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Cell type-specific responses of peripheral blood mononuclear cells to silver nanoparticles

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ABSTRACT

Silver nanoparticles (Ag-NP) are increasingly used in biomedical applications because of their remarkable antimicrobial activity. In biomedicine, Ag-NP are coated onto or embedded in wound dressings, surgical instruments and bone substitute biomaterials, such as silver-containing calcium phosphate cements. Free Ag-NP and silver ions are released from these coatings or after the degradation of a biomaterial, and may come into close contact with blood cells. Despite the widespread use of Ag-NP as an antimicrobial agent, there is a serious lack of information on the biological effects of Ag-NP on human blood cells. In this study, the uptake of Ag-NP by peripheral monocytes and lymphocytes (T-cells) was analyzed, and the influence of nanosilver on cell biological functions (proliferation, the expression of adhesion molecules, cytokine release and the generation of reactive oxygen species) was studied. After cell culture in the presence of monodispersed Ag-NP (5–30 μg ml^{-1} silver concentration), agglomerates of nanoparticles were detected within monocytes (CD14+) but not in T-cells (CD3+) by light microscopy, flow cytometry and combined focused ion beam/scanning electron microscopy. The uptake rate of nanoparticles was concentration dependent, and the silver agglomerates were typically found in the cytoplasm. Furthermore, a concentration-dependent activation (e.g. an increased expression of adhesion molecule CD54) of monocytes at Ag-NP concentrations of 10–15 μg ml^{-1} was observed, and cytotoxicity of Ag-NP-treated monocytes was observed at Ag-NP levels of 25 μg ml^{-1} and higher. However, no modulation of T-cell proliferation was observed in the presence of Ag-NP. Taken together, our results provide the first evidence for a cell-type-specific uptake of Ag-NP by peripheral blood mononuclear cells (PBMC) and the resultant cellular responses after exposure.

1. Introduction

Nanoparticles are defined as particles in the size range of 1–100 nm in diameter [1]. They are increasingly used in various products, such as food, electronics, cars and clothing, as well as in different biomedical applications. It is generally expected that the use of nanoparticles will increase in the future. Currently, silver nanoparticles (Ag-NP) have one of the highest degrees of commercialization among the nanomaterials, mainly due to their characteristic antimicrobial properties [2]. Silver compounds have already been used clinically to reduce skin infections in the treatment of burns (e.g. silver sulfadiazine) and to prevent bacterial colonization on various surfaces, such as catheters and prostheses [3–7]. The advantage of Ag-NP is the dramatic increase in the specific surface area for particles with a size in the range of a few nanometers. Such an extended specific surface area will lead to an effective silver ion release in parallel with a low total silver concentration in the coated or impregnated object or implant [8]. Previous studies have demonstrated that the silver ions released from silver coatings or compounds are the bioactive component. These ions (Ag^+) exert antimicrobial effects on a wide spectrum of microorganisms, including *Staphylococcus aureus*, *Escherichia coli* and many fungi [9–11]. In this context, Ag^+ has been reported to interact with a variety of microbial molecules, such as DNA [2], cell wall components or the sulfhydryl groups of metabolic enzymes [7,9], causing the interruption of bacterial replication, membrane permeability and different metabolic enzymes in the bacterial electron transport chain [12]. Considering the increasing use of nanosilver products, it is crucial to understand the biological effects of Ag-NP on human cells.

Nanoparticles may enter the human body through inhalation, ingestion, injection for therapeutic purposes and dermal contact

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[13]. It has been shown that injected Ag-NP are translocated to the circulatory system and distributed throughout the main organs of the body, especially the kidney, liver, spleen and brain [14]. In previous studies, we have demonstrated that polyvinylpyrrolidone (PVP)-functionalized Ag-NP (50 nm metallic core) exerted cytotoxic effects on tissue cells, such as mesenchymal stem cells (hMSC), at high concentrations, but also induced cell activation at sublethal concentrations [15]. Thus, the PVP polymer itself did not exert any influence on the toxicity. Furthermore, we have shown that these Ag-NP can enter hMSC as nanoparticles and then agglomerate inside the perinuclear region within the endolysosomal cell compartment [16].

After the implantation of nanosilver-containing or -coated biomaterials, blood cells will come into close contact with the surfaces of these materials. In the blood, leukocytes represent a cellular host defense system that is able to release various inflammatory mediators after cell activation. It has been shown that cells respond to the exposure of metallic nanoparticles by the generation of reactive oxygen species (ROS), which in turn initiate inflammation, as indicated by the release of pro-inflammatory cytokines [17–19]. The induction of intracellular oxidative stress is a mechanism that can trigger several biological responses (e.g. inflammation) [20].

Therefore, we investigated the biological effects of well-characterized Ag-NP at non-toxic concentrations on human peripheral blood mononuclear cells (PBMC). Because silver ions (silver acetate) are continuously released from Ag-NP, and silver ions are believed to be responsible for biological effects, we also analyzed the effects of silver ions on these cells. PBMC mainly consist of lymphocytes (e.g. T-cells) and monocytes, and represent a well-defined subpopulation of host defense cells [21]. We specifically investigated the uptake of nanosilver into these cells by light microscopy, focused ion beam milling and flow cytometry (side scatter signal). Subsequently, the cell response after ingestion of Ag-NP was analyzed by cytokine release, the expression of activation/adhesion molecules and the generation of ROS.

