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Effects of Protein Corona on Intracellular Uptake and Cytotoxicity of PM_1

Dear Editor,

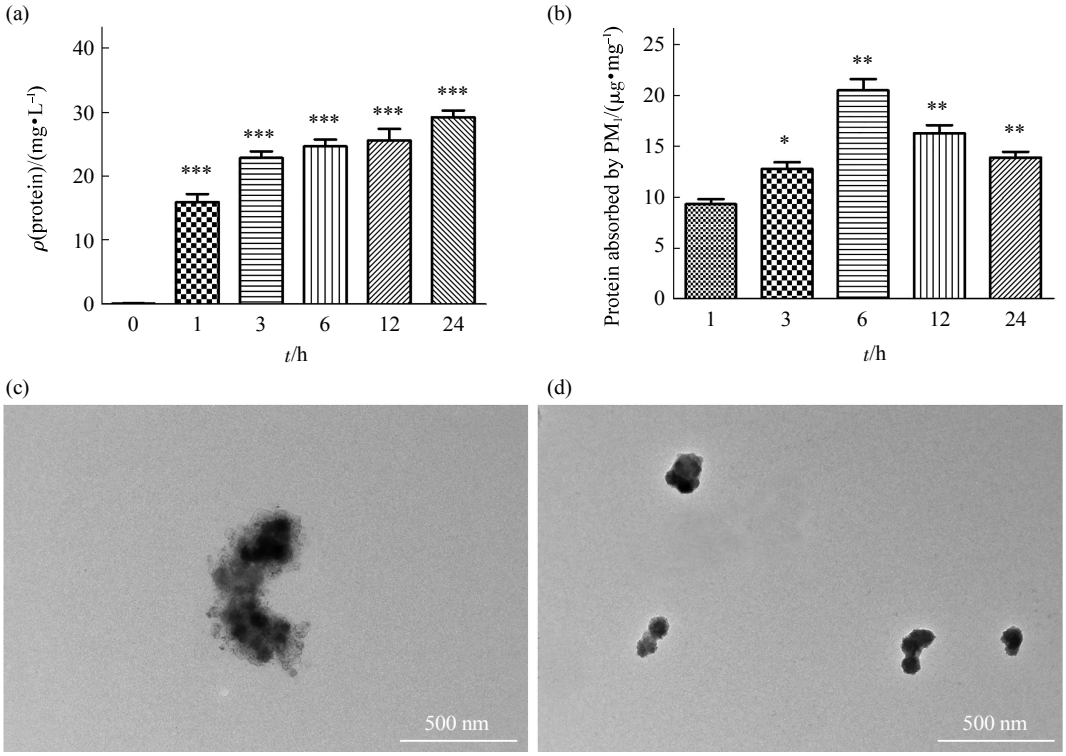
The epidemiological studies have demonstrated that both short-term and long-term exposure to high levels of ambient particulate matter (PM) lead to increased risk of mortality and morbidity of respiratory and cardiovascular diseases [1]. The effects of PM on respiratory epithelial cell function in the respiratory system are diverse and complex including toxicity by surface chemistry or inductions by soluble or bioavailable chemical compositions of particles [2]. Although it has been reported that the fine particles PM_1 (aerodynamic diameter less than 1 μm) is more harmful than $PM_{2.5}$ (aerodynamic diameter less than 2.5 μm), as PM_1 is more efficiently retained in the peripheral lung [3]. However, there is lack of understanding the pathological impacts of PM_1 on lung alveolar epithelial cells. Recently, several studies have demonstrated that proteins compete for the nanoparticle "surface" leading to a protein "corona" that largely defines the biological property of the particle [4-7]. Different reports have shown that protein corona or biological macromolecule adsorption is influenced by physiochemical nonmaterial properties and can affect the *in vivo* toxicity of the nonmaterial [8-9]. As such, evaluating and understanding the toxicity of airborne PM_1 coated with protein coronas is a critical step toward cytotoxic effects. The aim of this study was to investigate the effect of protein corona on intracellular uptake and cytotoxicity of PM_1 in human lung epithelial A549 cells.

