J Huazhong Univ Sci Technol [Med Sci]
DOI 10.1007/s11596-014-1276-2
34(3):314-321,2014

Size-dependent Biological Effects on Vascular Endothelial Cells Induced by Different Particulate Matters*

Wen-juan CHENG (程文娟)[1, 2], Yi RONG (荣 怿)[1], Ting-ming SHI (史廷明)[3], Ting ZHOU (周 婷)[1], Yue-wei LIU (刘跃伟)[1], Wei-hong CHEN (陈卫红)[1#]
[1]*Department of Occupational and Environmental Health, MOE Key Lab of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China*
[2]*Nanchang Center for Disease Control and Prevention, Nanchang 330038, China*
[3]*Hubei Center for Disease Control and Prevention, Wuhan 430079, China*

Summary: The contribution of particles to cardiovascular mortality and morbidity has been enlightened by epidemiologic and experimental studies. However, adverse biological effects of the particles with different sizes on cardiovascular cells have not been well recognized. In this study, sub-cultured human umbilical vein endothelial cells (HUVECs) were exposed to increasing concentrations of pure quartz particles (DQ) of three sizes (DQPM1, <1 μm; DQPM3–5, 3–5 μm; DQPM5, 5 μm) and carbon black particles of two sizes (CB0.1, <0.1 μm; CB1, <1 μm) for 24 h. Cytotoxicity was estimated by measuring the activity of lactate dehydrogenase (LDH) and cell viability. Nitric oxide (NO) generation and cytokines (TNF-α and IL-1β) releases were analyzed by using NO assay and enzyme-linked immunoabsorbent assay (ELISA), respectively. It was found that both particles induced adverse biological effects on HUVECs in a dose-dependent manner. The size of particle directly influenced the biological activity. For quartz, the smaller particles induced stronger cytotoxicity and higher levels of cytokine responses than those particles of big size. For carbon black particles, CB0.1 was more capable of inducing adverse responses on HUVECs than CB1 only at lower particle concentrations, in contrast to those at higher concentrations. Meanwhile, our data also revealed that quartz particles performed stronger cell damage and produced higher levels of TNF-α than carbon black particles, even if particles size was similar. In conclusion, particle size as well as particle composition should be both considered in assessing vascular endothelial cells injury and inflammation responses induced by particles.
Key words: particles; vascular endothelial cells; biological effects; size-dependent

Adverse health effects from exposure to ambient particles are one of the most important concerns of public health. Current epidemiological studies have reported that ambient particles exposure has been associated with increased risk of acute or chronic respiratory diseases as well as cardiovascular diseases. Crystalline silica, the commonest minerals on earth, is a common component of air particles. Long-term exposure to silica has been reported to increase mortality from cardiovascular diseases in a cohort study of 74 040 workers[1]. The standardized mortality ratio of ischemic heart disease (1.65, 1.35–1.99) was significantly elevated among workers exposed to respirable silica concentrations equal to or lower than 0.1 mg/m^3. Unlike crystalline silica, carbon black is a typical combustion-sourced component of ambient particles[2]. A unit increase in personal black carbon was reported by Zhao and his colleagues[3] to be associated with an increase in systolic blood pressure of 0.53 mmHg and diastolic blood pressure of 0.37 mmHg. The influencing factors by which ambient particulate matters induce the adverse effects of cardiovascular system are not very clear.

The aerodynamic equivalent diameter (AED) of particles was thought to be the main factor to decide the deposition and translocation of particles in the body[4]. The mass concern is taken on the particulate matter with diameter of less than 10 μm (PM_{10}). PM_{10} is classified according to diameter into three size fractions such as $PM_{2.5\text{-}10}$ (defined as coarse particles, 2.5<AED<10 μm), $PM_{2.5}$ (fine particles, AED≤2.5 μm), and UFP (ultrafine particles, ≤0.1 μm). A report from a task group of the International Commission on Radiological Protection (ICRP) displayed that the particles of 5–10 μm have higher deposition in pharynx nasalis and upper respiratory tract, while the particles of <5 μm can penetrate in bronchi and alveoli, and particles of <2.5 μm have deeper penetration in alveoli[5]. Recently, accumulating evidence has indicated that small size particles (especially for ultrafine particles) can move across the alveolar-blood barrier and then translocate into the systemic circulation, contact with cardiac tissue, brain and other organs distant from the lungs. Direct interactions of particles with cardiovascular system may cause cardiovas-

Wen-juan CHENG, E-mail: cwjaajx@163.com
#Corresponding author, E-mail: wchen@mails.tjmu.edu.cn
*This project was supported by grants from the National Basic Research Program of China (No. 2011CB503804) and the National Natural Science Foundation of China (No. 81372967).

cular injury[6–9]. However, particle size-dependent cell damage, oxidative stress and cytokine responses for cardiovascular system have not been well established.

In this study, we chose human umbilical vein endothelial cells (HUVECs) as target cells to observe the direct and indirect effects of particles on endothelial functions. Endothelial cells are thought to be important in the mechanisms of cardiovascular diseases induced by fine particles[10]. Different sizes of two types of particles: pure quartz (crystalline silica) and pure carbon black were selected because crystalline silica is commonest non-combustion-sourced particle and carbon black is a typical combustion-related particle. Both particles are main components in ambient particles. The objectives of this study were to quantify the biological effects of particle size on HUVECs and to compare the cell toxicity, oxidative damage and cytokine responses induced by quartz and carbon black particles through *in vitro* test.

