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Review article

Biomolecular coronas in invertebrate species: Implications in the environmental impact of nanoparticles

Laura Canesi[a,*], Teresa Balbi[a], Rita Fabbri[a], Annalisa Salis[b], Gianluca Damonte[b], Moritz Volland[c], Julian Blasco[c]

[a] Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Italy
[b] Centre of Excellence for Biomedical Research (CEBR), University of Genoa, Italy
[c] Instituto Ciencias Marinas de Andalucia, Consejo Superior de Investigaciones Científicas (ICMAN-CSIC), Cadiz, Spain

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ABSTRACT

The development of nanotechnology will inevitably lead to the release of consistent amounts of nanoparticles (NPs) in the environment. Invertebrates, that represent > 90% of animal species, widespread in different ecosystems, are emerging both as suitable target organisms and as models for evaluating the environmental impact of NPs.

Once released in different ecosystems, both NP intrinsic properties and those of the receiving medium will affect particle behavior. In particular, interactions with different biomolecules will lead to the formation of 'eco-coronas', that will influence NP bioavailability/uptake/toxicity in different environments, depending on the organisms present and the surrounding conditions. However, as shown in mammalian studies, the evaluation of the biological effects of NPs requires additional understanding of how, once within the organism, NPs interact at the molecular level with cells in a physiological environment, *i.e.* in biological fluids. Different types of NPs associate with serum soluble components, organized into a 'protein corona', which confers a biological identity to NPs and affects their interactions with target cells. In comparison, the study on NP-protein coronas in invertebrates is still at its infancy, since the protein composition of their extracellular fluids is largely unknown, given the large diversity of phyla and species. From the first demonstration of an AgNP protein corona in the body fluid of earthworms, NP-protein coronas are being characterized in marine invertebrates. The identification of protein coronas formed with different types of NPs (amino modified polystyrene- PS NH_2 and nano-oxides, n-CeO_2 n-TiO_2) in hemolymph serum of the marine bivalve *Mytilus galloprovincialis* is presented as a case study. The results indicate that, in *Mytilus* hemolymph, the formation of a biomolecular corona is partly NP-specific. The results obtained so far in terrestrial and marine invertebrates indicate that in each model, endowed with a peculiar protein repertoire, NP-coronas are characterized by unique protein components. The role of NP-protein coronas formed by lower organisms, and their possible contribution in evaluating the environmental impact of NPs are discussed.

1. Introduction

The development of nanotechnology will inevitably lead to the release of consistent amounts of nanoparticles (NPs) in the environment, with potential adverse effects on different organisms (Gottschalk et al., 2013; Liu et al., 2014; Caballero-Guzman and Nowack, 2016; Peng et al., 2017). Invertebrates, that represent > 90% of animal species, widespread in terrestrial, freshwater and marine ecosystems, are emerging both as suitable target organisms and as models for evaluating the environmental impact of NPs (Baun et al., 2008; Lee et al., 2010; Canesi et al., 2012; Canesi and Prochàzovà, 2013; Hayashi and Engelmann, 2013; Rocha et al., 2015; Canesi and Corsi, 2016). Most data on the biological effects of NPs are obtained from traditional ecotoxicity testing. However, evidence is accumulating on the complex interactions occurring between NPs and environmental media, affecting their behavior and consequent fate/bioavailability. These data underlined the importance to establish the criteria for reliability and relevance evaluation of quali-quantitative ecotoxicity data for nanomaterials (Petersen et al., 2015; Selck et al., 2016; Hund-Rinke et al., 2016; Hartmann et al., 2017).

In addition, information is needed on the interactions of NPs occurring at the molecular level in both the external and internal

* Corresponding author at: Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, 16132 Genoa, Italy.
E-mail address: Laura.Canesi@unige.it (L. Canesi).

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environment (*i.e.* exposure medium and biological fluids within the organisms), that will in turn affect NP uptake and toxicity (Canesi and Corsi, 2016). This will help understanding, from a more strictly biological perspective, the potential impact of NPs on target organisms in different environments. In this work, information on the possible interactions of NPs with biomolecules in the external environment, including proteins secreted by invertebrates, leading to the formation of 'eco-coronas' will be briefly summarized. Moreover, on the basis of mammalian studies, attention will be focused on the formation of 'NP-protein coronas' within biological fluids of invertebrates, and on first available data on their characterization in terrestrial and marine models. In particular, results obtained in the bivalve *Mytilus galloprovincialis* are reported as a case study. The role of NP-protein coronas in invertebrate species, and their contribution in evaluating the environmental impact of NPs are discussed.

2. NP behavior in environmental media: interactions with biomolecules leading to the formation of eco-coronas

Environmental implications of NPs are strongly linked to their intrinsic peculiar features (such as particle surface charge, size, shape, functionalization and coating, *etc.*), all properties that affect their interactions with the surrounding media and their consequent behavior (Klaine et al., 2008). Moreover, NP dispersion, aggregation and agglomeration are driven by the physico-chemical parameters of the receiving media (such as pH, ionic strength, temperature), as well as by the presence of natural colloids, both inorganic (*e.g.* ions, natural minerals), and organic. These latter (collectively termed as natural organic matter -NOM) include plant derivatives (mainly humic and fulvic acids) and complex mixtures of substances (polysaccharides, proteins, lipids, nucleic acids, *etc.*) produced by different organisms in their habitats (Keller et al., 2010; Petosa et al., 2010). These complex interactions will affect the fate of NPs in the water column, sediments and soils, and therefore their bioavailability, uptake and toxicity (Praetorius et al., 2014; Ren et al., 2016; Baalousha, 2017). Moreover, the presence of functional groups on the surface of NPs, more than their core composition (metal-based, carbon-based, *etc.*), may play a major role in sorption to biofilms in aqueous media, such as natural sea water (NSW) (Nevius et al., 2012). For instance, colloidal polymers produced by microorganisms (known as extracellular polymeric substances EPS) can influence aggregation and transformation of NPs in aqueous media, mainly as a function of the hydrophobic and electrostatic interactions (Kadar et al., 2014; Adeleye and Keller, 2016; Lin et al., 2016).

Overall, these interactions will lead to the formation of an environmental coating, or *'eco-corona'* around NPs and NP agglomerates/aggregates in both fresh and marine water, as well as in sediments and soils. However, there are still knowledge gaps on how different NPs released into the environment can interact with the enormous diversity of biological macromolecules present in different compartments. These interactions will result in consequent multiple effects on NP behavior, fate and biological impact (Canesi and Corsi, 2016; Ren et al., 2016; Baalousha, 2017).

The initially acquired *eco-corona* in each environmental compartment, in particular in the aquatic environment, will not only depend on general interactions between NPs and NOM or EPS. Aquatic organisms condition their surrounding medium in response to stress by releasing proteins and other biomolecules that are not present in reference exposure media. This is a well-established predator–prey response in aquatic food chains. The freshwater model *Daphnia magna* typically shows dramatic physiological responses (*i.e.* changes in feeding and reproduction rate) in response to changes in water quality or to the presence of signaling molecules from their predators (kairomones) (Stibor and Müller Navarra, 2000; Effertz and von Elert, 2014). Similarly, in response to metal exposure, secreted proteins bind metal cations, thereby reducing their toxicity (Calisi et al., 2014; Giner-Lamia et al., 2016). Actually, the term 'exoproteome' describes the protein content that can be found in the extracellular proximity of a given biological system. These proteins reflect the physiological state of the organism and are indicators of how living systems interact with their environments (Armengaud et al., 2012). In this light, proteins secreted by different organisms in response to NP exposure may specifically interact with different NPs, thus affecting their uptake and consequent toxicity. Recent evidence shows that specific NP-coating proteins can be secreted by different invertebrate species. Proteins released by *D. magna* neonates create an eco-corona around COOH- or NH_2- polystyrene NPs that caused destabilization of the NP dispersions over the subsequent 6 h (Nasser and Lynch, 2016). Secreted proteins identified by mass spectrometry included Type VI secretion system, a stress response protein, and QseC sensor protein used in cell-to-cell signaling. Independent of the protein composition, the amount of secreted protein increased over time, and this progressively increased particle instability and agglomeration. Interestingly, the eco-corona coated NPs resulted in a lower EC_{50} than equivalent uncoated NPs, and were less effectively removed from the gut. The Authors hypothesized that larger agglomerates are probably more attractive as a food source, leading to higher accumulation and increasing toxicity (Nasser and Lynch, 2016).

In the freshwater oligochaete worm *Limnodrilus hoffmeisteri*, exposed for 36 h to Few layer graphene (FLG) aqueous suspension, FLG body accumulation was demonstrated (Mao et al., 2016). Proteins secreted by the aquatic worm coated the FLG, thus increasing its stability and decreasing its size in suspension. Twelve types of secreted proteins were identified, although their biological role was not investigated. When the earthworm *Eisenia foetida* or *D. magna* were exposed to uncoated and protein-coated FLG, different accumulation was observed for either organism. In particular, exposure to coated-FLG increased and decreased the FLG body burden in *E. foetida* and in *D. magna*, respectively, in comparison to uncoated FLG. The agglomeration potential of FLG was the main factor affecting the body burdens of *D. magna*, while the soil remaining in the gut tract and interactions between the FLG and the gut epithelium may be more critical in earthworms (Mao et al., 2016).

Both examples indicate that the formation of an eco-corona around different types of NPs also depends on the organisms present and the surrounding conditions. These observations underline the importance of considering how interactions of NPs with proteins secreted by one organism may impact their consequent uptake and effects in other organisms. However, the results obtained so far indicate that the effect of protein eco-coronas on NP uptake and accumulation is mainly related to their impact on particle colloidal stability, rather than on specific epitope recognition. Although no data are available on particle coverage in eco-coronas, this may be also due to the low amount of protein secreted by the organisms in the surrounding environment, in contrast with biomolecular coronas formed from protein-rich biological fluids (see below). Future studies on eco-coronas may benefit from identification and quantification of secretory proteins in the corona with particular attention to the NP-protein ratio in the "conditioned" exposure medium.

3. Bio-coronas: the formation of NP-protein complexes in biological fluids

The importance of the possible interactions between NPs and the enormous diversity of biological macromolecules potentially present in different environmental compartments is being recognized. However, the evaluation of the possible biological impact of NPs requires additional understanding of how, once within the organism, NPs interact at the molecular level with cells in a physiological environment, *i.e.* in biological fluids. Studies in mammalian systems have taught us how different types of NPs can associate with serum soluble components, organized into a 'biomolecular corona' (bio-corona), which affects particle interactions with target cells (internalization and effects) (Cedervall et al., 2007; Fubini et al., 2010; Monopoli et al., 2012; Fleischer and Payne, 2014; Treuel et al., 2015; Tenzer et al., 2013;