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Pathophysiologic mechanisms of biomedical nanomaterials

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ABSTRACT

Nanomaterials (NMs) have been widespread used in biomedical fields, daily consuming, and even food industry. It is crucial to understand the safety and biomedical efficacy of NMs. In this review, we summarized the recent progress about the physiological and pathological effects of NMs from several levels: protein-nano interface, NM-subcellular structures, and cell–cell interaction. We focused on the detailed information of nano-bio interaction, especially about protein adsorption, intracellular trafficking, biological barriers, and signaling pathways as well as the associated mechanism mediated by nanomaterials. We also introduced related analytical methods that are meaningful and helpful for biomedical effect studies in the future. We believe that knowledge about pathophysiologic effects of NMs is not only significant for rational design of medical NMs but also helps predict their safety and further improve their applications in the future.

1. Introduction

In the past decades, a number of engineered nanomaterials (NMs) have been developed and widely used in environment, occupational/industrial places, daily consumer items, and biomedical fields (Oberdörster et al., 2005; Chen et al., 2013; Dreaden et al., 2011; Setyawati et al., 2015; Peer et al., 2007). Compared to bulk materials, NMs serve as more efficient platform in biomedical fields because they have a larger surface-to-volume ratio, more tunable size, shape, easier design of surface chemistry and constructed structures, which offer larger space and stronger capacity to adsorb and load more therapeutic molecules. For example, carbon NMs are commonly used in gene and drug delivery and also as near infrared region imaging agents, which cover several carbon members such as fullerenols and the derivatives, nanotubes, graphene, nanospheres, nanodiamond, and nanodots (Gong et al., 2013; Liu et al., 2012; Wang et al., 2015a; Yan et al., 2012; Yang et al., 2012b). Mesoporous silica NMs also have expanded surface for molecule adsorption and controllable release of them in temperature and pH-dependent manner, serving as a multifunctional nanocarrier (Gao et al., 2011; Tay and Leong, 2015; Zhang et al., 2012c). Furthermore, NMs can serve as biomimetic catalysts due to their good ability to transfer electrons to generate or quench free radicals (He et al., 2011; He et al., 2013; Hu et al., 2013; Wang et al., 2015a) and also exhibit strong reactivity as powerful platforms for anti-cancer (Min et al., 2012; Pan et al., 2015; Yin et al., 2009) and anti-bacterial application (Hu et al., 2013; Zhao et al., 2014).

Biomedical NMs have been used in biosensing, imaging, and photothermal and photodynamic therapies. For example, when gold nanoparticles are irradiated by photons at specific wavelength, photons can interact with surface and strong absorption and/or scattering of light will occur due to surface plasmonic properties (Chen et al., 2013). As a result, the nanoparticles are able to absorb photons and immediately convert them into heat for photothermal therapy (Huang et al., 2006; Zhao et al., 2015). Meanwhile, the ability of light absorbing and scattering of visible/near infrared light and X-ray enables them as promising imaging tools such as light scattering imaging, two-photon luminescence imaging, photoacoustic tomography, and X-ray computed tomography (Dreaden et al., 2011; Saha et al., 2012). The combination of hyperthermia and imaging makes NMs attractive nanoplatforms in disease theranostics (Gu et al., 2013; Wang et al., 2014; Zhang et al., 2012c).

As mentioned above, NMs have been used as nanocarriers, theranostic agents, tissue engineering materials, and antibacterial coatings for medical dressing, *etc*. Oral, dermal, inhalation, and injection are common exposure routines to biomedical NMs. For example, nanocarriers can not only be injected into blood to deliver genes and drugs but also be inhaled in the formulation of mist spray (Kleinstreuer et al., 2008). Moreover, NMs can undergo digestion in the gastrointestinal tracts and intake by intestinal epithelium and then may enter the blood (Elder et al., 2009). The exposure diversity influences approaches used to evaluate potential effects of nanoparticles. Thus, various interaction modes between NMs and biological systems should correlate to the exposure routines.

