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Review

Factors impacting the interactions of engineered nanoparticles with bacterial cells and biofilms: Mechanistic insights and state of knowledge

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ABSTRACT

Since their advent a few decades ago, engineered nanoparticles (ENPs) have been extensively used in consumer products and industrial applications and their use is expected to continue at the rate of thousands of tons per year in the next decade. The widespread use of ENPs poses a potential risk of large scale environmental proliferation of ENPs which can impact and endanger environmental health and safety. Recent studies have shown that microbial biofilms can serve as an important biotic component for partitioning and perhaps storage of ENPs released into aqueous systems. Considering that biofilms can be one of the major sinks for ENPs in the environment, and that the field of biofilms itself is only three to four decades old, there is a recent and growing body of literature investigating the ENP-biofilm interactions. While looking at biofilms, it is imperative to consider the interactions of ENPs with the planktonic microbial cells inhabiting the bulk systems in the vicinity of surface-attached biofilms. In this review article, we attempt to establish the state of current knowledge regarding the interactions of ENPs with bacterial cells and biofilms, identifying key governing factors and interaction mechanisms, as well as prominent knowledge gaps. Since the context of ENP-biofilm interactions can be multifarious—ranging from ecological systems to water and wastewater treatment to dental/medically relevant biofilms— and includes devising novel strategies for biofilm control, we believe this review will serve an interdisciplinary audience. Finally, the article also touches upon the future directions that the research in the ENP-microbial cells/biofilm interactions could take. Continued research in this area is important to not only enhance our scientific knowledge and arsenal for biofilm control, but to also support environmental health while reaping the benefits of the 'nanomaterial revolution'.

1. Introduction

Rapid progress in the field of nanotechnology in the past few decades has triggered the formation of an entire research field concerning the environmental health and safety (EHS) of engineered nanoparticles (ENPs). ENPs, especially certain metal-oxide nanoparticles, have been produced in large quantities and widely used in industry, agriculture, household goods, and consumer products, including sunscreens, cosmetics, electronics, food packaging, semiconductors, and even food products (Fairbairn et al., 2011; Godwin et al., 2009; Keller et al., 2010; Ma et al., 2013; Maurya et al., 2011; Odzak et al., 2014; Pang et al., 2012; Xia et al., 2013). Some of the commonly used ENPs in consumer products include TiO_2, ZnO, CeO_2, SiO_2, Al_2O_3, Fe_2O_3, Mn_2O_3, ZrO_2, and Fe_3O_4. (Amir et al., 2005; Mitrano et al., 2015; Piccinho et al., 2012; Joo and Zhao, 2017). Among them, the ones produced in the most significant quantities annually are SiO_2 (5500 tons/year), TiO_2 (3000 tons/year), and ZnO (550 tons/year) (Amir et al., 2005; Mitrano et al., 2015; Piccinho et al., 2012). Overall, the production of ENPs is expected to continue at the currently predicted pace of 58,000 tons/year through 2020 (Assessment, 2009). The scientific and research community, government agencies, and industry are well aware of the potential risks of ENP proliferation in the environment and are concerned about the associated EHS impacts. Thus, there have been considerable efforts in the last decade to assess potential EHS impacts of nanotechnology and to better understand the mechanisms of ENP interactions with the natural environment.

According to a report by the National Research Council (NRC) (Borm et al., 2006) on research strategy in the field of nano EHS, despite considerable progress there are still critical research gaps. One important gap noted by the NRC committee is identification of

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environmental compartments where nanoparticles can be distributed. It is clear that ENPs released into aqueous systems are distributed into several biotic and abiotic compartments (e.g., organisms or sediments). There is significant interest in understanding ENP exposure to aquatic biota (Griffitt et al., 2008; Manusadžianas et al., 2012) and any biomagnification (Judy et al., 2011; Werlin et al., 2011). One of the important biotic components that can be exposed to and store ENPs is microbial biofilms (Battin et al., 2009; Ferry et al., 2009; Kroll et al., 2014; Nevius et al., 2012). For example, an experimental study demonstrated that almost 60% of gold nanoparticles partitioned to biofilms in a laboratory-constructed estuarine mesocosm containing sea water, sediment, sea grass, microbes, biofilms, snails, clams, shrimp, and fish (John L. Ferry et al., 2009). Also, it is being increasingly recognized that biofilms represent an important biotic compartment in natural aquatic systems, yet little is known about ENP-biofilm interactions and thus such interactions constitute a key area for future research.

Microorganisms, being positioned at the base of the food web, are key drivers of biogeochemical cycles and natural ecosystem processes. As such, free-floating (or planktonic) microorganisms have been studied to investigate the microbes living in communities of biofilms. It is believed that more than 99% of microorganisms in nature reside in biofilms (Costerton et al., 1987) and that these microbial communities are omnipresent on all wetted surfaces – from the plaque that forms on teeth (Marsh, 2010) to the colorful biofilms found in hot springs in Yellowstone National Park (Sheehan et al., 2005). Thus, being a major mode of microbial existence in nature and having ubiquitous presence, biofilms significantly impact and contribute towards carbon fluxes at ecosystem and global scales (Paerl and Pinckney, 1996). Moreover, since biofilms are often used as a food source by marine animals, the biofilms can act as a via-medium for trophic transfer of ENPs to food webs (Abreu et al., 2007; Burns and Walker, n.d.; Lawrence et al., 2002).

