticles) (Kish et al., 2014).

In another recent in vitro study (Taylor et al., 2015 exposure to metal oxide nanoparticles such as titanium dioxide, zinc oxide and cerium dioxide (at doses of 3 μg/L, 0.01 μg/L, and 0.01 μg/L, respectively) introduced for a week in a model colon including the microbial community taken from a young healthy donor, induced changes in multiple phenotypic traits of the colonic bacteria including the production of short chain fatty acids, which are key metabolites in the control of energy homeostasis, and have been implicated in the development of obesity induced by microbiota changes (Schwiertz et al., 2010). Indeed, an increase of body weight mediated by effects on gut flora composition has been observed in vivo in pigs exposed to silver nanoparticles: these ENMs caused a decrease in intestinal coliform (Fondevila et al., 2009), which was in turn associated with an increase in body weight: Starting from the second week, a linear correlation between the administered dose of silver nanoparticles and the daily increase in body weight was observed in five weaned pigs which were given a diet including 0, 20 or 40 mg/kg of silver nanoparticles. A concomitant dose-related decrease in intestinal coliforms was also observed. Similar findings have been reported after exposure of the same animal model to copper-loaded silica nanoparticles (Wang et al., 2012). If this effect may perhaps be considered positive in agricultural animals (and in fact the authors propose silver nanoparticle as an alternative to antibiotics for this purpose) the same is certainly not true for humans, especially in the light of the obesity epidemics in the Western World.

Silver nanoparticles may have other important biological effects mediated by the gut microbiome. It has been recently shown that the subchronic administration of these nanoparticles to rats (9, 18 and 36 mg/kg body weight/day twice daily ~10 h apart) for 13 weeks caused a shift in the gut microbiome towards a larger proportion of gram negative bacteria which was in turn linked to changes in the gut associated immune response (Williams et al., 2015). It is of interest that the effects of silver nanoparticles on gut microbiome are qualitatively different than those exerted by silver ions, and are therefore probably not mediated (or only in part mediated) by the release of these ions (Das et al., 2014). In this study, the authors found that the human microbiota (evaluated in stool samples) was partially inhibited after 48 h exposure to 0–200 mg/ml of silver nanoparticles. A quantitatively similar antibacterial action was observed after exposure to silver ions (AgCl; 25–200 mg/ml), however the effects were qualitatively different.