1 MATERIALS AND METHODS

1.1 Particle Samples

Three sizes (diameter <1 μm, 3–5 μm, 5 μm; namely DQPM1, DQPM3–5, DQPM5) of pure quartz particles (DQ), obtained from Institute for Occupational Safety and Health of German Social Accident Insurance, two sizes of carbon black particles (diameter <1 μm and <0.1 μm; namely CB1, CB0.1) provided by Hubei CDC in China, were used as experimental particles in this study. The particles were weighted and autoclaved at 121ºC for 30 min to sterilize, and high-concentration suspensions were made with phosphate buffer saline (PBS). All particle samples were placed in an ultrasonic bath for 15 min to ensure a uniform suspension before administration to the cells.

Fig. 1 presents the size distribution of particle samples. Carbon black particles appeared as parts of aggregates rapidly after being stirred, especially for CB0.1.

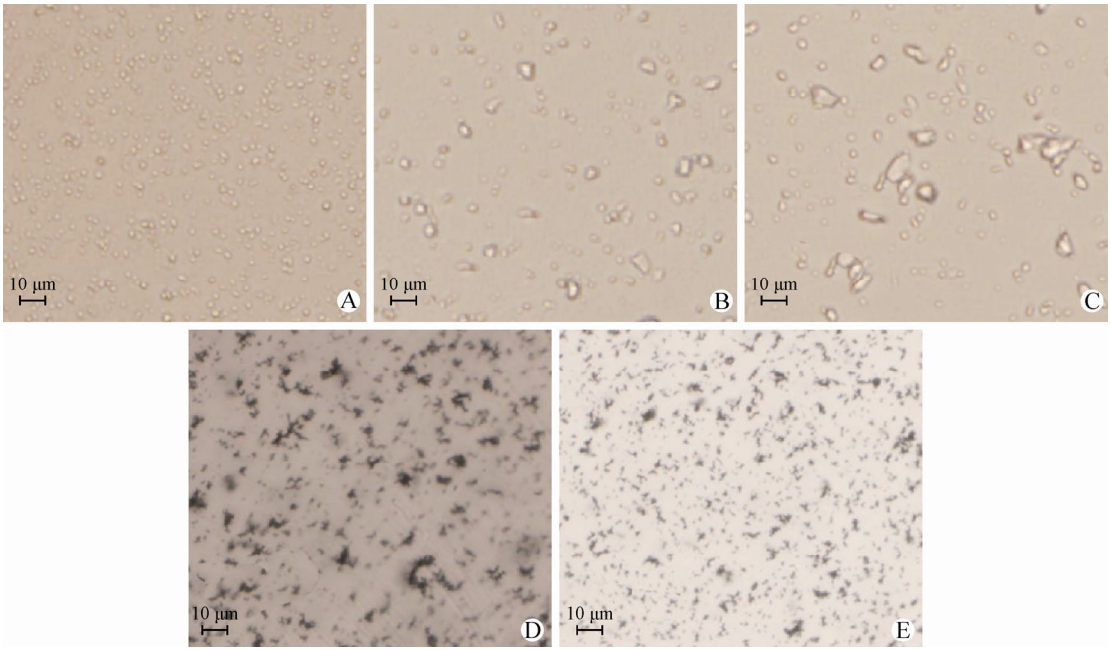

Fig. 1 Size distribution of particles (×400)
Every particle sample was made with PBS. Photographs were taken under inverted fluorescence microscope (Olympus ZX71, Japan) after particle samples were placed in an ultrasonic bath for 15 min. A: DQPM1; B: DQPM3-5; C: DQPM5; D: CB0.1; E: CB1

1.2 Culture of HUVECs

HUVECs obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Science (Shanghai, China) were cultured in DMEM cell media (Hyclone, USA) containing 10% heat-inactivated fetal bovine serum (FBS, Gibco, USA) at 37ºC and 5% CO_2. The cell suspension was plated on 96-well plates at a concentration of 1×10^5 cells/well with a volume of 200 μL for 24 h to stick to plates, then culture medium in each well was removed and replaced by DMEM without FBS for another 24 h. After culture medium refreshment, HUVECs were exposed to particle samples diluted with FBS-free DMEM in 25, 50, 100, 200 and 400 μg/mL for 24 h. Our pre-experiments found that HUVECs performed clearer biological response after exposure to particle samples for 24 h than that after exposure for 6, 12, 18 and 48 h. The concentration of 0 μg/mL was taken as a blank control. Conditioned media were collected and centrifuged at 10 000 × g for 10 min to remove dead cells and particles for subsequent analysis.

