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In vivo measurement of extravasation of silver nanoparticles into liver extracellular space by push–pull-based continuous monitoring system

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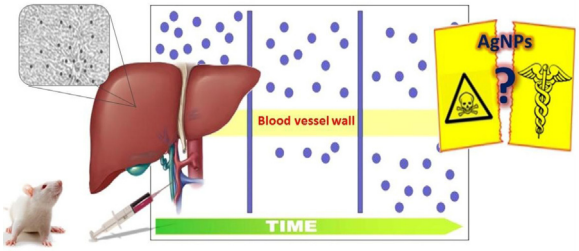
HIGHLIGHTS

- An analytical system composed of PPP sampling, in-tube SPE, and ICP-MS was developed.
- The system's detection limit and temporal resolution were 0.64 μg L^{-1} and 10 min.
- The transport kinetics of extracellular AgNPs in rat liver was investigated *in vivo*.
- Our results revealed the clearance of AgNPs may be blocked by a prior administration.

GRAPHICAL ABSTRACT



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ABSTRACT

With the increasing prevalence of silver nanoparticles (AgNPs) in various products, whether such AgNPs will introduce new injury mechanisms from new pathologies remains to be determined. From the toxicokinetic viewpoint, it is vital to have in-depth knowledge of their *in vivo* transport kinetics and extravasation phenomenon. By combining push–pull perfusion sampling, in-tube solid phase extraction, and inductively coupled plasma mass spectrometry, we used an *in vivo* push–pull-based continuous monitoring system to investigate *in vivo* transport kinetics of extracellular AgNPs in living rat liver with a detection limit and temporal resolution of 0.64 μg L^{-1} and 10 min, respectively. Before administration into living rats, the pre-incubation in DMEM with 10% FBS for 8 h was adopted as the optimized exposure condition for the used AgNPs. After repeated-dose treatments, we observed a higher concentration of AgNPs in the liver extracellular space, suggesting that AgNP clearance by the reticuloendothelial system (RES) may be blocked by a prior administration of AgNPs. Future studies on AgNP distribution in different liver compartments (blood stream, extracellular space and Kupffer cells/hepatocytes) are necessary for defining the risks and benefits of AgNP applications.

1. Introduction

Silver nanoparticles (AgNPs), reported as one of the most widely used nanosized materials (NMs) in consumer products (Muller and Nowack, 2008; PEN, 2013), have been used commercially with great success. The widespread use of AgNPs in myriad applications will, however, make it likely that humans would intentionally or unintentionally be exposed to engineered AgNPs through inhalation, ingestion, dermal, and injection. From the viewpoint of living systems' exposure to such particles, to clarify the circulation of NMs/AgNPs in the bloodstream, their site-specific extravasation as well as their metabolism and clearance are of primary importance (Wang et al., 2013).

With the advent of health risk assessment tools, physiologically based pharmacokinetic (PBPK) modeling was used to study the impact of the exposure scenario based on toxicokinetics or

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pharmacokinetics. However, to fully use the power of PBPK modeling for NMs/AgNPs, we must overcome several limitations, including the absence of some relevant animal models, lack of simple and rapid methods for minimizing the number of animals required for each study, the working time, and laborious sample preparation procedures (Li et al., 2010). Additionally, variations in physiology (*e.g.*, tissue volumes and blood flow rates), physicochemistry (*e.g.*, tissue-to-blood partition coefficients), and biochemistry (*e.g.*, metabolism rates and protein binding affinity) could potentially impact the transport kinetics. To improve our understanding of the underlying transport physiology, it has been indicated that the roles of circulatory mixing and vascular extravasation are vital in determining the biodistribution of xenobiotics (Weiss et al., 2011). Unfortunately, an understanding of the NM extravasation phenomenon was unavailable until intravital imaging was successfully developed for studying NM transport dynamics at the cellular level (Popovic et al., 2010; Chauhan et al., 2011; Cheng et al., 2012). The quantitative description of NM extravasation was not studied even after the spatial resolution of image-based technique was improved to subcellular scale. Because further refinement of the physiological and anatomical descriptions of the body (*e.g.*, at the extracellular level) would lead to more detailed and accurate PBPK models in the future, herein, we describe a facile technique for revealing the kinetics of and effect of extravasation on extracellular AgNPs in rat livers after intravenous (*i.v.*) administrations.