2. Materials and methods

2.1. Synthesis of silver nanoparticles

PVP-coated Ag-NP were synthesized by reduction with glucose in the presence of PVP, as previously described [16,22]. The PVP polymer stabilizes the particle nature of Ag-NP because pure Ag-NP are unstable with respect to agglomeration. The final silver concentration in all of the dispersions was determined by atomic absorption spectroscopy (AAS; Thermo Electron Corporation, M-Series). The hydrodynamic diameter and the zeta-potential of the dispersed particles were measured by dynamic light scattering (DLS) with a Malvern Zetasizer Nano ZS. The polydispersity index was below 0.3 in all cases, indicating the absence of larger agglomerates. Scanning electron microscopy (FEI Quanta 400 ESEM instrument) revealed that the Ag-NP used had a spherical shape, with a metallic core of 70 ± 20 nm (Fig. 1A); the hydrodynamic diameter of the nanoparticles was 75 ± 20 nm, as measured by DLS (Fig. 1B). The hydrodynamic diameter includes the polymer layer and the hydration shell; therefore, it is always larger than the pure metal diameter of the silver core, as determined by electron microscopy under high vacuum [23]. The zeta-potential was approximately −25 mV.

PVP (K30, Povidon 30; Fluka, molecular weight 40,000 g mol^{-1}), silver nitrate (Fluka, p.a.), and D-(+)-glucose (Baker) were used for the synthesis of the Ag-NP. Ultrapure water was prepared with an ELGA PURELAB Ultra instrument.

2.2. Isolation of PBMC

PBMC were isolated by a single-step procedure that was based on a discontinuous double-Ficoll gradient described by English and Andersen [24]. Briefly, ethylenediaminetetraacetic acid-anticoagulated peripheral blood (9 ml; Monovette®, Sarstedt, Nürnbrecht, Germany) obtained from healthy volunteers (covered by the approval of the local ethics committee #3036-07) were diluted with an equal volume of 0.9% NaCl and carefully overlaid on a double gradient formed by layering 10 ml of aqueous polysucrose/sodium diatrizoate, adjusted to a density of 1.077 g ml^{-1} (Histopaque 1077, Sigma–Aldrich, Taufkirchen, Germany), on 10 ml Histopaque 1119 (Sigma–Aldrich) in 50 ml Falcon tubes (BD-Biosciences, Heidelberg, Germany). The tubes were centrifuged at 700*g* for 30 min at room temperature. After centrifugation, two distinct leukocyte cell layers (PBMC and polymorphonuclear neutrophil granulocytes) were obtained above the bottom sediment of erythrocytes. The PBMC layer was carefully aspirated and transferred to a separate 50 ml tube, which was then filled with phosphate-buffered saline (PBS; Sigma–Aldrich) and centrifuged at 200*g* for 15 min at 4 °C. This method led to more than 95% pure and viable PBMC. Cell counting was performed using Tuerk staining solution (Sigma–Aldrich). The isolated cells were adjusted to 1×10^6 cells ml^{-1} in RPMI 1640 (GIBCO, Invitrogen GmbH, Karlsruhe, Germany) cell culture medium supplemented with L-glutamine (0.3 g l^{-1}), sodium bicarbonate (2.0 g l^{-1}), 10 % fetal calf serum (FCS; Gibco, Invitrogen GmbH), and 20 mM N-(2-hydroxyethyl)-piperazine-N′-(2-ethanesulfonic acid) (Sigma–Aldrich).

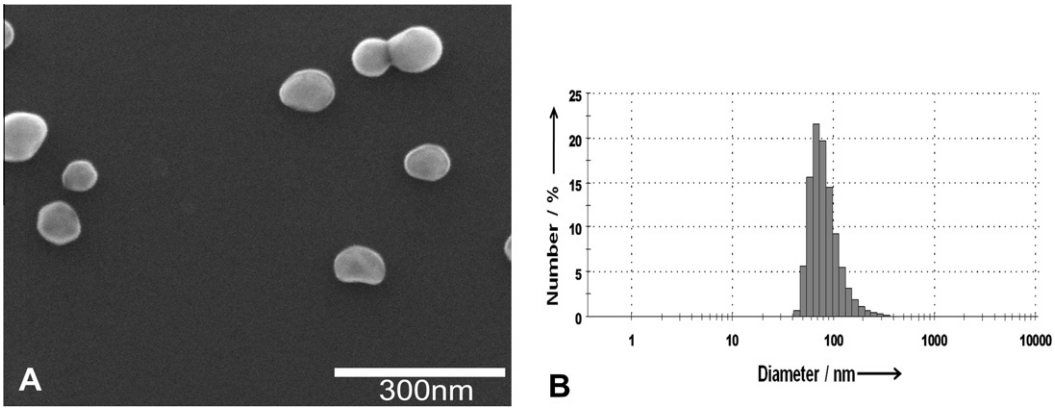

Fig. 1. Scanning electron micrograph of the spherical PVP-coated Ag-NP with a metallic core of 70 ± 20 nm (A). Dynamic light-scattering analysis of the Ag-NP, demonstrating a mean hydrodynamic diameter of 75 ± 20 nm. (B) The particle size distribution by number.