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Effects of starch-coating of magnetite nanoparticles on cellular uptake, toxicity and gene expression profiles in adult zebrafish

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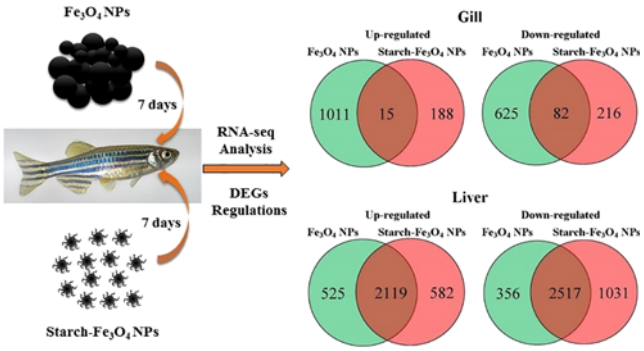
HIGHLIGHTS

- Starch coating affect biological responses to Fe_3O_4 nanoparticles (NPs).
- Responses of zebrafish to bare and coated NPs are analyzed by RNA-seq.
- Toxicity of Fe_3O_4 NPs is dependent on tissue and particle surface chemistry.
- Bare NPs cause more cytotoxicity to gill, and coated NPs trigger more harm to liver.
- Both bare and starched NPs could induce inflammation and oxidative stress.

GRAPHICAL ABSTRACT



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ABSTRACT

Engineered magnetite nanoparticles (Fe_3O_4 NPs) have been used in many fields. To prevent particle agglomeration, stabilizers or coatings are often required. While such coatings have been shown to enhance performances, the environmental impact or toxicity of stabilized or coated Fe_3O_4 NPs remain poorly understood. In an effort to understand the impacts of such coatings on the toxicity of Fe_3O_4 NPs, we used the transcriptome sequencing (RNA-seq) technique to characterize the gill and liver transcriptomes from adult zebrafish when exposed to bare and starch-stabilized Fe_3O_4 NPs for 7 days, demonstrating remarkable differences in gene expression profiles, also known as differentially expressed genes (DEGs) profiles, in both tissues. Bare Fe_3O_4 NPs exerted greater toxicity than starch-coated Fe_3O_4 NPs in gill; in contrast, starch-Fe_3O_4 NPs triggered more severe damage on liver, though both bare and stabilized NPs appeared to share similar regulatory mechanisms. Quantitative real-time polymerase chain reactions using six genes each for the two tissues verified the RNA-seq results. The surface coatings play an important role in determining the nanoparticle toxicity, which in turn modulate cell uptake and biological responses, consequently impacting the potential safety and efficacy of nanomaterials.

1. Introduction

Engineered magnetite nanoparticles (Fe_3O_4 NPs) have been widely studied or used in a number of fields including medical and environmental remediation areas due to their special physicochemical properties. For instances, a recent review by Revia and Zhang (2016) provided