For monitoring the kinetic profile of extracellular AgNPs, the push–pull perfusion (PPP) sampling technique was used for continuous collection of AgNP-containing extracellular fluid from rat livers. In addition, to eliminate severe interference resulting from the presence of large numbers of blood cells, proteins, and dissolved salts in the push–pull perfusate, we designed a simple and efficient, online in-tube solid phase extraction (SPE) procedure—based on hydrophobic interactions between polytetrafluoroethylene (PTFE) and AgNPs—for separating AgNPs from the complex push–pull perfusate. By combining the abovementioned devices and inductively coupled plasma mass spectrometry (ICP-MS), this online hyphenated system could be used for disclosing the effect of extravasation on the kinetic profile of liver extracellular AgNPs.

2. Materials and methods

2.1. AgNP preparation and characterization

Commercial AgNPs (particle size: <100 nm) with a relatively wide particle size distribution were purchased from Sigma-Aldrich (658804; St. Louis, USA) for being close to their exposure from conventionally environmental purpose (Benn and Westeroff, 2008). The AgNPs [10% (w/w)] were dispersed in pure ethylene glycol. A dialysis membrane with a molecular weight cut-off of 20-kDa was used to collect the innate silver ion content. The concentrations of silver ions and total silver in the purchased suspensions and the dialysates were determined by ICP-MS, respectively. Primary AgNPs were dispersed in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) with 10% fetal bovine serum (FBS; GIBCO). The primary particle size distributions of AgNPs in ethylene glycol and DMEM containing 10% FBS were determined using transmission electron microscopy (TEM), thereby estimating a mean diameter by ImageJ software; changes in their hydrodynamic diameter (HD) within 12 h were measured using dynamic light scattering (DLS; Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK). Furthermore, to evaluate the stability of these FBS pre-incubated AgNPs *in vitro*, their HD variations were monitored after dispersal in fresh rat plasma. Cells in rat blood, acquired *via* cardiac puncture, were removed by conventional centrifugation; the supernatant was filtered through a 0.1-μm filter cartridge (PALL, Port Washington, NY, USA) before FBS pre-incubated AgNP dispersion.

2.2. PPP sampling

A fabrication procedure similar to those reported previously was used for fabricating a modified probe (Kottegoda et al., 2002). A concentric push–pull probe was fabricated to allow the direct infusion of phosphate-buffered saline (PBS; P3813, Sigma), with anticoagulant and heparin sodium (10 IU mL^{-1}; B. Braun, Melsungen AG, Germany), into the desired sampling region *via* an outer infusion polyimide tubing (i.d./o.d., 140/180 μm) and for simultaneous sample collection *via* an inner withdraw fused silica tubing (i.d./o.d., 50/125 μm). The flow rate required for drawing samples from the sampling site was not set as an experimental variable, but was adjusted daily using two peristaltic microdialysis pumps (MAB 20, Microbiotech/se AB, Stockholm, Sweden) for maintaining the withdraw flow rate equal to the infusion flow rate. For estimating AgNP concentrations, it was necessary to calibrate the daily recovery of PPP sampling, which was accomplished by implanting the push–pull probe in DMEM (with 10% FBS) with 10 μg AgNPs L^{-1} and perfusing at selected sampling flow rates. The AgNP signal in the perfusate was measured by following the online hyphenated system; recoveries were calculated as the ratios of AgNP signals in the perfusate to those in DMEM with 10% FBS.

