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Research paper

Endothelial barrier dysfunction induced by nanoparticle exposure through actin remodeling via caveolae/raft-regulated calcium signalling

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ABSTRACT

The rapid development of modern nanotechnology has resulted in nanomaterial being use in nearly all applications of life, raising the potential risk of nanomaterial exposure alongside the need to design safe and effective materials. Previous work has demonstrated a specific effect of gold nanoparticles (GNPs) of approximately 20 nm on endothelial barrier function in vitro. To expand our understanding of this size-specific effect, titanium dioxide, silicon dioxide, and polystyrene nanoparticles (NPs) in this similar size range were studied. All tested nanoparticles were found to have minimal effects on cell viability, but exhibited a significant detrimental effect on endothelial barrier function. Nanoparticles in the size range of 20 to 30 nm were internalized by endothelial cells through caveolae/raft-mediated endocytosis, causing intracellular calcium elevation by approximately 30% at 2 h after administration, and triggering myosin light chain kinase (MLCK)-regulated actomyosin contraction. These effects culminated in an increase in endothelial monolayer permeability across all particle types within the 20–30 nm range. This nanoparticle exposure-induced endothelial barrier dysfunction may provide valuable information for designing safer nanomaterials or potential applications of this nanoparticle exposure-induced permeability effect in biomedicine.

1. Introduction

Engineered nanoparticles (NPs), due to their controllable physiochemical properties, are heavily studied and used (Sahoo et al., 2007). The increasing risk of exposure to engineered NPs has raised extensive concern over their effects on human health (Limbach et al., 2007; Simkó and Mattsson, 2010; Baun et al., 2008; Hassellöv et al., 2008). The endothelium at the inner lining of blood vessel walls serves as an important interface between circulating blood and surrounding tissues. The nature of the endothelium makes it a barrier that regulates the substance exchange between blood and the tissues that rely on the blood supply, supporting the entire biological system. When NPs enter the human body, accidentally or intentionally, blood circulation is the main route for their spread throughout the body. The endothelium is a critical barrier to interact with the nanoparticles, which makes endothelial barrier integrity a critical consideration when assessing the safety of nanoparticles (Engin et al., 2015). Even though much research has been done on the impact of nanoparticle exposure on endothelial barrier function (Brun et al., 2012; Setyawati et al., 2013; Guo et al., 2017), there still exists widespread uncertainty about the impact of nanomaterials due to the complexity of barrier function regulation and the physicochemical property-dependent interaction between nanoparticles and cells.

Previous work in our lab has shown that uncoated, 20 nm gold nanoparticles (GNPs) cause endothelial barrier dysfunction without affecting cell viability by altering the cytoskeleton structure, while smaller or larger GNPs did not have the same effect (Liu et al., 2017). Other recent research has also reported that nanoparticles in this size range are more likely to interact with cells (Brun et al., 2012; Setyawati et al., 2013), (Setyawati et al., 2014; Trickler et al., 2010; Liu et al., 2015). Studies by Setyawati, Magdiel I., et al. and Tay., et al. reported very similar size-dependent endothelial permeability induced by gold, TiO_2, and SiO_2 NPs in the 20 nm size range (Setyawati et al., 2013; Setyawati et al., 2017; Tay et al., 2017). To explore whether this size-specific effect was particle bulk material-dependent and further explore the underlying mechanism, other types of commonly used, organic and inorganic nanoparticles in the 20 nm size range were investigated here in addition to GNPs. Titanium dioxide (TiO_2), silicon dioxide (SiO_2),

Abbreviations: GNPs, gold nanoparticles; NPs, nanoparticles; MLCK, myosin light chain kinase; PEG, polyethylene glycol; TiO2, titanium dioxide; SiO2, silicon dioxide; PS, polystyrene; HUVEC, human umbilical vein endothelial cells; EGM-2, endothelial cell growth medium 2; SEM, scanning electron microscopy; NTA, nanoparticle tracking analysis; DI, deionized; DLS, dynamic light scattering; CCK-8, cell counting kit 8; ROS, reactive oxygen species; H2DCFDA, 2′,7′-Dichlorofluorescin diacetate; PBS, phosphate buffered saline; FITC, fluorescein isothiocyanate; FFT, fast Fourier transform; MLCP, myosin light chain phosphate

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and polystyrene (PS) NPs are widely used in the food and food packaging industries, cosmetic products, and biomedical applications, which makes them the most commonly used in human contact situations (Weir et al., 2012; Gondikas et al., 2014; Darvin et al., 2012; Danhier et al., 2012; Han et al., 2017). The concentrations of particles studied were surface area-matched to concentrations of GNPs previously studied, which represent physiologically-relevant in vivo doses of nanoparticles (Liu et al., 2017). TiO_2, SiO_2, and PS NPs in the 20 to 30 nm size range were studied for their impact on cell viability and endothelial barrier function, with a focus on understanding the underlying mechanism of particle-cell interactions.

2. Materials and methods

2.1. Cell culture

Human umbilical vein endothelial cells (HUVEC, Lonza, Walkersville, MD) were cultured in endothelial cell growth medium 2 (EGM-2, Lonza) supplemented with the EGM-2 SingleQuot Kit Suppl. & Growth Factors (CC-4176, Lonza)—and grown under standard conditions (37 °C, 5% CO_2, humidity).

