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Adsorption characteristics of nano-TiO_2 onto zebrafish embryos and its impacts on egg hatching

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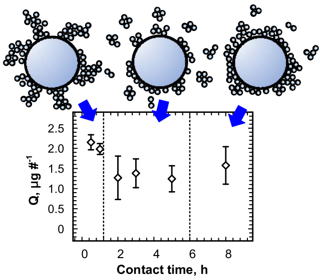
HIGHLIGHTS

- This work describes the adsorption behavior of TiO_2 nanoparticles by zebrafish embryos.
- Maximum capacity is estimated through Langmuir model.
- TiO_2 dose influences the stages of flocculation and aggregation on embryos.
- Effects of TiO_2 conformation on hatching properties are proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

The characteristics of nanoparticles (NPs) uptake may fundamentally alter physicochemical effects of engineered NPs on aquatic organisms, thereby yielding different ecotoxicology assessment results. The adsorption behavior of nano-TiO_2 (P-25) on zebrafish embryos in Holtfreter's medium (pH 7.2, I ~ 7.2 × 10^{-2} M) and the presence of sodium alginate (100 mg/L) as dispersant was investigated. Zebrafish embryos (total 100) were exposed to nano-TiO_2 at different concentrations (e.g., 0, 10, 20, 60, 120 mg/L) in batch-mode assay. The adsorption capacity of nano-TiO_2 on fish eggs was determined by measuring the Ti concentration on the egg surface using ICP-OES analysis. Results showed that the adsorption capacity increased rapidly in the first hour, and then declined to reach equilibrium in 8 h. The adsorption characteristics was visualized as a three-step process of rapid initial layer formation, followed by break-up of aggregates and finally rearrangement of floc structures; the maximum adsorption capacity was the sum of an inner rigid layers of aggregates of 0.81–0.84 μg-TiO_2/#-egg and an outer softly flocculated layers of 1.01 μg-TiO_2/#-egg. The Gibbs free energy was 543.29–551.26 and 100.75 kJ/mol, respectively, for the inner-layer and the outer-layer aggregates. Adsorption capacity at 0.5–1.0 μg-TiO_2/#-egg promoted egg hatching; but hatching was inhibited at higher adsorption capacity. Results clearly showed that the configuration of TiO_2 aggregates could impact the hatching efficiency of zebrafish embryos.

1. Introduction

Nano-manufacturing is rapidly growing with a vast number of

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high-tech applications such as electronics, medical devices, catalysts, antimicrobial fabrics, membranes, cosmetics (Aitken et al., 2006; Savolainen et al., 2010). Engineered nanoparticles (NPs) commercially utilized nowadays include metal oxides (e.g., titanium dioxide, zinc oxide, copper oxide, silica and iron oxide), and pure metals and non-metals (e.g., zero-valent iron, silver, carbon black and nanotube) (Jo et al., 2012). The unintentional release of nanoparticles into the environment has aroused public concerns because of the potential adverse impacts on ecosystems (Beddoes et al., 2015). There have numeral studies on the cytotoxic effects brought by NP-biological interactions that result in cellular membrane translocation, morphological disruption (Metzler et al., 2011; Chen and Bothun, 2013; Fan et al., 2014; Huang et al., 2014), production of highly reactive oxygen species enabling indirect membrane damages and cell death (Madl et al., 2014; Gong et al., 2012). Oberdorster et al. (1994, 2005) reported that small NPs (<100 nm) caused more adverse respiratory health effects than larger particles made from the same material in the post-exposure period. Erdem et al. (2015) studied the potential impacts of metal oxide nanoparticles (NPs) on Gram(+) *Bacillus subtilis* and Gram(−) *Escherichia coli* (K12) bacteria, in eight different nanosized titanium dioxide (TiO_2) suspensions and reported that TiO_2 NPs were found to be harmful to varying degrees under ambient conditions, with maximum antibacterial activity at primary particle in the size range of 16–20 nm.

Fish is a major group of the aquatic food chain and is most potentially exposed to NPs. Currently, there are a number of investigations on ecotoxicity addressing the transport mechanism and intake consequences of NPs at different growth stages of embryo, larva and mature fish (Zhu et al., 2010; Chen et al., 2011; Xiong et al., 2011). In the last decades, researchers have tried to standardize nano-ecotoxicological protocols for assessing the effects of NPs on the environmental health (Clemente et al., 2014). However, the mobility of NPs is affected by the dispersive conditions prevailing in natural colloids (i.e. temperature, pH, ionic strength, and organic matters) (Loosli et al., 2013), which in turn determines the acute harms of NPs to the aquatic environment. In other words, after entering an aquatic system, the fate of NPs depends upon whether they remain suspended or aggregate to ultimately remove themselves from the water column (Li et al., 2010; Petosa et al., 2010). The increase in hydrodynamic size following agglomeration, as well as changes in actual exposure concentration, underestimates the toxicity of NPs. Due to the different NPs and exposure conditions, there are discrepancies in toxicity results reported in the literature. For example, there were disagreements on the of EC_{50} values of TiO_2 toward the same organism (Nur et al., 2015). Actually, in the ecosystem natural organic matter (such as humic and fulvic acids) sometimes could coat the surface of NPs, which will render the NPs stabilized (Handy et al., 2008). There are extensive studies focused on the influence of NP exposure on the toxicity (e.g., lethality, oxidative stress, organ pathology) (Xiong et al., 2011; Chen et al., 2011), but little is known on the mechanism of the interactions between NPs and fish embryos and the effect of particle-egg interactions on embryo hatching.