1.3 Measurement of Cell Viability

Cell viability was measured after particles treatment by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, performed as method of Mosmann (1983). Briefly, 20 μL MTT (Gibco, USA) stock solution (5 mg/mL of PBS) was added to each well, and the plate was incubated at 37ºC in 5% CO_2 for 4 h. Then the culture medium of each well was quickly removed and 150 μL of dimethyl sulfoxide (DMSO, Sigma, USA) was added to dissolve the intracellular crystalline formazan crystals for 10 min at room temperature. After that, the absorbance (*A*) at wavelength of 490 nm was

read spectrophotometrically using a microplate spectrophotometer (Bio-Rad, USA). The results were expressed as a ratio of *A* value of the sample to that of blank control (100%).

1.4 Estimation of Cell Damage

Cell damage was estimated by measuring the activity of lactate dehydrogenase (LDH), and the LDH activity in 20 μL supernatants was tested by a commercial cytotoxicity kit (Nanjing Jiancheng Bio-tech Co. ltd., China). The results were expressed as U/L.

1.5 Assessment of Nitric Oxide (NO) Generation

Generation of NO was assessed using NO assay kit (Beyotime Bio-tech Co. ltd., China), according to the manufacturer's instructions. NO concentration in 50 μL supernatants was reported as μmol/L.

1.6 Analysis of Cytokine Release

Releases of TNF-α and IL-1β in the culture medium were analyzed using enzyme-linked immunoabsorbent assay (ELISA) kits (human, R&D systems, USA), according to the manufacturer's manual. Cytokine concentrations in 100 μL supernatants were reported as pg/mL.

1.7 Statistical Analysis

The results were expressed as $x\pm s$ of separate replicate experiments. Statistical significance of the dose-response relationships was determined by analysis of variances (ANOVA) followed by Dunnett-*t* test. Analysis of variances (ANOVA) followed by *q* test were used to analyze statistical significance in different experimental groups. All statistical analyses were performed using SAS 9.1 software (SAS Institute Inc., USA). $P<0.05$ was considered to be statistically significant.

2 RESULTS

2.1 Cell Viability

The results of HUVECs viabilities induced by quartz and carbon black particles are shown in fig. 2. Both particles induced a clear dose-dependent decrease in cell viability when particle concentrations increased from 25 μg/mL to 400 μg/mL. Significant decreases were observed when quartz concentrations were higher than 100 μg/mL or carbon black concentrations were higher than 25 μg/mL.

Among quartz samples, DQPM1 induced more decrease in HUVECs viabilities than DQPM3-5 and DQPM5 although there was no statistically significant difference (fig. 2A). Cell viabilities induced by CB0.1 were lower than those induced by CB1 at the concentrations equal to 100 μg/mL or lower. When particle concentration was 200 μg/mL or 400 μg/mL, cell viabilities induced by CB0.1 were higher than those induced by CB1. No significant difference was detected in HUVECs viabilities treated with CB1 and CB0.1 (fig. 2B). Cell viabilities induced by CB1 were lower than those induced by DQPM1 without statistically significant difference (fig. 2C).

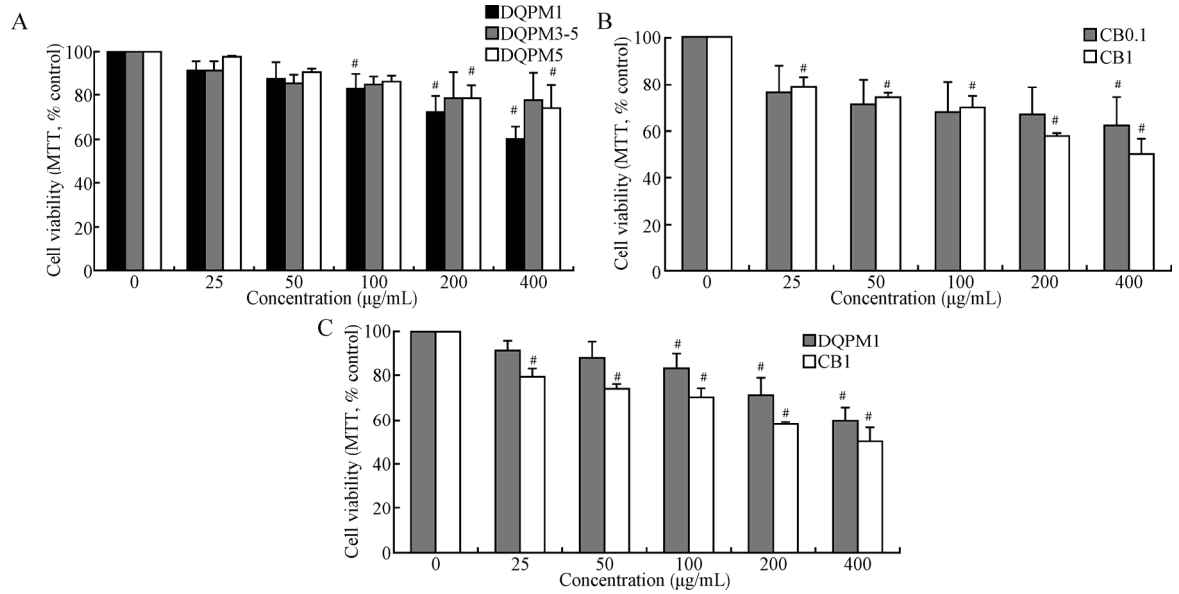

Fig. 2 Comparison of HUVECs viabilities after treated with quartz particles (A) and black particles (B) and comparison of cell viabilities induced by parallel size of quartz particles and carbon black particles (C)

HUVECs were exposed to 0, 25, 50, 100, 200 and 400 μg/mL particles for 24 h. The bar graph represents $\bar{x}\pm s$ of separate experiments (n=3). $^{\#}P<0.05$ *vs.* blank control with Dunnett-*t* test

2.2 LDH Levels Induced by Particles

Clear dose-response relationship was observed between three sizes of quartz and the enhancement of LDH levels after exposure of HUVECs to quartz for 24 h. The same trends were also observed when HUVECs were exposed to two sizes of carbon black particles (fig. 3).

