Recent progress towards microbiota-inclusive nanosafety research

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RECENT PROGRESS TOWARDS MICROBIOTA-INCLUSIVE NANOSAFETY RESEARCH

by Dr Bregje B.W. Brinkmann

All multicellular organisms host microbes on their tissues, and these microbes jointly make up the host-associated microbiota. Microbes are important to the health of organisms and play a crucial role at the exposure interface with nanoparticles. To illustrate this, you can imagine a nanoparticle to increase to the size of a football. Following the same size scale, a football would approximately increase to size of the Earth. Adding a microbe to this comparison, would mean that you add an object with roughly the size of a city bus. Massive as compared to the 'football-sized nanoparticle', tiny as compared to 'the Earth-sized football'. And there are many of them: according to current estimates for humans, 1 cm^2 of bronchial tissue is colonized by approximately 10^3 bacteria [1], 1 cm^2 of skin hosts around 10^6 bacteria [2], and 1 mL colonic content includes around 10^{11} bacteria [3]. Likewise, we observed dense microbial assemblages on the external surface of zebrafish eggs (Figure 1) [4].

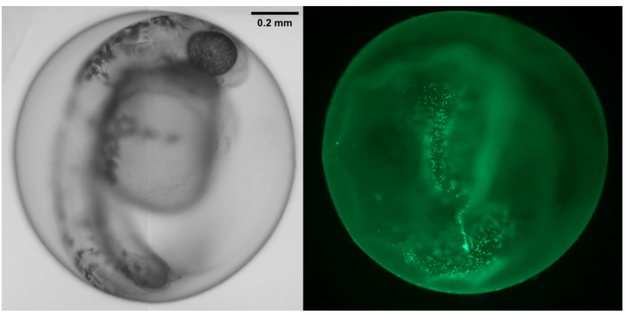

Figure 1. Dense microbial colonization on a zebrafish egg at one day post-fertilization.
Left: The zebrafish embryo develops inside of a protective (chorion) membrane;
Right: using a fluorescent dye (Syto-9), microbes can be observed on the chorion membrane.

Recent advances in the nanosafety field have shown that host-associated microbiota can significantly affect the toxicity of nanoparticles [5]. Mechanistically, this has mainly been explained by the intricate interactions between the host and microbes that colonize the exposure interface. Host-associated microbes aid in the digestion of food, help to fight off pathogens, protect against autoimmune inflammatory disease, and can even influence behavioral responses by signaling to the brains via the so-called gut-brain axis. This axis is formed by immune cells, hormones, and other biomolecules that functionally connect the intestinal nervous system with the central nervous system, and thereby facilitate the communication between the intestines and the brains [6]. These intricate interactions between microbiota and the host, however, can be disturbed by the detrimental effects of certain nanoparticles. In rodents, changes in the composition of microbiota caused by silica and titanium dioxide nanoparticles have already been shown to result in severe microbiota-dependent pathologies like lung inflammatory injury and colitis [7],[8],[9]. Interestingly, the opposite effects have been obtained by exposing animals with a perturbed microbiota community structure to these nanoparticles. In these cases, the nanoparticles restored the community structure of microbiota and cured the related pathologies [10].

At our lab in Leiden University, we have recently identified two additional mechanisms that contribute to microbiota-dependent nanoparticle toxicity [5]. In contrast to the above, these mechanisms concern the direct interactions between microbiota, the host and nanoparticles. In this Nanopinion, I will first explain the progress we have made in elucidating these interactions. Thereafter, I will discuss how the progress we have made can aid to more realistic safety assessment for nanoforms.

