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Mesoporous carbon nanomaterials induced pulmonary surfactant inhibition, cytotoxicity, inflammation and lung fibrosis

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ABSTRACT

Environmental exposure and health risk upon engineered nanomaterials are increasingly concerned. The family of mesoporous carbon nanomaterials (MCNs) is a rising star in nanotechnology for multidisciplinary research with versatile applications in electronics, energy and gas storage, and biomedicine. Meanwhile, there is mounting concern on their environmental health risks due to the growing production and usage of MCNs. The lung is the primary site for particle invasion under environmental exposure to nanomaterials. Here, we studied the comprehensive toxicological profile of MCNs in the lung under the scenario of moderate environmental exposure. It was found that at a low concentration of 10 μg/mL MCNs induced biophysical inhibition of natural pulmonary surfactant. Moreover, MCNs at similar concentrations reduced viability of J774A.1 macrophages and lung epithelial A549 cells. Incubating with nature pulmonary surfactant effectively reduced the cytotoxicity of MCNs. Regarding the pro-inflammatory responses, MCNs activated macrophages *in vitro*, and stimulated lung inflammation in mice after inhalation exposure, associated with lung fibrosis. Moreover, we found that the size of MCNs played a significant role in regulating cytotoxicity and pro-inflammatory potential of this nanomaterial. In general, larger MCNs induced more pronounced cytotoxic and pro-inflammatory effects than their smaller counterparts. Our results provided valuable information on the toxicological profile and environmental health risks of MCNs, and suggested that fine-tuning the size of MCNs could be a practical precautionary design strategy to increase safety and biocompatibility of this nanomaterial.

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Introduction

Mesoporous carbon nanomaterials (MCNs) possess desirable physicochemical properties, including chemical stability, high electric and thermal conductivity and strong optical absorption feature (Xu et al., 2014). Moreover, MCNs possess a well-organized pore network, with the pore size usually ranging from 1.5 to 10 nm (Xu et al., 2014). This intricate porosity generates a high surface area and high pore volume, which endow this material with enhanced capability in storing energy and chemotherapeutic drugs (Kim et al., 2008; Vallet-Regí et al., 2007). Owing to these pronounced physicochemical properties, MCNs have been squeezed into the cutting edge of nanotechnology. Studies have revealed promising applications of MCNs in a large array of biomedical branches, such as cancer nanotheranostics and biosensing. The development of MCN-centered biomedicine requires in-depth understanding about its biocompatibility, biosafety and potential health risks. Compared to other carbon nanomaterials, the current understanding on the biocompatibility and health effects of MCNs is rather limited. Thus, sufficient insights into its toxicity profile become a prerequisite for further development of MCN-based applications.

In general, the respiratory system is most susceptible to environmental exposure to airborne nanomaterials. Although relatively large particles can be cleared by the mucociliary escalator in airway, nanomaterials such as MCNs may penetrate deeply into the lung. The entire air–water interface of the lung is coated with an adsorbed pulmonary surfactant film (Zuo et al., 2008). The pulmonary surfactant is composed of approximately 90% phospholipids and 10% surfactant-associated proteins by weight Lopez-Rodriguez and Pérez-Gil, 2014). The major physiological function of the pulmonary surfactant is to act as the first-line host defense against inhaled particles and pathogens, and to reduce the alveolar surface tension against lung collapse. Deficiency or dysfunction of the pulmonary surfactant leads to pathological pulmonary conditions such as the respiratory distress syndrome and acute lung injury (Lewis and Veldhuizen, 2003).

The pulmonary surfactant film functions as the initial biological barrier against inhaled particles (Fan et al., 2011; Hu et al., 2013). Consequently, interacting with the inhaled particles may adversely affect the biological function of the pulmonary surfactant film. Accumulating evidence showed that various engineered nanomaterials inhibited the biological function of the pulmonary surfactant, *i.e.*, abolished its ability of reducing surface tension to near-zero values (Beck-Broichsitter et al., 2011; Fan et al., 2011; Sachan et al., 2012). It was found that the extent of surfactant inhibition caused by the inhaled nanomaterials largely depended on their physicochemical properties, such as the chemical, size, shape, surface area, hydrophobicity, and surface charge of the nanomaterials (Beck-Broichsitter et al., 2011; Fan et al., 2011; Sachan et al., 2012). After interaction and translocation across the pulmonary surfactant film, inhaled nanomaterials are in direct contact with the alveolar epithelium cells and macrophages, posing detrimental effects on cells and even causing inevitable cell death (Xia et al., 2016). Meanwhile, some invaded particles can be phagocytosed by resident macrophages (Xia et al., 2016). Therefore, macrophages form a group of important sentinels to sense and engulf invading particles (Thomas et al., 2013). Macrophages are also activated through sensing molecules to enhance pro-inflammatory responses by secreting inflammatory cytokines and recruiting additional inflammatory leukocytes (Sandberg et al., 2012).

In this study, we systematically evaluated the biocompatibility and potential toxicity associated with MCNs of various sizes. We first evaluated the potential adverse biophysical effect of MCNs on natural pulmonary surfactant. Then, we studied the toxic effects of MCNs through investigating cell viability, cellular uptake, oxidative stress, pro-inflammatory responses *in vitro* and *in vivo*, and lung fibrosis in mice. We found that MCNs caused pulmonary surfactant inhibition and induced significant cytotoxicity to macrophages. This study opened a new avenue to understand the adverse biological effects and environmental health risks of MCNs.

1. Materials and methods

1.1. Synthesis and characterization of MCNs

Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), 2-methylimidazole, cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), and PEG-Vitamin E (PEG-VE) were purchased from Sigma (USA). All reagents were in the analytical grade and were used without further purification. In brief, $Zn(NO_3)_2{\cdot}6H_2O$ (3 amounts at 16, 24 and 32 mmol were individually used for different sizes) was dissolved in 225, 300, and 450 mL methanol, respectively, and 2-methylimidazole (67.5, 101.25 and 135 mmol) were thereafter added into methanol accordingly. Then, two methanol solutions were mixed, and stirred for 2 hr at 25°C. For the intermediate sized MCNs (I-MCNs), 225 mL methanol solution containing 16 mmol $Zn(NO_3)_2{\cdot}6H_2O$ and 67.5 mmol 2-methylimidazole were individually added into the above solution. For the largest sized MCNs (L-MCNs), 150 mL methanol solution containing 8 mmol $Zn(NO_3)_2{\cdot}6H_2O$ and 33.75 mmol 2-methylimidazole were accordingly added into the above solution. For the smallest sized MCNs (S-MCNs), no solution was added. Finally, imidazolate framework (ZIF-8), a carbon precursor, was obtained through centrifugation and washing. Next, the carbon precursors were dispersed in 240 mL methanol (pH 11, adjusted by NaOH), and the CTAB solution (0.8 mmol/L) was added. TEOS (1.2 mL) was then added dropwise into the above mixed solution, and the resulting solution was stirred for 0.5 hr. The carbon precursor@$mSiO_2$ was separated by centrifugation, and washed with ethanol. Afterwards, the carbon precursor@$mSiO_2$ was pyrolyzed for 2 hr under N_2 atmosphere at 800°C, and was cooled naturally to room temperature. The samples were added into 4 mol/L NaOH solution to remove the $mSiO_2$ shell. The obtained samples were centrifuged and washed with water until the pH of supernatant reached 7.0. Finally, all samples were modified by PEG-VE (1 kD). The morphology and size of MCNs were characterized by H-7500 electron microscope (TEM) and SU8020 scanning electron microscope (SEM). The zeta potential and hydrodynamic diameter were measured using dynamic light scattering (DLS, Malvern, UK). In addition, carbon black (CB), purchased from Macklin, was used a reference carbonaceous material in the current study.

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