DQPM1 induced significantly higher LDH levels than DQPM5. On average, the consequential enhancement appeared at particle concentration of 400 μg/mL after HUVECs were exposed to these two sizes of quartz (fig. 3A). DQPM1 significantly elevated LDH in the supernatant when compared with CB1 (fig. 3C). In contrast with quartz, CB0.1 induced significantly higher LDH level than CB1 only at low particle concentration

of 50 μg/mL. When carbon black particles concentration reached 200 μg/mL or 400 μg/mL, the LDH levels in the supernatants of CB1 were higher than those of CB0.1, although they did not reach statistically significant difference (fig. 3B).

2.3 NO Generation Induced by Particles

In this study, we detected NO generation in the supernatants of HUVECs after they were exposed to quartz or carbon black particles directly in a dose-response manner (fig. 4). Both quartz and carbon black induced obvious generation of NO at the particle concentrations of 200 μg/mL and 400 μg/mL. No statistically significant difference in NO existed between DQPM1 and CB1, or among the groups of different sizes for quartz or carbon black particles.

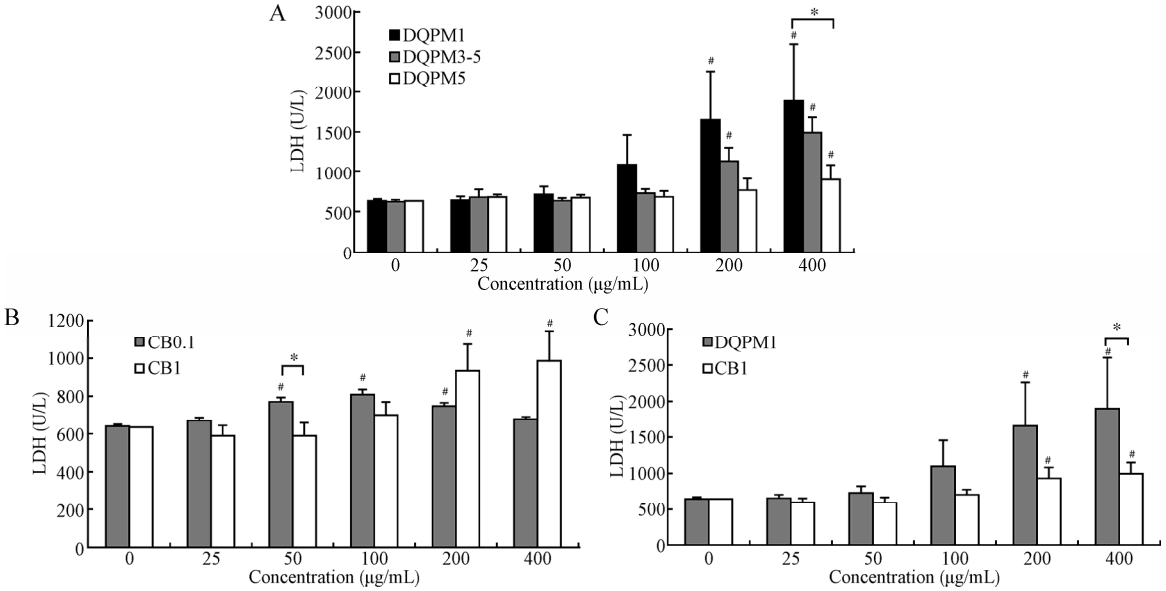

Fig. 3 Comparison of LDH levels detected in HUVECs exposed to three sizes of quartz particles (A) and two sizes of carbon black particles (B) and comparison of LDH levels induced by parallel size of quartz particles and carbon black particles (C)

The treatment of HUVECs was the same as above. The levels of LDH were determined by commercial cytotoxicity kit. Bar graphs represent $\bar{x}\pm s$ of separate experiments (n=3). $^{\#}P<0.05$ *vs.* blank control with Dunnett-t test; $^{*}P<0.05$ showing statistically significant differences among responses to three sizes of quartz particles in fig. 3A; $^{*}P<0.05$, CB0.1 *vs*. CB1 in fig. 3B; $^{*}P<0.05$, DQPM1 vs. CB1 in fig. 3C

