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Developmental toxicity of glycine-coated silica nanoparticles in embryonic zebrafish☆

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ABSTRACT

Nanoparticle (NP) surface coatings are known to influence the toxicity of engineered nanomaterials. This work examines the effect of glycine functionalization on silica NPs and investigates changes in viability and developmental defects in the organs of zebrafish embryos upon exposure. Silica NPs and glycine-functionalized silica NPs are synthesized and characterized. Exposure of zebrafish embryos to glycine-silica NPs affects the mortality percentage in a similar manner to soluble glycine. Developmental defects are observed in embryos exposed to soluble glycine, glycine-silica NPs, or silica NPs in comparison with the unexposed embryos. The damage is localized in the brain, heart, and liver of zebrafish embryos. These observations suggest a complex mechanism of toxicity, with glycine maintaining its toxic activity even when covalently bound on silica surface. Our results illustrate that surface modification of non-lethal particles can create different toxicity outcomes in the organs of exposed zebrafish embryos.

1. Introduction

Rapid growth in nanotechnology has led to increased manufacture and use of engineered nanomaterials (ENMs) in a large variety of applications and consumer products (Kessler, 2011; Roco, 2011). Reports dealing with environmental health and safety (EHS) assessment of ENMs have recommended caution in dealing with the rapid growth and implementation of nanotechnology products (Service, 2004) due to their potential to induce harmful effects which can vary with the composition, size, dose, and exposure environment (Mudunkotuwa and Grassian, 2015). Silica nanoparticles (SNPs) are one of the most extensively used ENMs, also listed as one of the top five nanomaterials in nanotech-based consumer products (Duan et al., 2013a). Approximatively 100 consumer products already available on the market are reported to contain SNPs (Vance et al., 2015). It is estimated that the annual production of SNPs worldwide could reach up to 5500 tons/year, making it one of the most produced nanomaterials (Piccinno et al., 2012). SNPs are produced by a variety of synthetic pathways and many are coated with surface ligands to enhance stability of the dispersion and modulate their physical and chemical characteristics (Tang et al., 2012; Chen et al., 2013a; Chen et al., 2013b). Understanding the toxic effects and biocompatibility of SNPs is essential for their rational design and use. SNPs may have increased residence times in the aquatic medium which affects their transport and persistence in the environment and impact live organisms (Garner and Keller, 2014). Assessing the environmental impact of SNPs is important, considering the increased incidence of release or disposal of SNPs directly into landfill, soil, water, and air (Keller et al., 2013).

An important aspect in understanding EHS risks associated with SNPs exposure is to determine the effect of surface modification when the NPs are placed in biological systems. Modification of NPs by organic coatings is used to produce uniform NPs and control their physicochemical properties to make them amenable to incorporation into applications (drug delivery systems, food packaging, water purification membranes, self-cleaning and antifouling materials) (Lin and Haynes, 2010; Service, 2010; Kango et al., 2013). Functionalization of SNPs is achieved by the covalent bond formation between the surface silanol groups and organic

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functionalities (Rahman and Padavettan, 2012; Chen et al., 2013a; Lu, 2013; Liberman et al., 2014) or through physical adsorption (Meng et al., 2004; Rimola et al., 2006). The presence of an organic coating on the NPs may change the toxicity outcome due to the changes in surface charge, reactivity, and transport characteristics (Donaldson and Poland, 2013; Mudunkotuwa and Grassian, 2015). Surface modification can also enhance adsorption and transport of toxins (Donaldson and Poland, 2013) or they can induce toxic effect by themselves, indirectly imparting toxicity to the NPs. While several works have reported toxicity studies of bulk SNPs, the effect of organic linkers on the toxicity of the NPs is mostly unknown. Characterization of surface coatings is important because their effect is critical in determining the impact of NPs in the environmental and biological systems.

Glycine is used to functionalize the surface of NPs for environmental remediation or therapeutic applications (Barick and Hassan, 2012; Ballav et al., 2014; Feitoza et al., 2014). Glycine/silica NPs are used extensively at large scale in industrial applications in the semiconducting industry (Speed et al., 2015). Therefore, toxicity studies of such systems are important for their environmental friendly use with minimum adverse consequences. Notably, the study of the glycine-SNPs system is relevant due to its broad application in slurry formulations in chemical mechanical planarization (CMP), a wafer polishing technique for chemical-mechanical removal of excess deposited materials during manufacturing of integrated circuit chips in the semiconductor industry (Zantye et al., 2004; Matijević and Babu, 2008). The CMP process uses a large quantity of SNPs and chemical additives that include glycine, among others. The study of SNP and glycine toxicity has environmental significance due to the large amount of waste generated by these processes, estimated to approximately 30–50 L of waste slurry per each 200 mm wafer (Belongia et al., 1999; Lin and Yang, 2004; de Luna et al., 2009).

