Science Bulletin xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Science Bulletin

journal homepage: www.elsevier.com/locate/scib

Article

Toxicity of silicon dioxide nanoparticles with varying sizes on the cornea and protein corona as a strategy for therapy

Dayu Sun [a,b,c], Linji Gong [d,e], Jing Xie [b,c], Xianliang Gu [b,c], Yijian Li [b,c], Qinglin Cao [b,c], Qiyou Li [b,c], Luodan A [b,c], Zhanjun Gu [d,e,*], Haiwei Xu [b,c,*]

[a] Department of Physiology, Third Military Medical University (Army Medical University), Chongqing 400038, China
[b] Southwest Hospital/Southwest Eye Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China
[c] Key Laboratory of Visual Damage and Regeneration & Restoration of Chongqing, Chongqing 400038, China
[d] Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China
[e] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 17 March 2018
Received in revised form 17 May 2018
Accepted 28 May 2018
Available online xxxx

Keywords:
Particulate matter
Silicon dioxide nanoparticles
Cornea
Toxicity
Foetal bovine serum

ABSTRACT

The human cornea is exposed directly to particulate matter (PM) in polluted air. This exposure can cause eye discomfort and corneal injury. Ultrafine PM (diameter <100 nm) is thought to be particularly harmful to health, but there is limited research investigating its toxicity to the eye. In this study, we evaluated toxicity differences among 30-, 40-, 100- and 150-nm silicon dioxide nanoparticles (SiO_2 NPs) on the cornea. A 24-hour in vitro exposure of primary human corneal epithelial cells (hCECs) to ultrafine (30 and 40 nm) SiO_2 NPs produced toxicity, as evidenced by cell membrane damage, reduced cell viability, increased cell death and mitochondrial dysfunction. In vivo exposure to the same nanoparticles produced observable corneal injury. These effects were more severe with ultrafine than with fine (100 and 150 nm) SiO_2 NPs. Common antioxidant compounds, e.g., glutathione, did not protect the cornea from SiO_2 NP-induced damage. However, foetal bovine serum (FBS) did significantly reduce toxicity, likely by forming a protective protein corona around the nanoparticles. This finding suggests that FBS (or its derivatives) may be a useful clinical therapy for corneal toxicity caused by ultrafine particulates.

© 2018 Science China Press. Published by Elsevier B.V. and Science China Press. All rights reserved.

1. Introduction

Air pollution has increasing impacts on human health, particularly in urban environments [1,2], and particulate matter is one of the primary types of potentially harmful pollutants [3,4]. "Particulate matter" (PM) describes a mixture of solid or liquid matter suspended in air. Systematic epidemiological studies have demonstrated that the concentration of PM correlates positively with total mortality [5], cardiovascular disease [6], respiratory disease [7] and cancer [8].

PM is commonly classified according to the maximum aerodynamic diameter of suspended particulates into PM_{10} (<10 μm; "coarse"), $PM_{2.5}$ (<2.5 μm; "fine") and $PM_{0.1}$ (<0.1 μm, that is <100 nm; "ultrafine"). The particle size of PM is closely related to its toxicity. For example, silicon dioxide (SiO_2) particles with <1 μm diameter dramatically increased cell toxicity and increase cytokine release by endothelial cells compared with 3–5 μm particles [9]. A recent epidemiological study has also shown that ultrafine particles ($PM_{0.1}$) but not fine particles ($PM_{2.5}$) are associated with total mortality and cardio-respiratory mortality in Erfurt, Germany, between 1995 and 2001 [10]. Furthermore, the life-span (which is the potential human exposure time) of $PM_{2.5}$ and $PM_{0.1}$ (days to weeks) is considerably longer than that of PM_{10} (hours to days) [11].