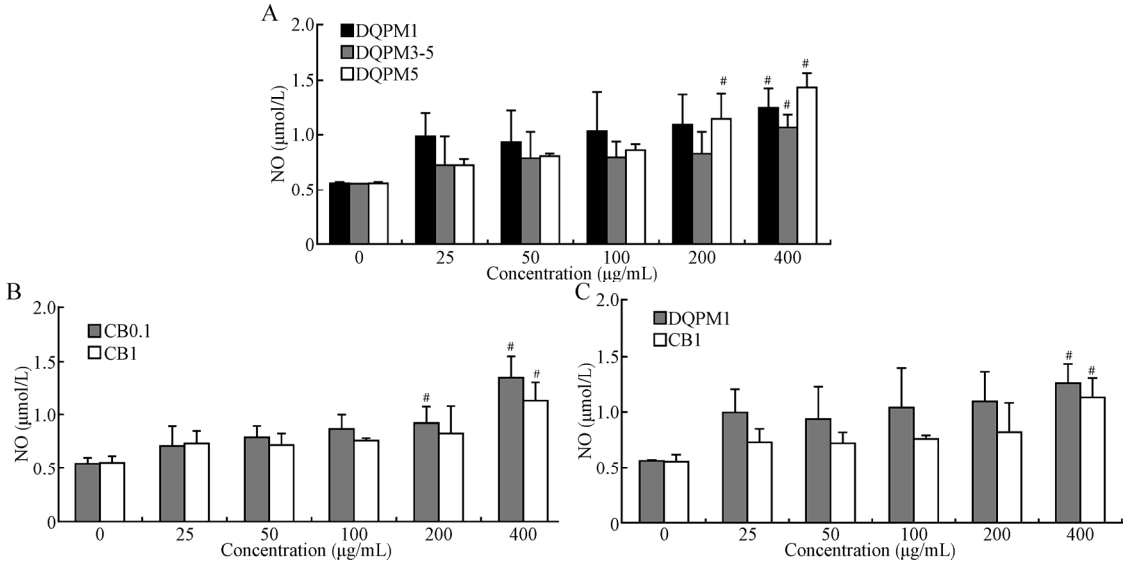

Fig. 4 Comparison of NO generation detected in HUVECs exposed to three sizes of quartz particles (A) and two sizes of carbon black particles (B) and comparison of NO generation induced by parallel size of quartz particles and carbon black particles (C)

The treatment of HUVECs was the same as above. The NO levels were assessed using NO assay kit. Bar graphs represent $\bar{x}\pm s$ of separate experiments (n=3). $^{\#}P<0.05$ *vs.* blank control with Dunnett-t test

2.4 TNF-α Release Induced by Particles

Particles-induced releases of TNF-α from HUVECs presented in dose-related manner for all samples (fig. 5). Quartz particles induced marked releases of TNF-α at

concentrations equal to 100 μg/mL or higher, while carbon black particles induced obvious releases of TNF-α at concentrations of 200 μg/mL and 400 μg/mL.

For quartz series, the smaller the particles were, the higher the TNF-α levels were at all particle concentrations. There were significant differences in TNF-α levels induced by different size groups at concentrations equal to 100 μg/mL or higher (fig. 5A). For carbon black particles, CB0.1 induced higher TNF-α releases from HUVECs than CB1 at all particle concentrations except 400 μg/mL, although no statistically significant difference was detected. Conversely, when particle concentration reached 400 μg/mL, the TNF-α level in HUVECs induced by CB1 was significantly higher than that induced by CB0.1 (fig. 5B).

As shown in fig. 5C, the effect of DQPM1 on TNF-α release was more powerful than that of CB1, and significant differences were observed at the concentrations from 100 μg/mL to 400 μg/mL.

2.5 IL-1β Release Induced by Particles

IL-1β is widely regarded as a pro-inflammation cytokine, which plays an important role in subsequent cytokine response. There was no detectable release of IL-1β from HUVECs after exposure to three sizes of quartz particles (fig. 6A), whereas both CB1 and CB0.1 contributed to the IL-1β releases alone at the highest 400 μg/mL particle concentration (fig. 6B). There was no statistically significant difference among the same particles of different size groups or between DQPM1 and CB1 (fig. 6A–C).