Therefore, it is imperative to investigate the impact of ENPs on free-floating 'planktonic' microbes, as well as the interaction of ENPs with microbial biofilms. In particular, the antibacterial effect of ENPs, resultant toxicity mechanisms, and factors influencing the interaction between ENPs and bacteria need to be more clearly explained. Similar to nanoparticle-bacteria interactions, nanoparticle-biofilm interactions are highly complex and depend on the varying configuration and characteristics of ENPs (*e.g.*, size, surface functionalization, shape, charge), biofilms (*e.g.* EPS composition, surface roughness, density, bacterial species), and the environmental conditions surrounding them (e.g., temperature, pH, ionic strength, water hardness, organic matter). The few existing studies provide some sparse information on observed ENP effects on biofilms, with several varying parameters and measurements across the studies, making it difficult to draw fundamental and mechanistic conclusions.

In this review, we revisit and analyze how different characteristics of ENPs influence bacterial toxicity and corresponding mechanisms in order to comprehend the interactions of ENPs and bacterial cells. Similarly, mechanisms impacting ENP-biofilm interactions are examined according to biofilm characteristics and environmental factors. Given the increasing concerns about antibiotic resistant bacterial strains being present in water and the resultant public health issues, as well as the impact of nanoparticle-bacterial interactions on the biological treatment efficiency of contaminants of concern, this review provides a timely forum to discuss future research by conducting a survey of recent literature on this critical theme. The overall objective of this study is to review the advances in knowledge on the interfaces of ENPs with bacterial cells and biofilms, with a specific goal of examining factors and mechanisms that govern the interactions of ENPs with bacterial cells and biofilms.

2. Factors and mechanisms impacting the interactions of ENPs with bacterial cells

ENPs widely used in consumer products have been extensively investigated for their antibacterial nature and toxicity upon exposure to bacterial cells. Mechanisms responsible for the extent of toxicity are primarily influenced by physicochemical properties of ENPs including particle size, surface charge, polymorphism, shape, exposure time, and concentration. In this section, toxicity chemistry, photochemistry, transport interfacial phenomena, and kinetics of ENP-bacterial cell interactions based on ENP physicochemical properties and other factors affecting antibacterial behavior, as well as their associated mechanisms, are reviewed and analyzed.

2.1. Physicochemical properties and toxicity

Physicochemical properties of ENPs, including surface charge, size, polymorphism, and shape, have demonstrated critical roles in ENP toxicity and interactions (Gajapathi et al., 2015; Khare et al., 2014; Suresh et al., 2013). These characteristics of ENPs also affect their solubility, and aggregation, thus controlling the extent of toxicity and resultant mechanisms through the amount of released metal ions, reactive oxygen species (ROS) generation, adsorption, and hydrolysis, etc. Apart from those factors, background solution media (culture media), pH, ionic strength, surface charge, and the presence of other contaminants can also influence bacterial cell interactions with ENPs.

2.1.1. ENP size & solvent characteristics

Several studies show higher toxicity levels at smaller particle sizes (Lin et al., 2014; Palanikumar et al., 2014). Different morphologies also affect the degree of toxicity through cell surface interactions (e.g., higher toxicity from rods and spherical TiO_2 morphologies) (Tong et al., 2013). Similarly, spherical and smaller particles of tin oxide (SnO_2) revealed higher antibacterial behavior and severe membrane damage (Chávez-Calderón et al., 2016). While physicochemical properties of ENPs affect the toxicity, the solvent characteristics such as culture media, pH, and ionic strength also play an important role in the cellular effects of ENPs by impacting ENP stability, solubility, and reactivity (Borm et al., 2006; Lison and Huaux, 2011; Nel et al., 2006). As an example, ENPs in culture media with a higher charge can affect the stability of ENPs due to higher electrostatic repulsion, thereby preventing aggregation, enhancing interactions with cell membrane, and increasing cellular uptake. This indicates that the types of bacteria can also affect toxicity due to their peculiar membrane compositions and differing surface charges (Laha et al., 2014; Suresh et al., 2013).

2.1.2. ENP surface modification or coating

Modification of ENP surfaces and/or different surface charges due to coating materials can result in discrepancies in toxicity kinetics and their mechanisms. Some ENPs—such as CaO, MgO, and ZnO—have shown superior antibacterial effects mainly due to ROS generation from their oxide surfaces (Bhuyan et al., 2015; Nagajyothi et al., 2014). Interestingly, according to a recent study (Esmaeili and Farrahi, 2016), iron oxide nanoparticles coated with chitosan were effective for removing heavy metals in a bioreactor where a two-stage treatment system consisting of bacteria and the coated iron oxide nanoparticles was used. Similarly, the effectiveness of coated ENPs (ZnO coated with an extract of *Pongamia pinnata* leaves) has been illustrated for treating pathogenic bacteria (Sundrarajan et al., 2015). In the study, coating materials enhanced antibacterial activity of ZnO on both *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) organisms.

In addition to the ENPs coated with leaves extract, another study (Jafarirad et al., 2016) considered a fruit extract of *Rosa canina* acting as a reducing and capping agent for ZnO nanoparticles to examine its cytotoxicity towards the A549 cell line. The *Rosa canina* exhibited increased toxicity to cells as concentrations of ZnO nanoparticles