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Preliminary investigation on cytotoxicity of fluorinated polymer nanoparticles

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ABSTRACT

As well-known persistent organic pollutants (POPs), organofluorine pollutants such as perfluorooctane sulfonate (PFOS) have been proven to be bioaccumulated and harmful to health. However, toxicological assessment of organofluorinated nanoparticles, which have emerged as a novel tool for biomedical and industrial applications, is lacking, to the best of our knowledge. To assess the biological effects and health risk of fluorinated nanoparticles, trifluoroethyl aryl ether-based fluorinated poly(methyl methacrylate) nanoparticles (PTFE-PMMA NPs) were synthesized with various fluorine contents (PTFE-PMMA-1 NPs 12.0 wt.%, PTFE-PMMA-2 NPs 6.1 wt.% and PTFE-PMMA-3 NPs 5.0 wt.%), and their cytotoxicity was investigated in this study. The *in vitro* experimental results indicated that the cytotoxicity of PTFE-PMMA NPs was mild, and was closely related to their fluorine (F) contents and F-containing side chains. Specifically, the cytotoxicity of PTFE-PMMA NPs decreased with increasing F content and F-containing side chains. After exposure to PTFE-PMMA NPs at a sublethal dose (50 μg/mL) for 24 hr, the phospholipid bilayer was damaged, accompanied by increasing permeability of the cell membrane. Meanwhile, the intracellular accumulation of reactive oxygen species (ROS) occurred, resulting in the increase of DNA damage, cell cycle arrest and cell death. Overall, the PTFE-PMMA NPs were found to be relatively safe compared with typical engineered nanomaterials (ENMs), such as silver nanoparticles and graphene oxide, for biomedical and industrial applications.

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Introduction

Fluorinated nanoparticles belong to a class of important nanomaterials, and have emerged as a promising tool for biomedical and industrial applications (Kislukhin et al., 2016; Kurczy et al., 2015; Pengo and Pasquato, 2015; Yao et al., 2014). Compared with other organic nanoparticles, organofluorinated nanoparticles are more tolerant to high temperature, ultraviolet radiation, high-frequency, acid–base corrosion, oxidation and other extreme conditions or extreme environments. This is because the bond energy of carbon–hydrogen bonds in basic organic nanoparticles is relatively weak (about 99 kcal/mol), and the introduction of carbon–fluorine bonds (>116 kcal/mol) into organic nanoparticles can improve their performance (Yao et al., 2014; Mathkar et al., 2013; O'Hagan, 2008). For example, fluorine (F)-containing synthetic rubber has been widely applied in modern aviation, military technology, space navigation, *etc.* (Souzy et al., 2004; Iacono et al., 2007). Additionally, isotopically labeled fluorine (*e.g.*, ^{18}F and ^{19}F) in fluorinated nanoparticles can provide many advantages for *in vitro* and *in vivo* bioimaging and medical therapy (Kislukhin et al., 2016; Kurczy et al., 2015; Que et al., 2016; Bailey et al., 2010; Hu et al., 2016).

Fluorinated compounds and materials have been widely used in our daily life, but their potential toxicity is far from being understood. Previous studies have demonstrated that chronic exposure to F and its compounds could induce damage to the brain, bones, kidney, teeth, *etc.* (Jha et al., 2011). For example, as typical persistent organic pollutants (POPs), perfluorooctane sulfonate (PFOS) is persistent, bioaccumulative and toxic to mammalian species. In animal studies, PFOS has been reported to cause cancer, endocrine disruption, neonatal mortality, *etc.* (Lau et al., 2004; DeWitt et al., 2009; Younglai et al., 2007). The biological toxicity and potential health risks of fluorinated nanoparticles have been poorly known until now, although various functional fluorinated nanoparticles have been developed in recent years, which have emerged as valuable tools due to their remarkable chemical and physical characteristics (Kislukhin et al., 2016; Bailey et al., 2010; Kurczy et al., 2015; Que et al., 2016).

As potential hazardous materials and environmental pollutants, engineered nanomaterials (ENMs) have received a lot of attention in the field of ecotoxicological science, which has rapidly developed in recent years (Handy et al., 2008). Before the practical and commercial application of new nanomaterials, evaluation of their biosafety evaluation and toxicity is becoming increasingly necessary and significant. For example, ENMs such as graphene-like nanomaterials have been extensively studied in order to better understand their biological effects and improve their biocompatibility (Zou et al., 2016; Xu et al., 2016; Zhu et al., 2017).

In order to investigate the toxicity of fluorinated nanoparticles and understand the role of F atoms in the carbon skeleton of organic polymers, trifluoroethyl aryl ether-based fluorinated poly(methyl methacrylate) nanoparticles (PTFE-PMMA NPs) were synthesized first based on our previous work (Yao et al., 2014; Tong et al., 2008). Then, the cytotoxicity of PTFE-PMMA NPs with various F contents was investigated as a pilot study. Finally, the underlying toxicological mechanisms involved in two representative cell lines (liver carcinoma HepG2 cells and monocyte macrophage J774A.1 cells) were studied.

1. Materials and methods

1.1. Synthesis and characterization of PTFE-PMMA NPs

The detailed synthesis processes and characterization of the fluorinated compounds and PTFE-PMMA NPs are described in supporting information (Appendix A. Figs. S1–S9). Through adjusting the ratio of methyl methacrylate (monomer A) and trifluoroethyl aryl ether-based methacrylate (monomer B), PTFE-PMMA-1 NPs (A:B = 3:1), PTFE-PMMA-2 NPs (A:B = 5:1) and PTFE-PMMA-3 NPs (A:B = 7:1) were synthesized with different F contents. To characterize the synthesized trifluoroethyl aryl ether-based methacrylate (monomer B) and PTFE-PMMA NPs, nuclear magnetic resonance (NMR) analysis was performed on a Bruker Avance 500 spectrometer (500 MHz) in $CDCl_3$. Tetramethylsilane (TMS) (^1H NMR) was used as an internal standard, and CF_3CO_2H was used as an external standard for ^{19}F NMR. Finally, the PTFE-PMMA-1 NPs, PTFE-PMMA-2 NPs and PTFE-PMMA-3 NPs were dispersed in dimethyl sulfoxide (DMSO) as stock solutions (16 mg/mL).

Transmission electron microscopy (TEM) analysis of PTFE-PMMA NPs was performed on a transmission electron microscope (H-7500, Hitachi Scientific Instruments, Japan). The TEM samples were prepared by depositing a small drop of solution onto a carbon-coated copper electron microscopy grid (Beijing Zhongjingkeyi Technology Co., Ltd., China) and were then dried at room temperature. The zeta (ζ)-potential and hydrodynamic diameter of PTFE-PMMA NPs in deionized (DI) water and cell culture media were assayed using a Zetasizer (Malvern Nano series, Malvern, UK).

1.2. Cell culture

Liver carcinoma HepG2 cells and monocyte macrophage J774A.1 cells were obtained from the Shanghai Cell Bank of Type Culture Collection of China, and used for *in vitro* experiments in this study. Cells were seeded in 96, 12 or 6-well plates with the density of 1×10^4, 2×10^5 or 4×10^5 cells/well, respectively. Then, the cells were cultured at 37°C and 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco BRL Life Technologies Inc., USA) supplemented with 10% fetal bovine serum (FBS) and 100 units/mL penicillin/streptomycin (Invitrogen).

1.3. Cellular localization of PTFE-PMMA NPs through TEM

For TEM analysis, J774A.1 cells were exposed to PTFE-PMMA-1 NPs, PTFE-PMMA-2 NPs and PTFE-PMMA-3 NPs at 50 μg/mL, respectively. After 24 hr, the cells were collected at 1000 r/min for 3 min, and repeatedly washed with phosphate buffer saline (PBS) three times. The harvested cells were fixed with 2.5% glutaraldehyde solution and embedded in epoxy resin. The ultrathin cell specimens (70 nm) were placed on the grids, stained with 1% lead citrate and 0.5% uranyl acetate and then examined with TEM. Intracellular localization of PTFE-PMMA NPs was studied using a high-resolution transmission electron microscope (JEOL JEM 2010F, Hitachi Scientific Instruments, Japan).

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