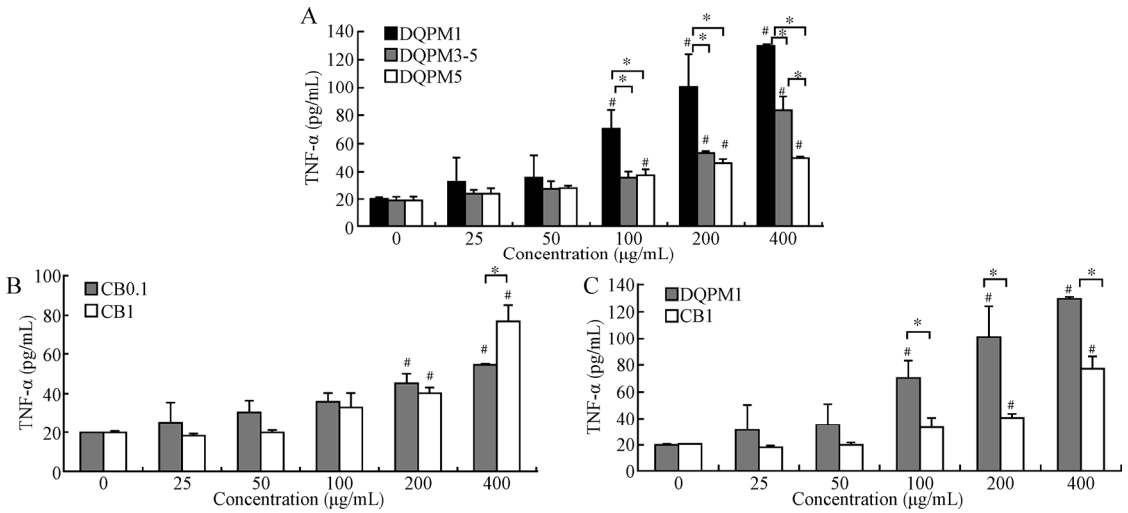

Fig. 5 Comparison of TNF-α release from HUVECs exposed to three sizes of quartz particles (A) and two sizes of carbon black particles (B) and comparison of TNF-α release induced by parallel size of quartz particles and carbon black particles (C)
The treatment of HUVECs was the same as above. Conditioned media were collected and the levels of TNF-α were analyzed by ELISA. Bar graphs represent $\bar{x}\pm s$ of separate experiments (n=3). $^{\#}P<0.05$ *vs.* blank control with Dunnett-t test; $^{*}P<0.05$ showing statistically significant differences among responses to three sizes of quartz particles in fig. 5A; $^{*}P<0.05$, CB0.1 *vs.* CB1 in fig. 5B; $^{*}P<0.05$, DQPM1 *vs.* CB1 in fig. 5C

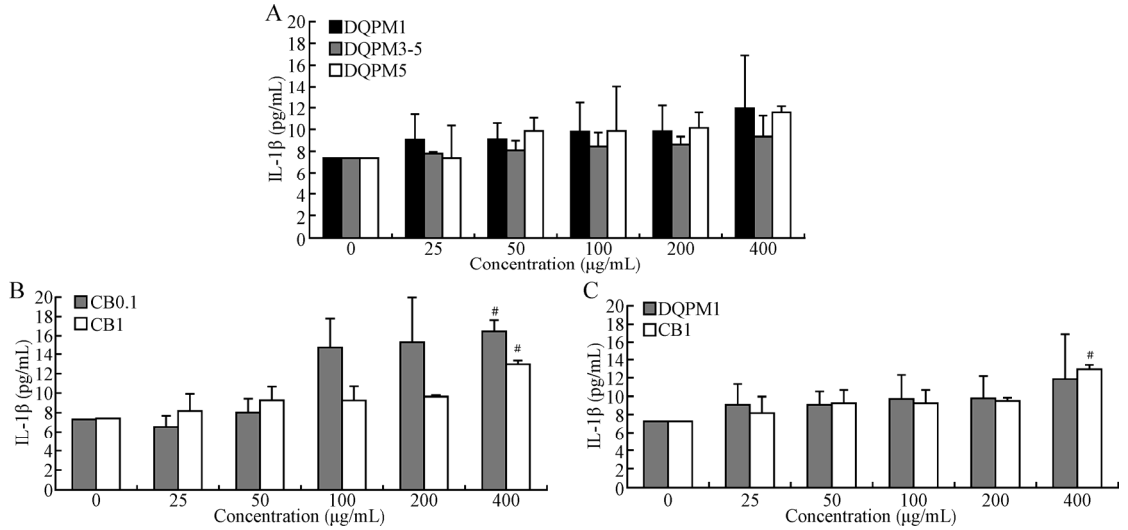

Fig. 6 Comparison of IL-1β release from HUVECs exposed to three sizes of quartz particles (A) and two sizes of carbon black particles (B) and comparison of IL-1β release induced by parallel size of quartz particles and carbon black particles (C)
The treatment of HUVECs was the same as above. Conditioned media were collected and the levels of IL-1β were analyzed by ELISA. Bar graphs represent $\bar{x}\pm s$ of separate experiments (n=3). $^{\#}P<0.05$ *vs.* blank control with Dunnett-t test

3 DISCUSSION

This study indicated that both quartz and carbon black particles induced adverse biological effects on HUVECs in a dose-dependent manner. The size of particles exerted important impact on their biological effects. For quartz particles with sizes from 1 to 5 μm, the smaller the particle size was, the stronger the cytotoxicity and more TNF-α release from HUVECs. For carbon black particles with sizes of 0.1 μm and 1 μm, CB0.1 was more capable of inducing adverse responses on HUVECs than CB1 only at particle concentrations lower than 100 μg/mL. Meanwhile, our data also revealed that quartz induced higher cytotoxicity and more TNF-α release than carbon black particles even if particle size was similar. However, there was no significant difference among the same particles of different size groups or between DQPM1 and CB1 for generation of NO or IL-1β.

The injury of vascular endothelial cells is the important causative factor of many cardiovascular diseases[10, 11]. Higher LDH activity and lower cell viability are indicative of more serious damage of cells. In the present study, we could not get quartz particle of 0.1 μm diameter because crystalline will be broken when it is cut into so small size. When diameter of quartz changed from 1 to 5 μm, we observed that LDH activity became decreased. The same trend was shown in carbon black particles: CB0.1 induced significantly higher LDH level than CB1 at particle concentration of 50 μg/mL. Whereas, interestingly, the LDH levels induced by CB1 became higher than those by CB0.1 when particle concentration was equal to 200 μg/mL or higher. The possible reason for this change might be attributed to that ultrafine particles have a tendency to aggregate at high concentrations. This may potentially reduce surface area, which is considered as a chief parameter to assess the toxicity of ultrafine particles[12, 13].