While glycine is a simple amino acid, excess concentrations can be toxic to vertebrates at later stages of development. Extracellular increases in glycine have been observed with loss of either the glycine transporter (GlyT1) (Gomeza et al., 2003) or the metabolic disorder nonketotic hyperglycinemia (NKH), which is due to a loss of glycine decarboxylase (GLCD) (Saudubray et al., 2012). Loss of the GlyT1 transporter leads to failure of clearing extracellular glycine in both mouse (Gomeza et al., 2003; Applegarth and Toone, 2006) and zebrafish (Cui et al., 2005), leading to motor defects, respiratory defects, and potentially death. Loss of GLDC in NKH prevents catabolism of glycine and results in severe postnatal neurological impairment (Saudubray et al., 2012). Increased glycine with loss of GlyT1 or GLDC is thought to interact with inhibitory glycine receptors and excitatory N-methyl-D-aspartate receptors in the brain (Gomeza et al., 2003; Applegarth and Toone, 2006). Extracellular glycine can be also converted to methylglyoxal, a toxic reactive aldehyde involved in the pathology of diabetes (Kim et al., 2015).

In this work, glycine-functionalized SNPs (glycine-SNPs) and their uncoated counterparts were synthesized and their physicochemical and surface properties were assessed. We used the zebrafish (*Danio rerio*) vertebrate model system to identify potentially toxic effects originating from glycine-SNPs and soluble glycine, in comparison with the bare NPs. To our knowledge, the impact of glycine-functionalized NPs on early stages of vertebrate development has not been reported to date.

2. Materials and methods

2.1. Synthesis of glycine functionalized SNPs

SNPs were synthesized using a sol–gel method based on the Stöber process (Lu, 2013). A mixture of ethanol (100 ml, Pharmco-AAPER), ammonium hydroxide (10 ml, J. T. Baker) and tetraethylorthosilicate (TEOS, 8.32 g, Fluka) was stirred at 60 °C for 3 h. A mixture of glycine (0.83 g - 10 wt% of the TEOS weight, Fluka) and 28% ammonium hydroxide (0.1 ml) dissolved in deionized water (DIW, 3.5 ml, Millipore, Direct-Q System) was added dropwise to the above suspension under vigorous stirring for 30 min. The resultant reaction mixture was then refluxed at 75 °C while stirring for 3 h. The product was separated, washed with ethanol six times and dried overnight at 60 °C. The modified particles were washed and centrifuged until the supernatant did not show any presence of free glycine, quantified using the ninhydrin reagent. Similarly, unmodified SNPs were synthesized and separated without glycine treatment.

2.2. Physicochemical characterization of NPs

High resolution transmission electron microscopy (HRTEM) was performed by A JEOL JSM-2010 instrument on the dispersed SNP aliquots directly placed on a copper HRTEM grid followed by drying under vacuum. Particle size distribution and zeta potential (ζ-potential) were measured at 25 °C with a Malvern zetasizer (nano ZS) particle and zeta potential analyzer. Samples were prepared in distilled water for analysis. Powder X-ray diffraction (PXRD) patterns were recorded on a Bruker D2 Phaser diffractometer equipped with a copper sealed tube ($\lambda = 1.54178$ Å) between 10° and 90°. Powder samples were dispersed on low-background discs for analysis. Fourier Transform Infrared spectroscopy (FTIR) spectra were obtained using a Thermo Nicolet iS10 FTIR Spectrometer. The samples were prepared in KBr powder (Sigma Aldrich) and pressed into a pellet form. Thermogravimetric analysis (TGA) was carried out using a Seiko Exstar TG/DTA 6200 analyzer coupled with a Pfeiffer Vaccum GSD 301 T2 mass spectrometer, working under helium atmosphere with a heating rate of 10 °C/min, from 20 °C to 800 °C. UV-VIS spectrophotometric measurements were performed with a HP Agilent 8453 UV-VIS spectrophotometer equipped with a 1 cm path length cell. SNPs, glycine-SNP and a series of pure glycine standards were tested for their glycine content using the ninhydrin test. 1 ml of ninhydrin reagent (0.2 wt%, Sigma Aldrich) and 1 ml of acetate buffer solution (sodium acetate and acetic acid, 0.1 M, pH = 5.6, J. T. Baker) were added to each sample (4 ml prepared in DIW). The tubes were covered with aluminum foil and vigorously mixed. The tubes were placed in boiling water bath for 15 min and then cooled down to room temperature in a cold water bath. The samples were then diluted by adding 1 ml of 50 v% ethanol/distilled water mixture and centrifuged. The absorbance of the supernatant was measured at 570 nm against the blank reference.

2.3. Fish stock and viability assay procedure

Fish maintenance and matings were performed as previously described (Westerfield, 1993). AB wild type fish were used for most procedures. Zebrafish eggs were collected and sorted out in E3 medium. E3 medium (pH 6.9–7.2) contains 5 mM sodium chloride (Fisher Scientific), 0.17 mM potassium chloride (LabChem, Inc.), 0.33 mM magnesium sulfate (Fisher Scientific) and 0.33 mM calcium chloride (Acros Organics) in DIW, without or with addition of 1-phenyl-2-thiourea (PTU, Sigma Aldrich) to inhibit the formation of pigment in embryos. At 24 h post-fertilization (hpf), the embryos were manually dechorionated and viable embryos were separated based on their developmental stage examined under an optical microscope (Nikon SMZ1000 Stereomicroscope). The exposure solutions were freshly prepared by dispersing appropriate amounts of glycine-SNPs or raw SNPs (up to 3 mg/ml), or dissolving glycine (up to 1 mg/ml) in E3 medium, without or with addition of PTU. 3 ml of testing solution was added to each of the three 20-embryo