Zebrafish (embryo) has recently been selected an appropriate *in-vivo* model for comparative studies of NP toxicity (Zhu et al., 2009; King-Heiden et al., 2009). The present work aimed at illustrating the attachment behavior of nano-particles, exemplified by TiO_2 (Degussa, P-25) on zebrafish embryos. It has been reported that the average concentration of engineered nanoparticles in the aquatic environment was in the range of 10 mg/L to 10 mg/L (Westerhoff et al., 2009) and 0.1–1.0 mg/L (Khosravi et al., 2012; Gottschalk et al., 2013). In order to establish the NP adsorption isotherms on fish eggs, the initial TiO_2 level used in the present study was in the range of 1.0–100 mg/L. Based on the “adsorption isotherm” established, it is then possible to predict the impacts of engineered nanoparticle, specifically, nano-TiO_2 on eco-logical systems such as fish eggs in terms of embryo hatchability.

The time-dependent profile of nano-TiO_2 particles trapped by embryos and the adsorption isotherm were obtained in Holtfreter's medium, which was a standard medium allowing the embryonic cells to survive during the exposure experimental period (Brun et al., 2014). In order to prevent the severe aggregate formation and have a better understanding of the amount of NPs trapped by eggs in medium of high ionic strength, alginate, a polysaccharide-based organic material found in natural waters, was chosen as a biologically relevant dispersant to stabilize nanoparticles (Loosli et al., 2013). It has been suggested that the NP agglomerates not penetrating the chorion can act as a “delivery vehicle” for nutrients when in contact with the embryo (Handy et al., 2008; Fent et al., 2010; Brun et al., 2014). Based on the hypothesis of NPs conformation varying with numbers of TiO_2 uptake, we elaborated also the impact of the configuration and state of nano-TiO_2 on the hatching rate and efficiency.

2. Materials and methods

2.1. Embryo production

A breeding stock of mature zebrafish (*Danio rerio*) with a mean age of about four months was used. Embryos were produced via spawning groups. Photoperiod was set to a 14:10 h of light-dark cycle (Faria et al., 2014) for at least two weeks and the temperature was held at 26 ± 1 °C. A total of 21 fish (male-to-female ratio = 2:1) were placed in spawning tanks for fertilization prior to the exposure experiments. Zebrafish embryos were obtained by natural mating. A spawn trap was placed into the tank for the collection of embryos spawned. To prevent the predation of eggs by adult zebrafish, the spawn traps were covered with inert wire mesh of appropriate mesh size (*ca.* 2 ± 0.5 mm). The selection criteria for embryos followed OECD guidelines on fish embryo acute toxicity test (OECD, 2013); basically, the overall fertilization rate of all eggs in batch tests should be $\geq$70% and survival rate of embryos in the dilution-water control should be $\geq$90% at the end of 96-h exposure period.

2.2. Chemicals

Adsorption experiments were conducted in the Holtfreter's medium (George et al., 2011) (pH 7.2, $I \sim 7.2 \times 10^{-2}$ M), comprised of 3.5 g of NaCl (UniRegion Bio-Tech, USA), 0.2 g of $NaHCO_3$ (Merck, Germany), 0.05 g of KCl (Merck, Germany), and 0.12 g of $CaCl_2 \cdot 2H_2O$ (NACALAL TESQUE, Inc., Japan) in 1 L of aqueous solution. Deionized water, purified with a laboratory-grade RO-ultrapure water system, (resistivity > 18.3 MΩ-cm), was used for the preparation of all solutions. Alginic acid (sodium salt, $(C_6H_8O_6)_n$, Acros, USA) was used as dispersant to stabilize the TiO_2 suspension. Alginate is extracted from the cell walls of brown seaweed that comprises 1,4-linked b-D-mannuronic acid and a-L-guluronic acid residues (Loosli et al., 2013). At low monovalent electrolyte concentration, alginate was used to prevent aggregation of nanoparticles upon pH variations. The z-average diameter of alginate was found constant at 178 ± 21 nm from pH 3 to 11.

2.3. Batch adsorption experiments

Fertilized embryos were mildly rinsed with the medium several times to remove any dirt and feces. At ambient temperature, the first cleavage of fish eggs took place after 15 min, and thus the batch exposure experiments should be carried out in 3 h after fertilization