Size distribution and chemical composition of PM_1 was measured. A dynamic light scattering measurement of PM_1 in serum-free DMEM medium showed an average size of 0.21 μm. Among the total of 19 metal elements determined, Fe (19.34 μg/mg), Al (28.64 μg/mg), Ca (16.15 μg/mg), K (11.65 μg/mg), Mg (7.53 μg/mg), Na (6.08 μg/mg) and Zn (2.83 μg/mg) were the most abundant elements in PM_1. The water soluble inorganic ions in PM_1 mainly includes NO_3^- (128 μg/mg), SO_4^{2-} (124 μg/mg) and NH_4^+ (47 μg/mg). The average concentrations of organic carbon (OC) and elemental carbon (EC) in the PM_1 were 148.61 μg/mg and 31.79 μg/mg.

In order to investigate whether A549 cells release the proteins into the culture medium, the cells were incubated in serum-free Dulbecco's modified Eagle's medium (DMEM) for 0, 1, 3, 6, 12 or 24 h, respectively. A time-dependent increase of protein concentration in the supernatant of DMEM was observed (Figure 1a). In order to determine whether PM_1 particles was covered or not by these proteins, PM_1 were incubated with the collected supernatant for 1, 3, 6, 12 or 24 h, respectively. The concentrations of protein on PM_1 were determined. Higher level of protein on PM_1 at 6 h of incubation was observed (Figure 1b). The transmission electron microscope (TEM) image confirmed that there is protein corona surrounding the PM_1 (Figure 1c).

In order to determine that the uptake levels were related to the presence of protein corona on the PM_1, TEM examination was performed to compare PM_1 intracellular localization. As shown in Figure 2a and 2b, uptake in cells exposed to PM_1 in the presence of protein corona was higher than that in the absence of protein corona, at the same exposure times. We also noted that the cell viability was significantly decreased after exposure of PM_1 with protein corona (Figure 2c) as compared to exposure of PM_1 without protein corona (Figure 2d). These results suggested that PM_1 particles exposed to cells in the presence of protein corona have higher internalization efficiency and a stronger cytotoxicity.

In order to clarify their nature and origin, liquid chromatography technique coupled with tandem mass spectrometry (LC-MS) has been used for their identification. A list of the most abundant proteins on the PM_1 was given in Figure 1e. The major components of the corona were cytosolic proteins, components of the cytoskeleton, and proteins normally associated with the cell membrane. These results can be related to the strong adhesion of the PM_1 on the cell membrane in serum-free conditions and are indicative of cell damage even after only 1 h of PM_1 exposure (Figure 2c). In summary, the observations suggest that the protein corona on PM_1 could play a major role in the induction of cell damage, which is probably associated with the adverse health effects of PM.



(e)

Protein name	Accession number	Cellular location and function
Alpha-2-HS-glycoprotein precursor	P02765	Location: Extracellular matrix. Function: Promotes endocytosis, shows affinity for calcium and barium ions.
Prosaposin	P07602	Location: Extracellular matrix. Function: Binding to lipid, protein or G-protein coupled receptor.
Moesin	P26038	Location: Cell membrane, apical cell membrane, cell projection and microvillus membrane. Function: regulation of cell shape and podosome assembly.
Radixin	P35241	Location: cell periphery and extracellular matrix. Function: establishment of protein localization, positive regulation of cell migration and cell shape.
Annexin A2	P07355	Location: extracellular matrix and extracellular region. Function: Positive regulation of vesicle fusion.
Alpha-actinin-4	O43707	Location: Cytoplasm and cell junction. Function: Involved in vesicular trafficking *via* its association with the CART complex.
Alpha-actinin-1	P12814	Location: Cell membrane and cell projection. Function: F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein.
Talin-1	Q9Y490	Location: Cell surface, extracellular region and intercellular junction. Function: It codistributes with integrins in the cell surface membrane in order to assist in the attachment of adherent cells to extracellular matrices and of lymphocytes to other cells. The N-terminus of this protein contains elements for localization to cell-extracellular matrix junctions. The C-terminus contains binding sites for proteins such as beta-1-integrin, actin, and vinculin.
Clathrin	Q00610	Location: Cytoplasmic face of intracellular organelles. Function: Involved in the intracellular trafficking of receptors and endocytosis of a variety of macromolecules.
Annexin A1	P04083	Location: Cell membrane and cell projection. Function: Promotes membrane fusion and is involved in exocytosis.

Fig. 1 The formation of protein corona on PM_1

A549 cells were incubated with serum-free DMEM for 24 h. (a) The concentration of protein secreted by A549 cells in serum free medium. (b) The concentration of protein adsorbed by PM_1 from the cultured A549 cells. (c, d) TEM image of PM_1 after 24 h of incubation with the culture supernatants (c) and serum free DMEM (d). (e) The most abundant proteins adsorbed on PM_1.

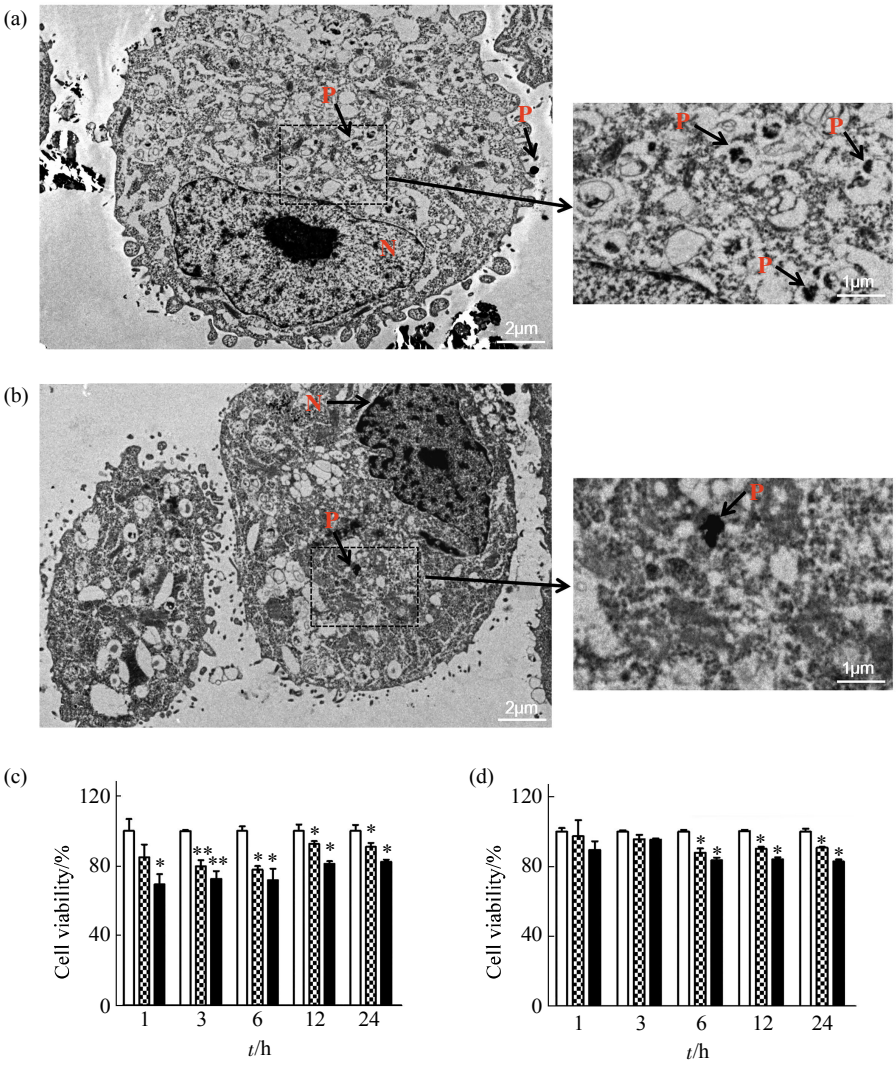


Fig. 2　Effect of protein corona on the interaction between PM_1 and A549 cells

(a, b) TEM images of A549 cells exposed to PM_1 with protein corona (a) and without protein corona (b). N means cell nucleus, P means PM_1. (c, d) The viability of A549 cells exposed to PM_1 with protein corona (c) and without protein corona (d). □: Control; ⚄: 100 μg/ml; ■: 200 μg/ml.

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