Numbers of investigations have shown that particle size and surface area determine the potential to elicit inflammatory responses and oxidative stress[14–19], which are considered as primary mechanisms to injure cardiovascular system. TNF-α and IL-1β have been suggested as important pro-inflammatory cytokines which play important roles in regulating the releases of some other cytokines and inflammation responses[20–22]. Our experiments found that both quartz and carbon black particles had powerful ability to stimulate TNF-α release from HUVECs, but the influence of size was different. For quartz series from 1 to 5 μm, the smaller the particle was, the higher TNF-α levels were induced at all particle concentrations. The results were in accordance with those obtained by Rong *et al*[20]. For carbon black particles, CB0.1 induced slightly higher TNF-α releases from HUVECs than CB1 at all particle concentrations except the highest concentration of 400 μg/mL. But the release of TNF-α in HUVECs induced by CB1 was stronger than that of CB0.1 at the concentration of 400 μg/mL. It again indicated that smaller sizes of particles were more potential to induce endothelial cells damage and inflammatory response when compared with those of larger sizes. The possible reason may be that small size particles have big surface area than large size particles with the same quality. Therefore, the number of atoms or molecules displayed and packaged on the surface of particles is increased exponentially as the size shrinks[23]. The size could make these chemicals more bioavailable at the contact sites of the particles with cells and tissues where inflammatory responses take place[17]. The decrease of TNF-α release at high concentration of CB0.1 might be that the particles become aggregate and decrease the surface area. This is normal phenomenon for ultrafine particles[24].

We observed that when the HUVECs were exposed to quartz particles directly, regardless of size, there was no obvious IL-1β secretion. Rong *et al*[20] also reported this phenomenon. They had detected that soluble mediators derived from macrophages after stimulation by quartz particles were more capable of producing the release of IL-1β in HUVECs compared with the effect of particles directly and particle size had influence on this response. Whereas, we also detected that both CB1 and CB0.1 could induce IL-1β releases only at the highest 400 μg/mL particle concentration, but particle size had no effect on this response. This indicated that compositions of carbon black particles may slightly contribute to IL-1 release in HUVECs.

This study found that both quartz and carbon black particles could induce marked generation of NO in HUVECs. This may be attributed to that these two types of particles have high relative content of pro-oxidant compounds and induce high oxidative stress. Porter *et al*[25] had reported that free SiO_2 on quartz particles could stimulate NO production. Xia *et al*[26] and Li *et al*[27] had demonstrated that relevant organic constituents such as polycyclic aromatic hydrocarbons (PAH), organic carbon and redox in particles, can induce oxidative stress in cells.

When compared with carbon black, we found that quartz particles produced higher cytotoxicity and TNF-α release of HUVECs when the particles size was similar. Liu *et al*[28, 29] and Zhang *et al*[30] had also reported that silica exposure could induce high mortality and morbidity from cardiopulmonary diseases by epidemiologic studies. The results indicated that chemical compositions of particles are decisive factor[31–34], and particle size has important influence on the pathogenicity of particles[35-37].

The limitation of this study is that no 0.1 μm quartz particle was determined because the structure of crystalline will be broken when quartz is crushed or cut into so small size.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

REFERENCES

1 Chen W, Liu Y, Wang H, *et al*. Long-term exposure to silica dust and risk of total and cause-specific mortality in Chinese workers: a cohort study. PLoS Med, 2012,9(4): e1001206

2 Wang R, Tao S, Balkanski Y, *et al*. Exposure to ambient black carbon derived from a unique inventory and high-resolution model. Proc Natl Acad Sci USA,