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a detailed account of Fe_3O_4 NPs used for cancer diagnosis treatment, and treatment monitoring; whereas Sharifi et al. (2015) and Wu et al. (2015) reported applications of superparamagnetic iron oxide nanoparticles (SPIONs) for in vivo molecular and cellular imaging, cell tracking, and drug delivery. Another important application of magnetic nanoparticles relates to lipid-magnetic nanoparticles hybrid systems for various therapeutic objectives, such as diagnostic imaging and drug delivery (Preiss and Bothun, 2011; Salvatore et al., 2016). In the environmental cleanup field, Fe_3O_4 NPs have attracted increasing attention for adsorption of trace contaminants from water and for in situ immobilization of toxic chemicals in soil and groundwater due to their high adsorption capacity and paramagnetic properties that enable the NPs to be easily separated from the environmental media (Su, 2017). For instance, Fe_3O_4 NPs are used for pollutant removal and mitigation of organics. Hernandez et al. (2015) reported degradation of methylene blue by Fe_3O_4 NPs; Li et al. (2015) claimed the effective removal of trace perfluorooctane sulfonates from water by mesoporous Fe_3O_4@SiO_2@CTAB–SiO_2; Ruan et al. (2015) used magnetite as a heterogeneous activator in degradation of trichloroethylene by persulfate. Fe_3O_4 NPs were also found effective for removal of metalloids. An et al. (2011) prepared a new class of starch-bridged magnetite NPs and observed unusually high adsorption capacity toward arsenate. Others reported that engineered Fe_3O_4 NPs can also remove heavy metal cations (e.g. Hg(II), Cr (VI), Cu(II), Pb(II) and Co(II)) (Shan et al., 2015; Villacís-García et al., 2015; Wanna et al., 2016), radionuclides (e.g. Sr(II), Th(IV) and U(VI)) (Ding et al., 2015; Tan et al., 2015), and rare earth elements (e.g. La, Ce, Pr and Nd) (Basualto et al., 2015). In addition, Fe_3O_4 nanocomposites have also been studied as antimicrobial agents for water disinfection (Pina et al., 2014).

However, bare or non-stabilized Fe_3O_4 NPs tend to form large aggregates, impeding their delivery and performances. To prevent the particle aggregation, various stabilizers or coating agents are often employed. Numerous studies have been conducted on modifying surface properties of SPIONs for medical uses, and it has been shown that the appropriate surface coating can facilitate size control, adsorption capacity, transportability/deliverability and biocompatibility of the materials (Gupta and Gupta 2005). For instance, An et al. (2011) reported starch-bridged Fe_3O_4 NPs offered 5 times higher adsorption for As(V) than bare Fe_3O_4 NPs within 1 h in simulated ion exchange brine. Liang et al. (2012) reported that starch-stabilized Fe_3O_4 NPs offered a 2.2-time greater As(V) uptake capacity than conventional non-stabilized Fe_3O_4 NPs. Furthermore, the researchers found that the stabilized NPs are deliverable into a sandy loam soil and may facilitate in situ immobilization of As(V) in soil (Liang and Zhao, 2014). Recently, Pan et al. (2017) modified the surface of Fe_3O_4 NPs by coating the particles with stearic acid (SA), oleic acid (OA), and octadecylphosphonic acid (ODP), and achieved improved capacity for U(VI) adsorption.

As uses of Fe_3O_4 NPs continue to grow, however, the potential environmental health impacts remain poorly understood. In particular, the fate and health risks of stabilized Fe_3O_4 NPs are of greater concern due to their much smaller size and environmental mobility. The uses of stabilizers or surface coatings on Fe_3O_4 NPs can not only prevent particle aggregation, but also regulate surface physical and chemical properties (e.g., particle size, surface charge, and functionalities) and affect the particle dissolution and environmental fate and transport behavior (He et al., 2009). Therefore, the surface coating is expected to play critical roles in assessing the environmental impacts and toxicity of NPs.

