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Plasmonic nanoparticles and their characterization in physiological fluids

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ABSTRACT

Nanoparticles possess unique properties beyond that of classical materials, and while these properties can be used for designing a dedicated functionality, they may also pose a problem to living organisms, to human health and the environment. The specific primary routes by which nanoparticles may interact with the human body include inhalation, injection, ingestion and application to the skin. Independent of the entry route, the particles inevitably encounter a complex physiological fluid populated with *e.g.* proteins, vitamins, lipids and salts/ions. Different consequences of such an encounter may include formation of a surface-bound protein layer, particle dissolution or aggregation, which are expected to have a crucial impact on cellular interaction. Understanding cellular responses to nanoparticle interactions starts with understanding particle behavior in physiological fluids. Nanoparticles are now available in practically any size, shape and functionalization, to promote distinct optical, magnetic, and physico-chemical properties, making the prediction of their behavior, in physiological fluids, not a trivial task. Characterization has therefore become of paramount importance. In this review, we give an overview about the diversity of physiological fluids as well as present an inventory of the most relevant experimental techniques used to study plasmonic nanoparticles.

1. Introduction

The development of "nano" started over 20 years ago, with nanoscale science and technology now having an increasing impact on many aspects of our daily lives. The enormous potential and rapid expansion of nanotechnology has resulted in an exploding array of engineered nanomaterials, which are increasingly being used for commercial purposes [1] but with clear differentiation across industry sectors. Typically, materials applications are reaching the markets first, with electronics and IT applications following closely behind [2]. Nanobiotechnology and nanomedicine have the potential to revolutionize diagnostic tools and treatment strategies, which are more personalized, efficient, or easier to administer. However, healthcare and life sciences applications have the longest time-to-market: approval by the regulatory agencies, technological adjustments in manufacturing [3], and potential risks associated with engineered nanomaterials are just some of the reasons for the comparatively slow development of nanomedicines.

Nanoparticles (NPs) or sources of NPs can be classified as natural or intentional/engineered and unintentional anthropogenic activities [4]. Humans can come in contact with engineered NPs in many different ways such as through the use of consumer products containing NPs (*i.e.* food and cosmetic products), at the working place (*i.e.* occupational exposure), during disposal of the products (*i.e.* incineration) or by the intended use of NPs in biomedical applications. The possible portals where those NPs may enter the human body are the lungs *via* inhalation, the gastro-intestinal tract *via* digestion, the skin, and blood vessels *via* intravenous injection [5]. No matter if the exposure is intended or occupational, NPs will interact at a certain time with the cells of the human body. One particular fraction of engineered NPs may be defined as 'designer' particles, having a desired functionality. These particles are synthesized mostly within the walls of academic and research centers laboratories generally aiming at obtaining uniform particles. In recent years, a lot of progress was made in the synthesis of such designer NPs, which have been designed in many sizes and morphologies [6–8] to produce distinct optical, magnetic, and physico-chemical properties.

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Over the past two decades, much research has been dedicated to the study of NP-cell interactions in order to advance nanomedical applications, while determining if the potential benefits of nanotechnology could be achieved without any adverse impact upon human health. More specifically, significant effort has been dedicated to elucidate the impact of physicochemical properties of NPs, such as their size, surface charge, hydrophobicity or shape, on their interaction with cells. This interaction is routinely assessed *in vitro* to reveal mechanistic insights [9–11]. Despite the overwhelming use of *in vitro* systems to evaluate cellular responses toward NPs, the impact of an omnipresent complex physiological fluid on the material is still insufficiently taken into consideration [12–14].

2. Physiological fluids

Upon contact with physiological fluids, NPs can interact with a wide range of biomacromolecules [15], with various consequences. In order to understand the behavior of NPs in physiological fluids it is imperative to summarize the composition of the non-cellular compartments, such as mucus (gastro-intestinal (GI) or respiratory tract) or aqueous lining layer covered by surfactant (lung parenchyma), the blood or the lymphatic fluid, as well as available methods to investigate the possible interactions.

2.1. Mucus

Mucus is a biopolymer-based hydrogel produced by specific cells such as goblet cells and its main function is the protection of epithelium against infectious agents. Mucus is rich in water (>95%) and glycoproteins but also contains enzymes, immunoglobulins, proteins and salts, with the composition varying between each mucosal surface [16,17]. If particles are ingested or inhaled, they can be deposited onto the mucus, and through subsequent displacement entrapped in mucin fiber mesh, depending on their size.

The interaction of NPs with the mucus can cause a change in particle size and zeta potential. The majority of experiments to study these parameters are usually performed using isolated mucus collected from animals [18]. Recently it has been shown that small-angle neutron scattering (SANS) is a powerful technique to investigate the interaction of mucus proteins/enzymes with NPs and enables to correlate the changes in comparison to the bare NPs with mucus permeation [19]. Recently, a combination of tools, such as cryo-scanning electron microscopy, capillary penetration, and optical tweezers, was applied to assess particle mobility in mucus and model hydrogels, and penetration of particles on various length scales. It has been shown that particle mobility is dependent on the highly rigid structures within the mucus mesh but also on the adhesive properties of the particles [20]. In addition to the physicochemical parameters, the muco-adhesive properties of NPs can be determined by applying rheological measurements [21]. Another possibility is to use the Ussing chamber. With it, it has been demonstrated that the diffusion efficiency of neutral NPs was higher in comparison to uncharged particles [22]. In addition, the pH of mucus varies according to the location within the body, and the interaction of NPs with the mucin polymers can be tuned by buffer conditions such as pH and ionic strength [23].

The respiratory tract is covered by pulmonary surfactant, which consists 85–90% of phospholipids and specific surfactant proteins (SP) [24]. The binding of those constituents onto the surface of a NP can be determined by thin layer chromatography for lipids and gel electrophoresis (SDS-Page) combined with liquid chromatography/tandem mass spectrometry (LC/MS/MS). By using a specific fixation method to stabilize the phospholipids and liquid lining layer, Raemy et al. have shown that it is possible to visualize NPs in the aqueous lining layer (Fig. 1), indicating that this is an important compartment where particle properties can change, depending on the environment [25].

The airway mucus varies in composition and amount along the airway tree. The airway epithelial goblet cells and submucosal glands secrete mucus, forming a two-layer mucus blanket over the ciliated epithelium, *i.e.* a low-viscosity sol layer covered by a high-viscosity gel layer and a glycocalyx [26]. Insoluble particles are trapped in the gel layer and are moved toward the pharynx (and ultimately to the GI tract) by the upward movement of mucus generated *via* metachronous beating of the cilia also known as mucociliary clearance. The aqueous lining layer height in the lung parenchyma is very thin, *i.e.* about 5 μm as reported for primary human epithelial type II cells in culture [27], therefore, the volume is also very small and makes it difficult to collect it *in vitro* or *in vivo* to determine the colloidal behavior of NP in this liquid. An alternative approach is to use artificial alveolar fluid, and it has been shown that incubation of this liquid with silver NPs induces aggregation

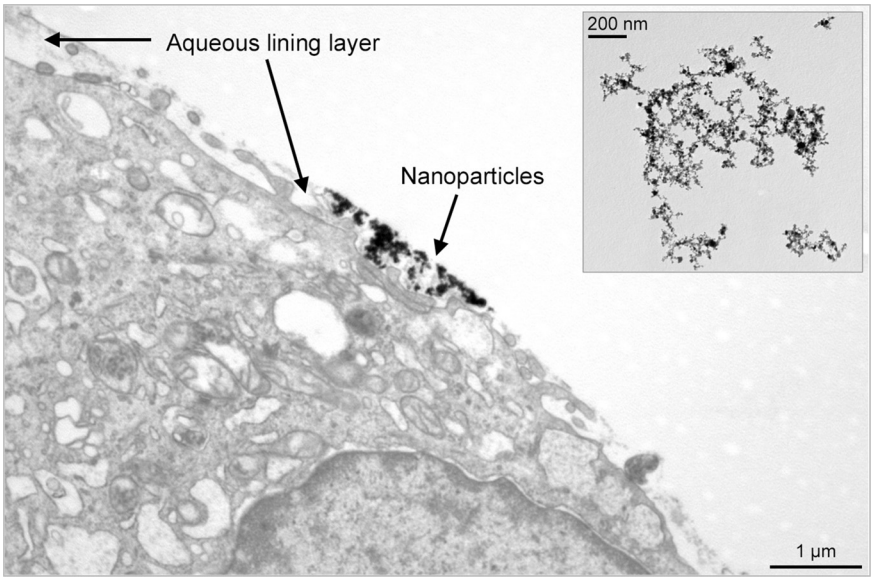

Fig. 1. TEM micrograph of a human epithelial II cell *in vitro* (A549 cells) incubated at the air–liquid interface and exposed to aerosolized NPs (inset). After 10 min of incubation the NPs are mainly localized in the aqueous lining layer formed by the cells before they are internalized.

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