2.2. Nanoparticles

All tested nanoparticles are commercially available. Titanium dioxide (TiO_2) (US3498) and silicon dioxide (SiO_2) (US3438) NPs were purchased from US Research Nanomaterials, Inc. (Houston, TX). Polystyrene (PS) NPs (FluoSpheres® Carboxylate-Modified, F8782) were purchased from ThermoFisher (Waltham, MA). For cell studies, stock nanoparticles were diluted appropriately to the testing concentrations in EGM-2 prior to use. In a recent review, the authors concluded an in vivo GNP dose range between 0.01 μg/g animal weight and 10 μg/g is suitable for biomedical applications without causing severe toxic reaction (Khlebtsov and Dykman, 2011). In a standard in vivo model, a 25 g mouse with a 1.5 mL blood volume, this GNPs dose range translates to between 0.167 and 167 μg/mL blood. Due to our focus on endothelial exposure, the tested concentrations of GNPs in the previous study were selected within this range to mimic reasonable physiologically relevant exposure. All tested concentrations of different nanoparticles in this study were normalized to match the concentrations of GNPs in the previous study based on the total surface area. Due to the complexity of the interactions between NPs, NPs and cells, as well as NPs with proteins in cell media, it is very difficult to quantify the amount of NPs that reached the cell surface (Aggarwal et al., 2009; Nel et al., 2009); concentrations of NPs tested in this study were expressed as μg/mL (NPs per unit solution volume) or μg/cm^2 (NPs per unit cell surface area).

2.3. Nanoparticle characterization

Scanning electron microscopy (SEM, Supra 55 VP, Zeiss, Thornwood, NY) was performed to obtain the dry particle size and morphology. The hydrodynamic size of nanoparticles was measured using nanoparticle tracking analysis (NTA, NanoSight NS300, Malvern, Westborough, MA) in both deionized (DI) water and EGM-2. Stock nanoparticles were diluted to an appropriate concentration for NTA and vortexed before measuring. Electrophoretic dynamic light scattering (DLS, Nano-ZS, Malvern) was used to measure zeta potential and hydrodynamic size of the nanoparticles. Stock nanoparticles were diluted, vortexed, and then 1 mL was transferred to a Malvern Capillary Zeta Potential cell for DLS measurements.

2.4. Cell viability assay

The effect of nanoparticle exposure on cell viability was quantified using Cell Counting Kit-8 (CCK-8, 96992, Sigma, St. Louis, MO). HUVEC were seeded in a collagen-coated 96-well cell culture plate (351172, Corning, Corning, NY) at 10,000 cells/cm^2 and cultured until fully confluent. Cells were exposed to TiO_2, SiO_2, and PS NPs at various concentrations for 24 h. Nanoparticles were removed, cells were rinsed, and 100 μL of CCK-8 solution (10% CCK-8 in EGM-2) was added into each well. After 2 h of incubation, the absorbance was measured at 450 nm using a plate reader (Synergy H1, BioTek, Winooski, VT). Cells exposed to 2% Triton™ ×100 (×100, Sigma) served as the negative control and cells given fresh EGM-2 served as the positive control. The results were presented as percentage of the positive control.

2.5. Reactive oxygen species (ROS) assay

The ROS level in HUVEC after nanoparticle exposure was measured using 2′,7′-Dichlorofluorescin diacetate (H2DCFDA, D6683, Sigma). HUVEC were seeded at 10,000 cells/cm^2 and cultured in a collagen-coated 96-well clear bottom black cell culture plate (3904, Corning) to confluence. Cells were exposed to TiO_2, SiO_2, and PS NPs at various concentrations for 24 h. Nanoparticles were removed, cells were rinsed, and the absorbance of nanoparticle-treated HUVEC was measured at 495 nm and 529 nm, the wavelengths of interest for H2DCFDA detection. Next, 100 μL of 20 μM H2DCFDA solution (in phosphate buffered saline (PBS) supplemented with Ca^{2+} and Mg^{2+}) was added into each well. After 30 min of incubation, H2DCFDA was removed, cells were rinsed, and then the fluorescence intensity of each well was measured using a plate reader (Synergy H1, BioTek) with excitation and emission wavelengths of 495 nm and 529 nm, respectively. Data were compensated for absorbance caused by the presence of nanoparticles.

2.6. Fluorescein isothiocyanate (FITC)–dextran permeability assay

HUVEC were seeded onto collagen-coated Transwell® (3413, Corning) inserts at 100,000 cells/cm^2 and cultured until confluent, then exposed to TiO_2, SiO_2, and PS NPs at the concentrations that yield the same total surface area in EGM-2 for 2 h, the apical chamber solution was replaced with 1 mg/mL 70-kD FITC-Dextran (46945, Sigma) in PBS supplemented with Ca^{2+} and Mg^{2+}, and the bottom chamber solution was replaced with PBS. The fluorescence signal intensity of the bottom chamber was then measured after 1 h of incubation using a plate reader (Synergy H1, BioTek) with excitation and emission wavelengths of 485 nm and 530 nm, respectively.

2.7. Actin alignment measurement

Actin microfilament rearrangement in HUVEC exposed to nanoparticles was quantified using a method described in our previous study (Liu et al., 2017). Succinctly, actin microfilament images were transformed to frequency domain images using a fast Fourier transform (FFT) with bandpass to exclude low intensity, high frequency noise. The waveforms of the transformed images were divided into 18 angle bands; spanning from 0 to 180° for easier distribution analysis. Total pixel intensities of each angle band were calculated, and then the coefficient of variation of the intensity distribution among all 18 angle bands was calculated. The coefficient of variation was then converted into an alignment index for easier interpretation (van der Meer et al., 2010). An index of 0 corresponds to an even distribution over all angle bands (no alignment), while an index of 1 corresponds to a total distribution in one angle band (complete alignment). Samples were analyzed after 2 h of exposure. All samples were imaged on an inverted wide field fluorescence microscope (Eclipse Ti, Nikon, Melville, NY). All image analysis was performed with NIH IMAGEJ software and MATLAB® (Mathworks, Natick, MA).

2.8. Caveolae/raft-dependent endocytosis inhibition

Various studies of the uptake of NPs by cells have shown that the