The eye is directly exposed to PM when it contacts polluted air. The transparent cornea forms the front part of the ocular surface and acts as a protective barrier to external agents, such as PM. The cornea composed (from inside to outside) of the corneal endothelium, Descemet's membrane, corneal stroma, Bowman's layer and corneal epithelium, which is later coated in a tear film. Recent studies have evaluated the toxicity of direct PM and dust exposure to the cornea. Indoor dust from air conditioner filters can induce toxicity in primary human corneal epithelial cells (hCECs) [12,13]. $PM_{2.5}$ from ambient outdoor air can trigger autophagy in a hCEC line [14], while PM_{10} can damage the tear film and destroy the cornea in a mouse model, leading to changes that are compatible with dry eyes in humans [15]. However, little is known

* Corresponding authors at: Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China.
E-mail addresses: zjgu@ihep.ac.cn (Z. Gu), haiweixu2001@163.com (H. Xu).

https://doi.org/10.1016/j.scib.2018.05.037
2095-9273/© 2018 Science China Press. Published by Elsevier B.V. and Science China Press. All rights reserved.

Please cite this article in press as: Sun D et al. Toxicity of silicon dioxide nanoparticles with varying sizes on the cornea and protein corona as a strategy for therapy. Sci Bull (2018), https://doi.org/10.1016/j.scib.2018.05.037

regarding the corneal toxicity of ultrafine PM, the effect of PM size on this toxicity or the best therapeutic methods to combat this toxicity.

Evaluating the toxicity of ambient urban PM of different sizes is hindered by the difficulty of separating pure $PM_{0.1}$ and $PM_{2.5}$ from total PM. Therefore, nanoparticles (NPs) such as SiO_2 NPs or titanium dioxide NPs, which are comparable to ambient PM, are often used in experimental models [16,17]. SiO_2 NPs are arguably a useful model because the fibrous particles in ambient fine PM is composed of 99% silicon [18], and silicon dominates the content of traffic-related PM, as it originates as wear particles from tires and road surfaces [19]. SiO_2 NP exposure also commonly occurs in industry and daily life and during the production of electronics [20], cosmetics [21], food [22] and nanomedicines [23,24].

In this study, we assessed toxicity to the cornea of $PM_{0.1}$ versus $PM_{2.5}$ using ultrafine (30 and 40 nm) and fine (100 and 150 nm) SiO_2 NPs, respectively. Primary hCECs exposed in vitro to ultrafine SiO_2 NPs demonstrated reduced cell viability, increased cell death and mitochondrial damage. These NPs also produced corneal damage in vivo in rats. Antioxidant agents did not reduce this toxicity. However, treatment with foetal bovine serum effectively reduced toxicity both in vitro and in vivo, likely via the formation of a protective protein corona around the nanoparticles.

2. Materials and methods

All procedures performed in studies involving human tissues were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All applicable institutional and/or national guidelines for the care and use of animals were followed.

2.1. Synthesis and characterization of SiO_2 NPs

The materials used to synthesize green fluorescein-doped silica nanoparticles (FITC-SiO_2 NPs) were purchased from Alfa Aesar, Ltd. China). These included 3-aminopropyltriethoxysilane (APTES, 98%), fluorescein isothiocyanate (FITC, 95%), ammonium hydroxide (NH_4OH, 28–30% as NH_3), and tetraethyl orthosilicate (TEOS, 98%). Absolute ethyl alcohol was acquired from the Beijing Chemical Reagent Company (China). All materials were used as received and without further purification. Deionized water was used in all experiments.

To prepare FITC-SiO_2 NPs for visible cellular internalization, we first prepared a FITC-APTES stock solution as follows: 20 mg FITC was stirred with 12 mL APTES in 10 mL ethanol overnight, in the dark at room temperature to prepare APTES-modified FITC. The prepared FITC-APTES precursor solution was stored in the dark at -20 °C for later use.

FITC-incorporated silica nanoparticles (30-, 40-, 100- and 150-nm size) were synthesized via a modified Stober process, as follows. An excess of FITC-APTES precursor solution was slowly added to a mixture containing a known amount of ethanol, TEOS and deionized water with vigorously magnetic stirring. Next, an ammonia solution (dispersed in ethanol) was added to catalyse the reaction, and the mixture was stirred continuously in the dark at room temperature. Various sizes of silica nanoparticles were produced by controlling the reaction times and dosage of the reactants. The resulting suspension was centrifuged and washed with ethanol and water several times to remove free FITC-APTES and ammonium ions.