The first mechanism that we uncovered, concerns the signaling between microbiota and the host organism. Hosts can sense diverse microbial constituents (called 'microbe-associated molecular patterns', or shortly MAMPs) using receptors of the Toll-Like Receptor (TLR) family. Depending on their location, and the kind of MAMPs involved, TLRs can either initi-ate pro-inflammatory signaling cascades, resulting in the clearance of invading pathogens, or can dampen immune responses, increasing the tolerance to symbiotic microbes of the resident microbiota. In a series of experiments with zebrafish larvae, we have recently shown that these interactions can also affect the sensitivity of hosts to immunotoxic nanoparticles, like silver nanoparticles (nAg) [11]. We found that zebrafish larvae without functional TLR2 receptors, are more sensitive to the pro-inflammatory effects of nAg than larvae with functional TLR2 receptors. However, for germ-free larvae that lack microbiota, this protective effect of TLR2 receptors against nAg toxicity could not be observed. Based on this finding, and the related results presented in [11], we could conclude that signaling between resident microbiota and the host organism lowers the sensitivity of hosts to pro-inflammatory nanoparticles like nAg.

The second mechanism that we studied, concerns the adsorption of microbial metabolites to the nanoparticle surface. To a large extent, immune responses like the above depend on what external particle surface the host 'sees' [12]. Hence, we constructed models to predict the adsorption affinity of microbial metabolites to the nano surface. We specifically focused on metabolites that are produced, modified or regulated by the dense microbiota in the intestines. Our quantitative structure-activity relationship (QSAR) models predicted a general pattern of higher adsorption affinities to carbon nanoparticles than to metal nanoparticles. Small, aliphatic metabolites like short-chain fatty acids formed an interesting exception to this pattern, and exhibited higher predicted adsorption affinities to metal nanoparticles than to carbon nanoparticles. Altogether, the QSAR results suggested that microbial metabolites can adsorb to the particle surface via n - n stacking and hydrogen-bond interactions. Molecular dynamics simulations, which are based on physical models rather than statistical relationships, supported these QSAR results well [13]. These results indicate what biomolecules will potentially form the 'microbial fingerprint' on the surface of ingested nanoparticles that travel through the intestines.

So, where do these 'city buses' take us in the nanosafety field? Perhaps to multiple places. There are several opportunities to include this mechanistic insight into extrapolation strategies that support 'microbiota-inclusive' nanosafety predictions. For example, the evolutionary conservation of TLR2 can be used to predict similar effects for other host organisms comprising these receptors. Likewise, the specificity of microbiota-mediated interactions to the core material (carbon vs. metal) and biological activity (i.e. immunotoxicity) of nanoforms, can be employed for microbiota-inclusive nanosafety strategies. Firstly, regarding the core material, the distinct adsorption interactions for carbon and metal nanoparticles can be used to inform the safe and sustainable by design process for nanomedicines. For example, the adsorption of beneficial biomolecules to the nano surface will lower the concentrations that remain available to the host. If a nanomedicine is designed for patients with inflammatory bowel diseases, who already have lower concentrations of short-chain fatty acids in their intestines, choosing a carbon rather than a metal surface might reduce such undesired adsorption interactions. Secondly, considering the microbiota-dependent effects for immunotoxic nanoparticles, these results indicate that extra care should be taken for nanoforms exerting both antimicrobial and immunotoxic effects. In these cases, the loss of protective microbiota might sensitize hosts over chronic or repeated exposure regimes. As precautionary strategy to account for these potentially chronic effects, nanotoxicity data for germ-free organisms could be included in nanosafety databases. This would allow to perform a worst-case hazard assessment for immunotoxic effects in germ-free organisms. Combined, these strategies can further progress the important transition towards microbiota-inclusive nanosafety assessment.

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Biography of the author

Bregje Brinkmann is postdoctoral researcher at Leiden University's Institute of Environmental Sciences (CML), the Netherlands. Bregje obtained her doctoral degree in 2022 for her research on the effects of host-associated microbiota on nanomaterial toxicity [5]. With her postdoctoral research, she aims to contribute to extrapolation strategies (read across, cross-species extrapolation, acute to chronic toxicity extrapolation) for advanced materials. This work is part of the project 'EcoWizard' (ERC-C 101002123) awarded to professor Martina Vijver. Since 2023, Bregje is also Member of the Editorial Board of the journal Aquatic Toxicology.

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