2014,111(7):2459-2463
3 Zhao X, Sun Z, Ruan Y, *et al*. Personal black carbon exposure influences ambulatory blood pressure: air pollution and cardiometabolic disease (AIRCMD-China) study. Hypertension, 2014,63(4):871-877
4 Kajiwara T, Ogami A, Yamato H, *et al*. Effect of particle size of intratracheally instilled crystalline silica on pulmonary inflammation. J Occup Health, 2007,49(2): 88-94
5 International Commission on Radiological Protection. Human respiratory tract model for radiological protection. Ann ICRP, 1994,24(1-3):1-482
6 Kreyling WG, Semmler-Behnke M, Seitz J, *et al*. Size dependence of the translocation of inhaled iridium and carbon nanoparticle aggregates from the lung of rats to the blood and secondary target organs. Inhal Toxicol, 2009,21(Suppl 1):55-60
7 Oberdörster G, Sharp Z, Atudorei V, *et al*. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol, 2004,16(6-7):437-445
8 Wallenborn JG, McGee JK, Schladweiler MC, *et al*. Systemic translocation of particulate matter-associated metals following a single intratracheal instillation in rats. Toxicol Sci, 2007,98(1):231-239
9 Geiser M, Kreyling WG. Deposition and biokinetics of inhaled nanoparticles. Part Fibre Toxicol, 2010,7:2
10 Zhao J, Xie Y, Qian X, *et al*. Acute effects of fine particles on cardiovascular system: differences between the spontaneously hypertensive rats and wistar kyoto rats. Toxicol Lett, 2010,193(1):50-60
11 Pan JQ, Li JC, Tan X, *et al*. The injury effect of oxygen free radicals *in vitro* on cultured pulmonary artery endothelial cells from broilers. Res Vet Sci, 2007,82(3): 382-387
12 Ramgolam K, Chevaillier S, Marano F, *et al*. Proinflammatory effect of fine and ultrafine particulate matter using size-resolved urban aerosols from Paris. Chemosphere, 2008,72(9):1340-1346
13 Oberdörster G. Pulmonary effects of inhaled ultrafine particles. Int Arch Occup Environ Health, 2001,74(1):1-8
14 Ho M, Wu KY, Chein HM, *et al*. Pulmonary toxicity of inhaled nanoscale and fine zinc oxide particles: mass and surface area as an exposure metric. Inhal Toxicol, 2011, 23(14):947-956
15 Valavanidis A, Fiotakis K, Vlachogianni T. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2008,26(4):339-362
16 Perrino C, Catrambone M, Esposito G, *et al*. Characterisation of gaseous and particulate atmospheric pollutants in the East Mediterranean by diffusion denuder sampling lines. Environ Monit Assess, 2009,152(1-4):231-244
17 Araujo JA, Barajas B, Kleinman M, *et al*. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. Circ Res, 2008,102(5):589-596
18 Ariola V, D'Alessandro A, Lucarelli F, *et al*. Elemental characterization of PM10, PM2.5 and PM1 in the town of Genoa (Italy). Chemosphere, 2006,62(2):226-232
19 Mills NL, Törnqvist H, Gonzalez MC, *et al*. Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. N Engl J Med, 2007,357(11):1075-1082
20 Rong Y, Zhou T, Cheng W, *et al*. Particle-size-dependent cytokine responses and cell damage induced by silica particles and macrophages-derived mediators in endothelial cell. Environ Toxicol Pharmacol, 2013,36(3): 921-928
21 Zhou T, Rong Y, Liu Y, *et al*. Association between proinflammatory responses of respirable silica dust and adverse health effects among dust-exposed workers. J Occup Environ Med, 2012,54(4):459-465
22 Guo J, Gu N, Chen J, *et al*. Neutralization of interleukin‐1 beta attenuates silica‐induced lung inflammation and fibrosis in C57BL/6 mice. Arch Toxicol, 2013, 87(11):1963-1973
23 Oberdörster G, Oberdörster E, Oberdörster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect, 2005, 113(7):823-839
24 Totlandsdal AI, Skomedal T, Låg M, *et al*. Pro-inflammatory potential of ultrafine particles in mono- and co-cultures of primary cardiac cells. Toxicology, 2008,247(1):23-32
25 Porter DW, Millecchia LL, Willard P, *et al*. Nitric oxide and reactive oxygen species production causes progressive damage in rats after cessation of silica inhalation. Toxicol Sci, 2006,90(1):188-197
26 Xia T, Korge P, Weiss JN, *et al*. Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity. Environ Health Perspect, 2004,112(14): 1347-1358
27 Li N, Sioutas C, Cho A, *et al*. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. Environ Health Perspect, 2003,111(4):455-460
28 Liu Y, Chen W. A SAS macro for testing differences among three or more independent groups using Kruskal-Wallis and Nemenyi tests. J Huazhong Univ Sci Technolog Med Sci, 2012,32(1):130-134
29 Liu Y, Steenland K, Rong Y, *et al*. Exposure-response analysis and risk assessment for lung cancer in relationship to silica exposure: a 44-year cohort study of 34,018 workers. Am J Epidemiol, 2013,178(9):1424-1433
30 Zhang X, Wang H, Zhu X, *et al*. Cohort mortality study in three ceramic factories in Jingdezhen in China. J Huazhong Univ Sci Technolog Med Sci, 2008,28(4): 386-390
31 Schins RP, Lightbody JH, Borm PJ, *et al*. Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents. Toxicol Appl Pharmacol, 2004,195(1):1-11
32 Beck-Speier I, Kreyling WG, Maier KL, *et al*. Soluble iron modulates iron oxide particle-induced inflammatory responses via prostaglandin E(2)synthesis: *In vitro* and *in vivo* studies. Part Fibre Toxicol, 2009,6:34
33 Soukup JM, Becker S. Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin. Toxicol Appl Pharmacol, 2001,171(1):

20-26
34 Araujo JA, Nel AE. Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. Part Fibre Toxicol, 2009,6:24
35 Donaldson K, Tran CL. Inflammation caused by particles and fibers. Inhal Toxicol, 2002,14(1):5-27
36 Brandenberger C, Rothen-Rutishauser B, Blank F, *et al*. Particles induce apical plasma membrane enlargement in epithelial lung cell line depending on particle surface area dose. Respir Res, 2009,10:22
37 Schwarze PE, Øvrevik J, Hetland RB, *et al*. Importance of size and composition of particles for effects on cells *in vitro*. Inhal Toxicol, 2007,19(Suppl 1):17-22

(Received Apr. 14, 2014; revised May 8, 2014)