To focus on the safety and performance efficacy of biomedical NMs, it is necessary to use proper approaches to study the interaction between NMs and biological entities in different levels including biomolecules, cells, tissues, biological barriers, and individuals (Nel et al., 2009;

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Zhao et al., 2008). On one hand, physicochemical parameters such as size, shape, surface chemistry, hydrophobicity and hydrophilicity, chirality, aggregation, degradability, and catalytic ability influence the interaction and biological responses (Nel et al., 2009; Wang et al., 2015c). Ideal NMs may be designed to target organelles, cells, and tissues where they have good performance for therapy and diagnosis. The desired functions of biomedical NMs include the treatment with diseased cells or organs, the repression in cancer, the controllable release of drugs and genes, and theranostics under certain external stimuli (Peer et al., 2007; Sanhai et al., 2008). However, NMs may induce some side effects by activating or depress some signal pathways, inducing oxidation stress and inflammation, changing metabolism, damaging tissues or organs, and causing toxicity (Deng et al., 2011; Hu et al., 2013; Zhou et al., 2012; Nel et al., 2009; Zhang et al., 2013; Zhou et al., 2014a,b,c). On the other hand, the behavior and fates of NMs in the body are modulated by microenvironment. During the exposure of NMs *in vivo*, protein adsorption is the early event that may change the structure and function of proteins. As a result, the adsorbed protein may serve as a natural shield to protect from the damage by toxic NM surface (Ge et al., 2011; Hu et al., 2011; Monopoli et al., 2011; Wang et al., 2013b). Adsorption of proteins in the blood may also overlay the functional surface to lose target ability of designed NPs that induces a major accumulation by macrophages (Larson et al., 2012; Walkey et al., 2012). Protein adsorption may also activate inflammatory responses (Deng et al., 2011). Moreover, acidic environment such as in the gastrointestinal tract and the endosomes/lysosomes play crucial roles in causing the dissolution of metal and metal oxide NMs to induce toxicity and oxidative damage (Jiang et al., 2015; Magdiel et al., 2015; Wang et al., 2011; Zhu et al., 2012a).

In this review, we focus on some aspects of the pathophysiologic effects from the interaction of inorganic NMs with biological systems. In details, we will review the protein adsorption on NMs, the interaction between biological membrane and NMs, the interaction between NMs and biological barriers, and their influences on signaling pathways. Finally, we summarize some up-to-date techniques in studying the biomedical effects of NMs.

2. Protein adsorption that affects biomedical effects

NMs can adsorb proteins once they are exposed in physiological fluids (Monopoli et al., 2012). Surface free energy of NMs is higher than bulk materials with unsaturated electron structures when the ratio of surface-to-bulk atoms and surface curvature increases. Protein adsorption can reduce the surface energy. For example, with complex protein components, NMs injected in the blood can adsorb albumin, fibrinogen, transferrin, immunoglobulin, lipoproteins, and complements to form multiple protein coronas on NMs (Monopoli et al., 2011; Tenzer et al., 2013). These proteins have distinct adsorption stability and amount on the surface due to the physicochemical properties of NMs and proteins. As we know, protein adsorption and dissociation is kinetic process. Hard corona has long term lifetime and slow rate to be exchanged by others, while soft corona has a low adsorption stability that is easy to be replaced by other proteins around. The corona stability, structure, composition, quantity, and surface coverage on NMs are important to understand the states of protein adsorption (Lundqvist et al., 2008). The interfacial status can be characterized by experimental methods such as NMR (Brancolini et al., 2012), CD (Ge et al., 2011), HPLC-MS (Walkey et al., 2012), TEM (Wang et al., 2011), AFM (Ge et al., 2011), X-ray absorption structures and theory prediction with computer simulation (Wang et al., 2013b).