2.3. Online monitoring system

The online hyphenated system, shown in Fig. 1, mainly comprised an in-tube SPE-based sample pretreatment device for facilitating analysis of the AgNPs in the complex biological matrix, and an ICP-MS instrument. The flow-injection SPE-based device comprised two six-port valves (C2-2348D, Valco, Lucerne, Switzerland), an eight-port valve (C22Z-3186E, Valco, Lucerne, Switzerland), and a piece of PTFE tubing. All valves were programmed and controlled by a personal computer through a serial valve interface (SIV-110, Valco, Lucerne, Switzerland). Following PPP sampling, the push–pull perfusate was immediately online-mixed with DMEM (with 10% FBS) containing heparin sodium (10 IU mL^{-1}) for minimizing blood coagulation and the possible hydrodynamic impedance in the PTFE tubing arising from of clots. Then, the mixture was delivered to the tract of the PTFE tubing (200-cm – long; 0.007 in. i.d.; Alltech, Virginia, USA) for extracting the AgNPs and separating the biological matrix. As the AgNPs were retained on the PTFE tubing's walls, 2% HNO_3 was used to detach and dissolve them for their introduction into ICP-MS and consequent determination. An Agilent 7500a ICP-MS system (Agilent, CA, USA) was used. Signals were obtained during acquisition of ion intensities at m/z 107 and 109 for setting up the calibration curves required for quantifying AgNPs in the push–pull perfusate. The optimized sample loading flow rate, sample pH, operation sequence of the online SPE pretreatment system as well as the instrumental operation conditions selected for achieving optimal sensitivity and low background noise, were integrated and provided [see Fig. S1, S2, and S3, Table S1 and S2 (Supplementary data)].

2.4. In vivo experiments

Adult male Sprague-Dawley (SD) rats (200–250 g) were obtained from the Laboratory Animal Center of the National Science Council of the Republic of China (Taiwan). These animals, which were specifically pathogen-free, were acclimatized to their environmentally controlled quarters (25 °C; 12-h light/12-h dark cycle) for at least 3 days prior to the experiment and were made to fast overnight prior to sacrifice. All experimental procedures were conducted in conformity with the guidelines and under approval of the Institutional Animal Care and Use Committee at National Tsing-Hua University (approval number: 09735). The rats were initially anesthetized with urethane (1600 mg kg^{-1} body weight, intraperitoneal injection); they remained anesthetized throughout the experimental period. A middle laparotomy was performed, and the liver hilum was exposed. The push–pull probe perfused with heparinized saline solution (flow rate: 5 μL min^{-1}) was implanted around the center of the left lateral lobe. Initially, the obtained perfusate contained obvious blood components owing to intrusion of the push–pull probe. After around 1 h, as the tissue stopped bleeding and returned to a state of homeostasis, a AgNP dosage of 30 μg kg^{-1} body weight was administrated intravenously, and the level of extracellular AgNPs in the liver was monitored at 10 min intervals. As the monitored concentration stabilized (130 min post-administration), a second, equal dosage of AgNPs was administered for investigating the effect of repeated stimulation on physiological response. During the monitoring of extracellular AgNPs *in vivo*, blood samples (*ca.* 40 μL) were collected from the jugular vein at various time points post-administration. Upon completion of the animal experiments, the rats were euthanatized with CO_2. Shortly thereafter, five pieces of liver tissue (*ca.* 0.5 g) were collected randomly from each rat, and blood was blotted from the tissue surface. After sample collection and preparation, replicate samples (0.5 g of liver tissue and 40 μL blood) were digested with concentrated HNO_3 using a microwave oven (MARS 5, CEM Corporation, Matthews, NC, USA). The presence of AgNPs in liver and blood samples was determined using off-line routine analysis methods.

2.5. Statistical analysis

The data are reported as the mean ± SEM. Comparison of the results between the two AgNPs administrations were carried out by Student's t-test and considered to be significantly different when $p < 0.05$.

3. Results and discussion

3.1. Characterization of AgNPs

The characteristic of the used AgNPs was summarized in Table 1. Fig. 2 shows the TEM images of AgNPs dispersed in different media. As indicated in Fig. 2, the AgNPs were approximately spherical in