Original Article

Combined Toxicity of an Environmental Remediation Residue, Magnetite Fe_3O_4 Nanoparticles/Cr(VI) Adduct

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Abstract

Objective This paper aims to elucidate the combined toxicity of magnetite nanoparticles/Chromium [MNPs/Cr(VI)] adducts.

Methods The HEK293 cell was exposed to either Cr(VI) or MNPs, or their adducts MNPs/Cr(VI). The cytotoxicity was evaluated by assessing the cell viability, apoptosis, oxidative stress induction, and cellular uptake.

Results The toxicity of formed adducts is significantly reduced when compared to Cr(VI) anions. We found that the cellular uptake of MNPs/Cr(VI) adduct was rare, only few particles were endocytosed from the extracellular fluid and not accumulated in the cell nucleus. On the other hand, the Cr(VI) anions entered cells, generated oxidative stress, induced cell apoptosis, and caused cytotoxicity.

Conclusion The results showed minor effects of the nanoadducts on the tested cells and supported that magnetite nanoparticles could be implemented in the wastewater treatment process in which advantageous properties outweigh the risks.

Key words: Magnetite Fe_3O_4; Chromium; Adduct; Toxicity

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INTRODUCTION

Chromium is a major industrial pollutant and its environmental level keeps increasing due to the extensive usage in leather tanning, stainless-steel production, and electroplating[1]. Chromium can accumulate in human's food chain to impact human physiology, and causes many diseases. It may result in severe health problems ranging from simple skin irritation to lung carcinoma when contact with chromium[2]. The removal of highly toxic Cr(VI) has been proposed using membrane filtration[3], chemical agents and precipitation[4], ion exchange resins[5] among many others. However, these techniques are costly, energy-intensive and not efficient for removing Cr(VI) at low concentration. With the rapid development of nanotechnology, nanoparticles have been widely used in wastewater purification[6-9].

The studies on magnetite nanoparticles (MNPs) have shown good potential in the removal of metals *via* surface adsorption. This benefits its physicochemical properties, such as the high ratio of surface-to-volume that results in a better and more efficient on adsorption ability. Magnetite-supported adsorbents can be easily separated from the treated water using an external magnet. Therefore an efficient, economic, scalable synthesis of MNPs has been widely demanded in wastewater purification industry[10].

Although nanomaterials (NPs) have shown a profound impact on the practical applications, their

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potential biological and environmental toxicity have not been sufficiently studied yet. The adsorption of pollutants to nanoparticles alters the properties for both pollutant and nanoparticle, resulted adducts may cause an immediate threat to human health and ecology. For instance, the toxicity effects of titanium dioxide NPs + glucose to rats (Chen et al.[11]) showed that oral exposure of NPs + glucose induced more evident toxicity than NPs alone due to the effects of excessive glucose and the interactions between NPs and glucose. Wang et al.[12] have reported that vitamin C promoted the toxicity of ZnO NPs to gastric epithelial cell line and neural stem cells because the vitamin C accelerated uptake of Zn ions and the dissolution of ZnO NPs. Both indirect mechanisms and synergistic or inhibitory effects can enhance or suppress the expected responses from the specific classes of pollutants. Therefore, the biological impacts of nanoparticles-adducts need to be cautious evaluated. Recently, significant research efforts have been made toward the investigation of nanoparticle toxicity, very little attention has been paid to nanoadducts though the nanoadduct formations has been broadly used in environmental remediation which also has potentials to cause pollution in even broader areas. Thus, the impact of nanoadducts also needs to be thoroughly studied and it is just as important as the study of nanomaterials and pollutants.

The objective of the present study was to evaluate the effect of MNPs/Cr(VI) adducts on human embryonic kidney cell line HEK293 by assessing cell viability, apoptosis, oxidative stress induction, and cellular uptake. HEK293 was used as a model because Cr(VI) is known to induce nephrotoxicity[13]. they are essential in defining the toxicological response of the *in vitro* culture models to nanoparticle adducts exposure. Our results indicated that the cytotoxicity of the MNPs/Cr(VI) adducts was remarkably reduced compared to Cr(VI) anions. And the cellular uptake of MNPs/Cr(VI) adducts was rare. The particles were endocytosed from the extracellular fluid and could not enter into the cell nucleus. In this case, MNPs/Cr(VI) adduct formation significantly reduces its associated cytotoxicity.

MATERIALS AND METHODS

Synthesis of Magnetite Fe_3O_4 and MNPs/Cr(VI) Adduct

Magnetite Fe_3O_4 nanoparticles (MNPs) were synthesized by coprecipitation of ferric and ferrous ion according to Laurent et al.[14]. A stock of solution of Cr(VI) at concentration of 500 mg/L was prepared by dissolving a known quantity of potassium dichromate ($K_2Cr_2O_7$) in ultrapure water. MNPs/Cr(VI) adduct was prepared by mixing appropriate amount of MNPs with Cr(VI) solutions of varying concentration from 0 to 30 mg/L in 15 mL centrifuge tubes. After formation of MNPs/Cr(VI), MNPs/Cr(VI) adduct was washed by centrifugation and re-dispersion in distilled water for three times.

Characterization

The size of morphology of MNPs and MNPs/Cr(VI) adducts were characterized by scan electron microscopy (SEM, FESEM 6700F, JEOL, Japan). Their hydrodynamic size was analyzed using a dynamic light scattering instrument (DLS, Nano ZS 90, Malvern, UK). Samples were sonicated prior to DLS determination. The structure phases of MNPs and MNPs/Cr(VI) adducts were analyzed using X-ray diffraction (XRD, Bruker D8, Germany).

Cell Lines, Reagents, and Culture Conditions

The human embryonic kidney cell line HEK293 was purchased from the Cell Resource Center of Shanghai Institute of Biological Sciences, Chinese Academy of Sciences (Shanghai, China). HEK293 cells were cultured with DMEM media, supplemented with 10% FBS (Gibco, Grand Island, NY, USA), 100 IU/mL penicillin, and 100 μg/mL streptomycin, incubated at 37 °C under 95% air and 5% CO_2. The cells were regularly monitored with an inverted light microscope (Leica, DMI3000B) and the culture medium was changed every day. The cells were normally subcultured at a ratio of 1:3 every 2 days to sustain the exponential growth phase.

Cell Viability

HEK293 cells were placed on 96-well cell culture plates with 5×10^3 cells in 200 μL medium per well. The plates were incubated at 37 °C for 12 h under a 5% CO_2 atmosphere and humid chamber until the cells were adhered to the surface of the cell culture dish. Twenty μL of each followings, Cr(VI) at 0, 10, 20, and 30 μg/mL, MNPs at 4 g/L, and synthesized MNP/Cr(VI) adduct, were added to individual wells respectively. Blank medium wells serviced as negative control in all cases. The culture plates were further incubated for 24 h.

Cell mitochondria dehydrogenase activity, which