Chemosphere 159 (2016) 628–637

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Impact of fluorescent silicon nanoparticles on circulating hemolymph and hematopoiesis in an invertebrate model organism

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HIGHLIGHTS

- SiNPs selectively entered circulating hemocytes and induced apoptosis.
- The hematopoiesis of hematopoietic organs reduced by SiNPs.
- Hematopoietic organs secreted hemocytes return to normal after Self-healing.

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
3 June 2016
Accepted 13 June 2016
Available online 25 June 2016

Handling Editor: Tamara S. Galloway

Keywords:
Silicon nanoparticles
Hematocyte invasion
Hematopoiesis
Damage

ABSTRACT

Silicon nanoparticles (SiNPs) have attractive potential applications in biological and medical fields, and yet their impact on animals is still controversial, and there have been no reports of their effects on hematopoiesis. In this study, the effects of SiNPs on hemocytes and hematopoiesis were investigated by administering SiNPs *via* a vascular injection into an invertebrate model, the silkworm. Our results show that the ability of SiNPs to enter different types of circulating hemocytes and their impact on those hemocytes differed significantly. Rapid accumulation of SiNPs was observed in granulocytes, oenocytoids, and spherulocytes, which have immune functions in the circulating hemolymph, whereas SiNPs did not easily enter prohemocytes, which can differentiate into granulocytes, oenocytoids, and spherulocytes and replenish them. The SiNPs that entered the hemocytes initiated autophagy and apoptosis *via* the lysosomal/mitochondrial pathway. High-dose SiNPs weakly stimulated lysosomal activity in hematopoietic organs, but did not lead to a significant increase in reactive oxygen species or severe autophagy or apoptosis in the organ tissues. We suggest that the damage caused by high-dose SiNPs to hematopoiesis is self-healing, because few SiNPs entered the hematopoietic stem cells in the circulating hemolymph, so the damage to the hematopoietic tissues was limited.

1. Introduction

Research into silicon nanoparticles/nanospheres (SiNPs) has attracted extensive attention in the fields of biosensors and biological imaging because of their excellent biocompatibility, their superior electronic, optical, and mechanical properties, and their abundant sources (Ji et al., 2015; Peng et al., 2014; Park et al., 2009; Howes et al., 2014; Zhong et al., 2013; McVey and Tilley, 2014; Ding et al., 2002; Pavesi et al., 2000). SiNPs have great potential application in biomedical fields, such as targeted drug delivery and tumor therapy, because they can be loaded with a variety of

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http://dx.doi.org/10.1016/j.chemosphere.2016.06.057
0045-6535/

molecules, including chemical drugs, proteins, and peptides, their synthesis can be controlled, and their loading volumes are large (Erogbogbo et al., 2011; Wei et al., 2013; Su et al., 2012; He et al., 2009, 2010; Zhong et al., 2012; Cheng et al., 2014). *In vitro* experiments have shown that SiNPs do not display the cytotoxicity typical of other quantum materials, such as CdTe quantum dots (QDs), which seriously damage cellular proteins, lipids, and DNA (Lovrić et al., 2005), resulting in cell death (Derfus et al., 2004; King-Heiden et al., 2009). Luminescent porous silicon nanoparticles (LPSiNPs) showed no obvious toxicity to cultured human HeLa cells *in vitro* (Park et al., 2009). *In vivo* experiments have shown that after the intravenous injection of high-dose (20 mg/kg) LPSiNPs in *Mus musculus*, the SiNPs were absorbed and degraded nonspecifically by organs rich in the reticuloendothelial system, such as the liver and spleen, and were excreted via the kidneys in the silicate form, with no apparent biotoxicity (Park et al., 2009).

However, in contrast, other studies have shown that silica nanoparticles are cytotoxic (Yu et al., 2011; Lison et al., 2008), causing the hemolysis of mammalian erythrocytes (Liu et al., 2013; Pavan et al., 2014). In an *in vitro* experiment, silica nanoparticles induced the generation of reactive oxygen species (ROS) in HepG2 cells, which triggered autophagy and cell death (Yu et al., 2014). Silica nanoparticles inhibited angiogenesis in mice by inducing endothelial cell (Duan et al., 2014). Treatment with silica nanoparticles for three months caused liver damage in mice (Liu et al., 2011). Early studies found that the innate immune response was activated through when the NALP3 inflammasome sensingsensed of silica in mice (Dostert et al., 2008). Recently, Elsabahy and Wooley (2015) summarized the reports from several laboratories and found that although nanoparticles displayed no obvious toxicity *in vitro* or *in vivo*, they might still trigger components of the immune system, such as interleukins, eotaxin, granulocyte-colony-stimulating factor, granulocyte–macrophage-colony-stimulating factor, monocyte chemotactic protein, macrophage inflammatory protein, and tumor necrosis factor, causing serious adverse reactions, suggesting that nanoparticles are potentially toxic to the blood and immune systems. Therefore, research into the biosafety of SiNPs is currently insufficient and discrepancies and disputes exist. Therefore, further in-depth studies are required with more representative tissues and system models, especially the immune system.