It is crucial to study the interaction of NMs with proteins because the protein-NM complex takes part in various biological process, influences physiological functions, and may induce pathological changes. In details, the coated proteins can serve as a natural shell to prevent cell membrane from contacting toxic surface of NMs, which decreases cell membrane damage and the induced necrosis. Recent studies have shown that hydrophobic NMs such as carbon nanotubes (Fig. 1a–d) (Ge et al., 2011) and graphenes (Hu et al., 2011), surfactant-modified gold nanorods (AuNRs) (Fig. 1e–l) (Wang et al., 2013b), and silica NPs (Lesniak et al., 2013) can directly adsorb and highly adhere on cell surface to cause the cell membrane rupture and necrosis in the serum-free medium. Interestingly, these NMs don't cause acute damage to the cell membrane after protein adsorption in serum-containing system. The reason is that hydrophobic surface or cationic surfactants can directly interact with lipid layers in cell membrane by extracting lipids or inducing defects that increases membrane permeability to form acute damage. In the presence of serum, a plenty of serum proteins can adsorb on the surface of NMs *via* physical forces or chemical binding. The protein layers may serve as organic coatings, block toxic surface of NMs, and decrease the direct contact between cell membrane and NMs. Actually, the properties of NMs determine the toxicity, while protein binding will change the physicochemical properties of NMs that influence the toxicity outcomes. Sometimes, protein coatings serve as an organic shield to the surface in order to prevent the acute toxicity originating from the surface properties of NMs. Furthermore, the adsorption may change structures of immunity-associated proteins and then influence their functions. Fibrinogen (Fg) is a major human plasma protein that doesn't provoke inflammatory responses without specific stimulus. When Fg interacts with poly(acrylic acid)-coated gold nanoparticles (PAA-GNPs), the secondary structures of Fg will be unfolded that can activate Mac-1 receptor pathway of monocytes to produce pro-inflammatory effects (Deng et al., 2011). In addition, protein-NM adsorption influences cellular uptake and targeted recognition. Bovine serum albumin (BSA), immunoglobulin G (IgG), and transferrin (Tf) are the most abundant proteins adsorbed on the same positively charged GNPs. Among three types of GNPs with different protein coatings, BSA-adsorbed GNPs have the least cellular uptake by human cervical carcinoma cells (HeLa) (Zhu et al., 2012b). As an abundant glycoprotein in the extracellular matrix and serum, vitronectin was found to adsorb on TiO_2 NPs surface, resulting in clathrin-dependent mode as a dominant endocytosis pathway (Tedja et al., 2012). Moreover, proteins can adsorb on NMs non-specifically to mask or replace the target ligands on NMs and prevent cell surface receptors to recognize target NMs, which limits the efficacy of target therapy (Salvati et al., 2013). As a result, the designed NMs cannot selectively accumulated in diseased tissues or cells but be cleared by the scavenger cell, macrophages (Larson et al., 2012; Walkey et al., 2012).

NMs can also recognize and contact membrane receptors to affect receptor-associated signaling pathways. Deng et al. found that fibrinogen is one of the major proteins bound to PAA-GNPs in human plasma to form fibrinogen/PAA-GNP complexes that changes the secondary structure of fibrinogen and thus selectively bind to Mac-1 receptors on the surface of monocytes. As a result, the complexes induce inflammatory responses by activating NF-κB pathway and promoting the release of cytokines. However, neither fibrinogen nor PAA-GNPs activates NF-κB pathway to release related cytokines (Deng et al., 2011). NMs can also interact with receptor protein to modulate cell differentiation. A recent work has reported that carboxylated MWCNTs can bind to bone morphogenetic protein receptors (BMPR) to form CNT-corona. As a result, CNT corona promotes the differentiation of mouse myoblast cells into myocytes (Zhang et al., 2012b). The electrostatic, hydrophobic and pi–pi stacking interactions involve in the formation of CNT-corona. Binding of CNTs to BMPR2 suppresses BMPR signaling activity that prevents the phosphorylation of BMPR1 and decreases the expression of id genes, but activated myogenin-dependent pathways (HEB-MyoD) to initiate the differentiation into muscles (Zhang et al., 2012b).

3. Nanomaterials and their biological membrane interaction, intracellular trafficking and biomedical effects

Cell membrane and the inner membrane for organelles take part in the process of internalization, translocation, accumulation, and