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Regular Article

Application of melatonin-loaded poly(N-isopropylacrylamide) hydrogel particles to reduce the toxicity of airborne pollutes to RAW264.7 cells

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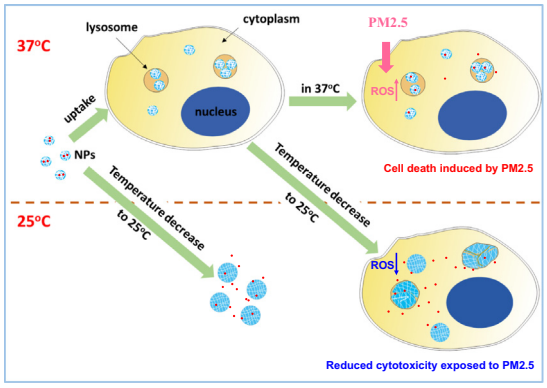
GRAPHICAL ABSTRACT

Thermo-responsive PNIPAM microgel particles were used to encapsulate anti-oxidant drug melatonin to reduce the cytotoxicity of Raw264.7 cells induced by the extractant of PM2.5 pollutes by suppressing the intracellular ROS level.



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ABSTRACT

The adverse effect of airborne PM2.5 pollutes on human beings and the environment, most likely due to heavy metal leaching, has received extensive attention recently. It is urgently required to develop a simple and effective method to suppress the toxicity of PM2.5 pollutes. In this study, the heavy metal content of PM2.5 pollutes around Zhejiang university were firstly identified. Their cytotoxicity was confirmed, by inducing significantly enhanced intracellular reactive oxygen species level. Poly(N-isopropylacrylamide) (PNIPAM) submicron hydrogel particles with tunable crosslinking densities and thermo-responsive swelling/shrinking properties were then prepared by adjusting the crosslinking density. Anti-oxidant drug melatonin (MLT) was encapsulated into the obtained PNIPAM nanogel particles with the drug loading efficiency larger than 50%, achieving thermo-responsive drug release profile. The PNIPAM/MLT particles had a strong ability to reduce the cytotoxicity to Raw264.7 cells induced by the extractant of PM2.5 pollutes, as well as to suppress the intracellular reactive oxygen species (ROS) level and secretion of tumor necrosis factor alpha (TNF-α), especially when the cells were treated under 25 °C for 3 h after ingestion of the PNIPAM/MLT particles. This concept-proving study demonstrates the potential to use the thermo-responsive PNIPAM/MLT particles to suppress the toxicity of airborne PM2.5 pollutes, which is a paramount requirement for human health.

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1. Introduction

Particulate matters (PM), classified as coarse (2.5–10 μm aerodynamic diameter, PM10), fine (0.1–2.5 μm aerodynamic diameter, PM2.5), and ultrafine (⩽0.1 μm aerodynamic diameter) particles, are heterogeneous mixtures of gases, liquid and solid particles of different origins and sizes suspended in air [1,2]. Fine particles remain suspended in the atmosphere for days and even weeks. They are able to deposit in the extrathoracic airways or penetrate deeper into the smaller airways and alveoli, where they can remain for weeks or months. Particles can be eliminated by the mucociliary system in the conductive airways and/or undergo phagocytosis by macrophages, when deposited in the alveolar regions [3,4]. More importantly, components leached from PM2.5 can enter the circulation and cause arterial plaque deposits, vascular inflammation and atherosclerosis, leading to heart attack or other cardiovascular problems [5–8]. It is estimated that PM2.5 ambient air pollution is responsible for approximately 0.8 million premature deaths and 6.4 million of life lost every year worldwide [9,10].

The mechanisms involved in the relationship between PM2.5 and adverse health effects are not well understood. Nevertheless, it is believed that an association between inflammatory process and oxidative stress exists because PM2.5 consists of a lot of heavy metal ions, sulfate, nitrate, carbon materials and harmful virus/bacteria [11–17]. For example, PM2.5 inhalation or instillation promotes inflammatory responses in animals and humans, characterized by cytokine release, increased oxidative stress and vascular permeability with concomitant neutrophil recruitment as well as increased expression of genes related to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation such as tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) and Interleukin 6 (IL-6) [18–21]. In particular, many types of heavy metals such as Fe, Zn, Pb, As, Cd, Cr, Cu and Ni are identified in the examined PM2.5 collected from China [11,22]. Since it is well acknowledged that release of toxic heavy metal ions and subsequently induced reactive oxygen species (ROS) in cells are responsible for cell death and tissues/organs injury, chelating agents of metal ions and ROS scavenger may be useful for suppressing the toxicity of PM2.5. For example, Cherny et al. employed the Cu/Zn-selective chelators to bind copper, iron, and zinc ions from β-amyloid plaques, and thereby the copper and iron-dependent neurotoxicity was alleviated [23–25]. Besides, elimination of ROS by antioxidants such as mannitol, vitamin C, polyphenols and peroxidases would protect the cells and largely reduce the toxicity of nanomaterials [26,27]. In our previous study, a hydrophobic antioxidant and a chelation agent curcumin was encapsulated into bovine serum albumin (BSA) particles, which suppressed the cytotoxicity generated by CuO nanoparticles as a result of decrease of both the intracellular reactive oxygen species (ROS) level and Cu^{2+} concentration [28,29]. However, the potential toxicity of curcumin at high concentration limits the overall detoxing effect.