The issue addressed in this paper is whether SiNPs exert adverse effects on hemocytes, which perform the main innate immune functions in animals, or affect hematopoiesis in the hematopoietic tissues. The hemocytes of invertebrates are widely regarded as an excellent model in which to study the congenital immune response and DNA damage (Liu et al., 2014; Carmona et al., 2011; Irving et al., 2005; Cherry and Silverman, 2006). Therefore, we chose the silkworm, an invertebrate model, to investigate the hemocyte defensive response and the impact of nanoparticles on hematopoiesis (Liu et al., 2014; Tan et al., 2013). Because the visceral tissues of silkworms are almost infiltrated in the hemolymph (Grigorian and Hartenstein, 2013), differences in the results of experiments conducted *in vitro* and *in vivo* can be compared. The QDs infused into the hemolymph and were directly in contact with hemocytes and diffused throughout the entire larvae immediately (Liu et al., 2014), which was much quicker than in mammals (Liu et al., 2013). The indirect impact of QDs on hemocytes and hematopoiesis by acting on other tissue cells is significantly weakened (Liu et al., 2014), thus avoiding the limitations of mammalian tissue barriers and eliminating the errors caused by the inhibition of angiogenesis by drugs (Tsoi et al., 2013).

Silkworms can also stably replicate the processes of hemocyte formation in and release from hematopoietic organs *in vitro*, and so have the advantage over mammalian model systems of allowing research into hematopoietic toxicology to be conducted both *in vivo* and *in vitro* (Liu et al., 2014; Nakahara et al., 2003).

2. Experimental section

2.1. Synthesis of silicon nanoparticles

Silicon nanoparticles (SiNPs) were synthesized according to the method of Zhong et al. (2013). Trimethoxysilane (100 ml) was added to 400 ml of a saturated solution of nitrogen containing 18.6 g of trisodium citrate dihydrate, and stirred for 10 min. The resulting solution contained the SiNP precursors. The SiNPs were further incubated at 160 °C for 15 min. Excessive trimethoxysilyl and trisodium citrate dihydrate were removed by dialysis with a molecular sieve with a molecular weight cut-off of 1 kDa. As shown in Fig. S1, the particle diameters of the prepared SiNPs were 1–3 nm. They emitted blue fluorescence at an excitation wavelength of 405 nm and an emission wavelength of 450–510 nm, and the maximum emission wavelength was ~460 nm. Thus, the characteristic spectrum was consistent with that of SiNPs reported previously (Zhong et al., 2013).

2.2. Preparation of the test organism

Bombyx mori of the Daozao strain was reared on fresh mulberry leaves at 25 °C with a photoperiod of 12 h light:12 h dark. SiNPs were administered by vascular injection to 48-h-old fifth-instar larvae of similar sizes (1.675 ± 0.210 g). The total volume of solution injected was 10 μl. The control organisms were injected with the same volume of pure water. The almost maximum water-soluble concentration of SiNPs (390 μg/ml) and dilutions of 39 μg/ml and 3.9 μg/ml were injected. Therefore, doses of 3.9 μg (390 μg ml^{-1} × 10 μl), 0.39 μg (39 μg/ml × 10 μl), and 0.039 μg (3.9 μg/ml × 10 μl) SiNPs per individual were used to assess their toxic effects on hematopoiesis. All of the animal experimental procedures in this study were performed in accordance with Soochow University Guidelines for the Welfare of Animals.

2.3. Assessment of hemocytes and hematopoiesis

Using the method of Liu et al. (2014), hemolymph (100 μl) was collected from the pereiopods and placed in a centrifuge tube at 1.5, 3, 6, 12, 24, 48 and 72 h after SiNP injection. Three larvae were sampled at each time point. The centrifuge tube was then shaken rapidly and the hemocyte count estimated with a blood counting chamber under a microscope (Olympus BX51, Japan). The characteristic blue fluorescence of the SiNPs was detected at an excitation wavelength of 405 nm and an emission wavelength of 460 nm. The entry of the SiNPs into the cells was observed in optical sections under a laser scanning confocal microscope (Leica TCS SP5, Heidelberg, Germany).

The hemopoietic organs were carefully removed using surgical forceps and washed rapidly with precooled physiological saline and culture medium. A hanging drop culture was performed for each hemopoietic organ with the method of Nakahara et al. (2003) and Liu et al. (2014). Grace's insect tissue culture medium supplemented with 10% *Bombyx mori* hemolymph was used with appropriate amounts of antibiotics. The volume of the culture medium was 10 μl and the culture temperature was 26 °C.

2.4. ROS staining

The levels of ROS were measured with Reactive Oxygen Species Assay Kita ROS kit (S0033-1, Beyotime, Jiangsu, China). The hemopoietic organs were collected in diethylpyrocarbonate