To further remove ammonium ions and reduce the cytotoxicity of the synthesized FITC-SiO_2 NPs, the suspension was dialyzed against purified water for one day using a dialysis tube (3.0 kDa molecular cut-off). Finally, the synthesized FITC-SiO_2 NPs were re-dispersed in sterile deionized water and stored in a dark place for future use.

The size and morphology of the synthesized FITC-SiO_2 NPs were observed using a field emission transmission electron microscope (JEM-2100PLUS, JEOL Ltd., Japan) and a scanning electron microscope (S-4800, Hitachi, Japan). The green fluorescence of the synthesized FITC-SiO_2 NPs was observed under ultraviolet illumination (365 nm wavelength).

2.2. Human corneal epithelial cell culture

Human corneal epithelial cells (hCECs) were cultured from corneal explants, which were sourced from the Southwest Hospital Eye Bank, as previously described [25]. Briefly, corneal epithelium of aborted foetal origin was washed in cold phosphate buffered saline (PBS) containing 100 international units (IU) mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. Corneal tissue was cut into 5 m m × 5 mm pieces and seeded onto six-well plates pre-coated with human vitronectin (Gibco, Thermo Fisher, USA). Explants were placed in a CO_2 incubator for 10 min; 40 μL foetal bovine serum (FBS) was applied, and the explants were left overnight for further attachment. The following day, hCECs were cultured in a medium consisting of Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12; HyClone, GE Healthcare, China), 10% FBS (Gibco), insulin-transferrinsodium selenite (1 μg mL^{-1}, Gibco), hydrocortisone (0.5 μg mL^{-1}, Sigma-Aldrich, USA), cholera toxin (0.1 μg mL^{-1}, Sigma-Aldrich), epidermal growth factor (EGF; 2 ng mL^{-1}, PeproTech, UK), penicillin (100 IU mL^{-1}) and streptomycin (100 μg mL^{-1}, HyClone). When the attached cells reached sub-confluence, they were passaged at 1:4 to expand in culture.

2.3. Immunostaining

Cells on coverslips were fixed with 4% paraformaldehyde (PFA) for 20 min and washed three times with PBS. Fixed cells were incubated with the primary antibody, goat anti-cytokeratin 12 (anti-CK12; 1:500, Abcam, UK), overnight at 4 °C. This step was followed by incubation with the secondary antibody, donkey anti-goat Alexa Fluor® 568 (1:500, Thermo Fisher). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich). Fluorescence staining was visualized and acquired with a confocal laser-scanning microscope (Zeiss LSM 800, Carl Zeiss, Germany).

2.4. SiO_2 NP treatment and experimental therapeutic treatment

The hCECs were seeded at 1×10^4 cells/well in a 96-well plate overnight. After 12 h, the growth medium was removed, and cells were treated with SiO_2 NPs at concentrations between 0 and 200 μg mL^{-1} (depending on the precise experiment) in a DMEM base medium (HyClone) for 24 h (Fig. S1a online). Four diameters of SiO_2 NP were tested: 30, 40, 100 and 150 nm.

We also tested several compounds for their ability to reduce or prevent the toxicity of SiO_2 NPs, namely: glutathione (GSH; 2, 5, 10 mmol L^{-1}; Sigma), resveratrol (3,5,4′-trihydroxy-trans-stil bene) (RSV; 5, 10, 20 μmol L^{-1}; Sigma) and curcumin (CUR; 5, 10, 20 μmol L^{-1}; Sigma). The hCECs were either pre-treated with each of these compounds for 1 h prior to SiO_2 NP treatment, or the drug was introduced into the medium for the 24 h SiO_2 NP exposure period (Fig. S1b online). Finally, we tested the effect of introducing FBS into the medium, at concentrations ranging from 1% to 100%, for the 24-h SiO_2 NP treatment period (Fig. S1c online).

Please cite this article in press as: Sun D et al. Toxicity of silicon dioxide nanoparticles with varying sizes on the cornea and protein corona as a strategy for therapy. Sci Bull (2018), https://doi.org/10.1016/j.scib.2018.05.037

Download English Version:

https://daneshyari.com/en/article/8917200

Download Persian Version:

https://daneshyari.com/article/8917200

Daneshyari.com