Melatonin (MLT, 5-methoxy-N-acetyl-tryptamine), which can be produced by animals, plants, fungi and bacteria, is known as an excellent anti-oxidant molecule [30–33]. For example, melatonin can suppress the production of ROS induced by UV irradiation in leukocytes [34]. Tocharus et al. reported that MLT could attenuate the toxicity of methamphetamine which causes oxidative stress and inflammation due to the overproduction of both ROS and reactive nitrogen species (RNS) [35]. Wang et al. demonstrated that MLT suppressed migration and invasion of glioma cells via inhibition of oxidative stress pathway [36]. Besides, Galano et al. showed that MLT and its metabolites can be used as chelating agents of Cu^{2+} to turn off the ROS production and reduce the oxidative stress [37]. Although melatonin is generally considered to be safe for most adults when taken by mouth or injected into the body for a short-term, it can cause some side effects including headache, short-term feelings of depression, daytime sleepiness, dizziness, stomach cramps, and irritability [38–40]. Therefore, a drug delivery system might be advantageous to minimize these side effects of MLT.

Stimuli-responsive materials have attracted great interest because their physical and chemical properties can be mediated in response to external stimuli such as temperature [41–44], magnetic field [45,46], ultrasonication [47], light[42,48], pH [49], enzyme [50,51], redox [52–54]. In particular, thermo-responsive polymeric particles have been widely used as smart drug delivery vehicles, because temperature is easier to control and the change of temperature usually will not cause significant toxicity to cells and tissues in a certain range [55]. Thermo-sensitive particles with lower critical solution temperature (LCST) can load and protect drugs when the particles are in a collapse state at a temperature higher than LCST. At a temperature below LCST, the polymer networks undergo collapse to swelling transition to release the loaded substances [56].

Poly(N-isopropylacrylamide) (PNIPAM) is a typical thermo-responsive polymeric material with a LCST at 32 °C and has received extensive attention [57–63]. In this study, cross-linked PNIPAM particles are prepared and used as carriers of MLT. Their impacts on the suppression of cytotoxicity induced by PM2.5 extractant are studied in terms of cell viability, intracellular ROS production and TNF-α secretion by using murine macrophage cells (RAW264.7) as model.

2. Experimental section

2.1. Materials

N-Isopropylacrylamide (NIPAM), poly(ethylene glycol) diacrylate (PEGDA, Mw 575 Da), ammonium persulfate (APS), melatonin (MLT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT) and bacterial lipopolysaccharide (LPS) from Escherichia coli O111:B4 were purchased from Sigma-Aldrich. NIPAM was sequentially recrystallized from toluene and hexane before use. Sodium dodecyl sulfate (SDS) was purchased from Haotian Co., Ltd., China. Acrylic acid (AAc) was purchased from Sinopharm Group Co., Ltd., China and purified by reduced pressure distillation prior to use. Water was purified via a Milli-Q® System equipped with a quantum™ cartridge, and had a resistivity of 18.2 MΩ/cm. Other chemicals were of analytical grade and used as received.

2.2. Collection of PM2.5

PM2.5 was collected using Libra Plus 20 (A.P. BUCK, USA) in front of the Department of Polymer Science and Engineering in the campus of Zhejiang University, Hangzhou, China, from March to April of 2015. The particulates were collected on SiO_2 fiber filters (Munktell, Sweden) and weighed before use. The filter with certain amount of PM2.5 was immersed into water, and the particles were released with the assistance of ultrasonication (30 min). The solution containing PM2.5 was centrifuged at 12,000*g* for 5 min. The supernatant was sterilized by passing through a 220 nm filter and kept under 4 °C until use. The metal ions in the supernatant were quantified using inductively coupled plasma–mass spectrometry (ICP-MS, XSENIES, USA). We used the concentration of PM2.5 to represent the extractant obtained.