Several studies have addressed the toxicity of coated Fe_3O_4 NPs. For instance, the genotoxicity of Fe_3O_4 NPs was evaluated with different surface coatings (PEG or polyethylene glycol, PEI or polyethylenimine) using three standard genotoxicity assays. The results suggested that the mutagenicity of Fe_3O_4 NPs varies with different surface coatings. PEG-coated Fe_3O_4 NPs exhibited mutagenic activity but no chromosomal and clastogenic abnormalities; while PEI-coated Fe_3O_4 NPs showed no genotoxicity in all three assays (Liu et al., 2014). Fe_3O_4 NPs coated with a dopamine-based ligand were found less toxic to RAW264.7 cells even though the particle dispersibility was increased in aqueous solutions (Wang et al., 2015). Sulfhydryl-modified Fe_3O_4@SiO_2 core/shell NPs showed low toxicity in mouse fibroblast (L-929) cell lines and no hemolytic activity, indicating good biocompatibility of this nanocomposite (Guo et al., 2015). Dimercaptosuccinic acid (DMSA)-coated Fe_3O_4 NPs were found non-toxic but accumulated in the spleen, liver and lung tissues in rats (Ruiz et al., 2015). Superparamagnetic Fe_3O_4 core-shell nanostructure microspheres showed good biocompatibility in the in vitro cytotoxicity tests (Yu et al., 2016). In another study, bare SPIONs were found nontoxic to cells via cell viability assays (Mbeh et al., 2015a); however, SPIONs coated with positively ($-NH^{3+}$) or negatively ($-COO^{-}$) charged shells alternated biocompatibility as their surface charge changed, negatively charged SPIONs are more biocompatible than both the positively charged SPIONs and the bare SPIONs (Mbeh et al., 2015b). Moreover, Berry et al. (2003) found that dextran-coated Fe_3O_4 NPs could cause cell death and reduce cell proliferation similar to that caused by uncoated iron oxide particles at 50 μg/mL, though dextran-coated Fe_3O_4 NPs showed more prominent membrane disruptions. Mahmoudi et al. (2010) reported that uncoated magnetite particles induce greater toxicity than the polyvinyl alcohol (PVA)-coated particles on a mouse fibroblast cell line. Based on the latest information, it is evident that the coatings on Fe_3O_4 NPs can greatly affect the toxic effects of the nanoparticles. Nonetheless, most of the Fe_3O_4 NPs toxicity studies are conducted within biomedical fields, and very limited toxicity information is available related to coatings used in the environmental remediation settings (e.g. starch).

In recent years, gene expression analysis has gained more interest in toxicological studies. This is due to the fact that gene regulation would be initiated when cells are subjected to toxicant exposure to protect cellular structures and repair damage (Causton et al., 2001). Studying gene expression profile can provide toxicity information of contaminants and predict their potential effects at higher biological levels (Garcia-Reyero et al., 2008). In addition, it can also provide a sensitive detectable endpoint for toxicity, and thus can serve as an early warning sign of a particular material. RNA-seq using the next-generation sequencing (NGS) has been widely used to serve this purpose. Namely, it could be used to study the effects of chemicals or pollutants on organisms with the benefit of the increased sensitivity.

In this study, we used zebrafish (*Danio rerio*) as a model organism to evaluate in vivo toxicity of bare and starch-coated Fe_3O_4 NPs in an aquatic environment. Zebrafish is known to be sensitive to various environmental pollutants, and has close homology with the human genome (Westerfield, 2000). Only limited studies are reported on the uses of Zebrafish embryos and adults for assessment of iron oxide NPs toxicity or accumulation. Zhu et al. (2012) used zebrafish embryos to evaluate the toxicity of uncoated α-Fe_2O_3 NPs at concentrations of 0.1, 0.5, 1, 5, 10, 50, 100 mg/L with the primary particle size of 30 nm at different exposure times, and they concluded that ≥10 mg/L of iron oxide NPs can induce developmental toxicity in these embryos, mortality, hatching delay, and malformation. Later, Zhang et al. (2015) used adult zebrafish for studying fish accumulation of iron oxide NPs upon continuous exposure to 4 mg/L and 10 mg/L of commercial nano-Fe_2O_3 and nano-Fe_3O_4, and they found high accumulation of both types of NPs in the fish body in the first 28 days. However, neither of these studies elucidated the related cyto- and genotoxicity mechanisms.

To our knowledge, this is the first toxicological study on the toxic effects of starch-coated Fe_3O_4 NPs on zebrafish. We selected gill and liver as the external and internal target organs to test the toxic effects via the latest RNA-seq technique. Our aim was to investigate the coating effects on cellular uptake, toxicity and gene expression profiles on zebrafish during aqueous exposure of Fe_3O_4 NPs. We therefore characterized the nanoparticle properties and measured tissue burden, evaluated the Differentially Expressed Genes (DEGs) profiles as well as gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG)