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In vivo testing of gold nanoparticles using the *Caenorhabditis elegans* model organism

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ABSTRACT

Gold nanoparticles (AuNPs) are present in many man-made products and cosmetics and are also used by the food and medical industries. Tight regulations regarding the use of mammalian animals for product testing can hamper the study of the specific interactions between engineered nanoparticles and biological systems. Invertebrate models, such as the nematode *Caenorhabditis elegans* (*C. elegans*), can offer alternative approaches during the early phases of nanoparticle discovery.

Here, we thoroughly evaluated the biodistribution of 11-nm and 150-nm citrate-capped AuNPs in the model organism *C. elegans* at multiple scales, moving from micrometric to nanometric resolution and from the organismal to cellular level. We confirmed that the nanoparticles were not able to cross the intestinal and dermal barriers. We investigated the effect of AuNPs on the survival and reproductive performance of *C. elegans*, and correlated these effects with the uptake of AuNPs in terms of their number, surface area, and metal mass. In general, exposure to 11-nm AuNPs resulted in a higher toxicity than the larger 150-nm AuNPs. NP aggregation inside *C. elegans* was determined using absorbance microspectroscopy, which allowed the plasmonic properties of AuNPs to be correlated with their confinement inside the intestinal lumen, where anatomical traits, acidic pH and the presence of biomolecules play an essential role on NP aggregation. Finally, quantitative PCR of selected molecular markers indicated that exposure to AuNPs did not significantly affect endocytosis and intestinal barrier integrity.

Statement of Significance

This work highlights how the simple, yet information-rich, animal model *C. elegans* is ideally suited for preliminary screening of nanoparticles or chemicals mitigating most of the difficulties associated with mammalian animal models, namely the ethical issues, the high cost, and time constraints. This is of particular relevance to the cosmetic, food, and pharmaceutical industries, which all have to justify the use of animals, especially during the discovery, development and initial screening phases. This work provides a detailed and thorough analysis of 11-nm and 150-nm AuNPs at multiple levels of organization (the whole organism, organs, tissues, cells and molecules).

1. Introduction

Many of the products used in our daily life contain nanoparticles, gold nanoparticles (AuNPs) in facial creams [1], silver nanoparticles in preservatives [2], or zinc oxide nanoparticles and titanium dioxide nanoparticles in colorants and sunscreens [3]. For example, colloidal AuNPs hold great promise in cosmetics and as therapeutic and diagnostic agents due to their inertness (which limits their toxicity to cells) and their unique optical and photothermal properties. The latter can be controlled and tuned by changing the size, shape and surface functionalization of AuNPs [4,5].

AuNPs have been selected by the Working Party on Manufactured Nanomaterials of the Organization for Economic Cooperation and Development (OECD) as an example of

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manufactured nanomaterials which are either in commercialization or likely to enter the market in the near future [6]. Indeed, some AuNPs formulations have already been proposed as novel tools for *in vitro* and *in vivo* molecular imaging and drug delivery [7]. AuNPs have, for example, attracted interest as carriers to enhance the oral absorption of drugs and vaccines that are either poorly absorbed or are susceptible to gastrointestinal degradation [8]. However, to our knowledge, only AuNPs used for local heat generation in the plasmonic photothermal therapy of atherosclerosis and cancer have, to date, reached clinical stage (ClinicalTrials.gov Identifiers NCT01270139, NCT01679470, NCT00848042, NCT00436410) [9]. In addition, AuNPs are present in day to day products such as anti-ageing creams and masks, toothpastes, and are even marketed as food supplements according to the Consumer Products Inventory, which is compiled by the Project on Emerging Nanotechnologies [10]. However, the regulations on nanoparticles are being tightened and the use of animal-free alternatives to evaluate new materials is being actively promoted. For instance, in March 2013, EU regulations on Cosmetics and Household Products banned the use of animals to assess the safety of these products. The North American Food and Drug Administration (FDA) also supports the development and use of alternatives to animal testing to assess the safety of cosmetic products [11]. Hence, there is a pressing need to develop new platforms and approaches to evaluate AuNPs-containing products, in particular to hasten the early stages of safe nanoparticle development in the cosmetic, food, and pharmaceutical industries.

In this context, animal models such as *Caenorhabditis elegans*, *Drosophila melanogaster* or *Danio rerio* allow scientists to obtain primary data on engineered nanomaterials in a simple biological system, and in doing so they face less strict regulations and ethical issues compared to research conducted with mammals. These model organisms are compatible with high-throughput screens, even microfluidic technologies, which in turn accelerate the path of novel materials to the market [12,13]. *C. elegans* is a worm which exhibits 60–80% genome homology with humans and shares a multitude of biological traits in terms of physiology, anatomy, and metabolism (Fig. 1) [14,15]. The use of these 1-mm long animals, which naturally live in decaying organic matter in the soil, allows a cost-effective initial biological assessment of nanomaterials within chemical laboratories [16]. In addition, their transparency, small size, prolific and short life-cycle, and few requirements of maintenance facilitate the study of the interaction between nanomaterials and a multicellular organism [16]. The external part of the worm, the cuticle, can be used as a skin model given that its function and composition is analogous to human skin (Fig. 1A) [14,17,18]. The *C. elegans* intestine also shares a similar cellular architecture with higher animals with respect to cell polarity of the intestinal cells (enterocytes) including the presence of apical and basolateral domains, cell junctions, and the presence of microvilli forming the brush border (Fig. 1B). Setting aside some differences in the composition, both mammals and *C. elegans* encompass a pertrophic-like layer that protects the microvilar surface of the gut [14,19–23]. Furthermore, the mechanisms of transport of biomolecules through biological barriers are highly conserved [24–26]. Therefore, *C. elegans* offers promising features and valuable tools to evaluate the delivery of topical and oral nanomaterials before moving to more complex model organisms [16,27]. *C. elegans* can be used to track NPs through different biological barriers (dermal and intestinal) and multiple levels of organization (the whole organism, organs, tissues, cells and molecules).

Here, we report how advanced optical techniques such as two-photon luminescence microscopy (TPLM) [28], together with an array of materials science characterization techniques and state-of-the-art electron microscopy protocols [29] can be applied to study and quantify the nano/bio interaction between monodisperse small and large citrate-coated AuNPs in *C. elegans*. The biodistribution of AuNPs inside the worm was evaluated by microscopy at multiple scales, moving from micrometric to nanometric resolution and from organs to cells. These analyses were further complemented by investigating the effect of AuNPs on the survival and reproductive performance of *C. elegans*, which were correlated with the uptake of AuNPs. We determined NP status inside *C. elegans* using absorbance microspectroscopy and related the plasmonic properties of AuNPs with NP confinement in different anatomical areas within *C. elegans*. Finally, we selected several transcriptional markers to study whether AuNP exposure affects endocytosis and intestinal barrier integrity.

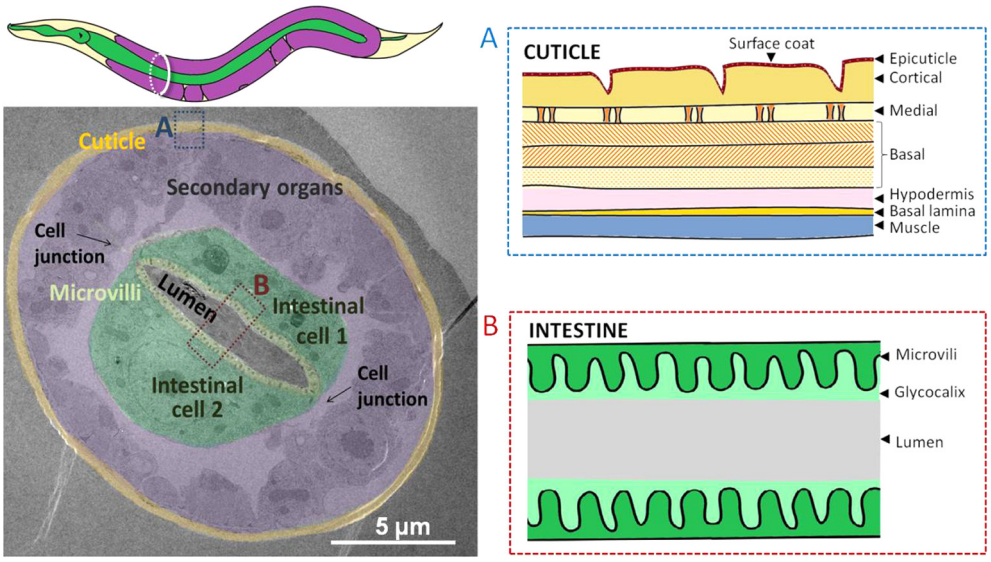

Fig. 1. Anatomy of *C. elegans*: the alimentary system, the cuticle, and secondary organs, including the reproductive system. Left panel shows a modified TEM image of a *C. elegans* cross-section, as marked in the drawing above. Right panel shows a magnification of A) the cuticle and B) the intestine, detailing their parts.