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Toxicity of silver nanoparticles on fertilization success and early development of the marine polychaete *Hydroides elegans* (Haswell, 1883)

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Abstract

Background: The silver nanoparticles (AgNPs) are used in the production of numerous commercial and medical products and are found to have adverse effects on animals. The sessile marine polychaete worm, *Hydroides elegans*, was examined for the influence of AgNPs on external fertilization and early developmental stages.

Results: Toxicity of AgNPs at various concentrations were examined on germ cells, fertilization rate, and early developmental stages. The EC_{50} was evaluated as 3 nM AgNPs; however, the survivability varied at each developmental stage, reducing with increasing stages of development, indicating a bioaccumulation effect of AgNPs. The lag in time of development suggested an impediment in dividing cells and the nuclear dysfunction assessed by DNA damage using comet assay.

Conclusion: The observations documented have provided an insight on hazardous effect of AgNPs on susceptible cells and reproductive efficiency on other organisms including human.

Keywords: Silver nanoparticles, *Hydroides elegans*, Development assay, Sperm toxicity, Fertilization

Background

The silver nanoparticles (AgNPs) possess bactericidal activity (Bryaskova et al., 2010; Lara Villegas, Vanesa Ayala-Nu, Carmen Ixtepan Turrent, & Padilla, 2010) with cytotoxic and genotoxic effect on microbes and cells (Arora, Jain, Rajwade, & Paknikar, 2008; Fenech, 2006). The commercial exploitation of AgNPs in the production of pharmaceutical and cosmetic products lead to its effluent waste disposed in aquatic system. The AgNPs are a cause of concern on risk of toxicity at different levels of the food chain and developmental processes of the aquatic organisms (Chen & Schluesener, 2008; Ahamed, Alsalhi, & Siddiqui, 2010; Benn & Westerhoff, 2008). The reported toxicity of AgNPs include developmental deformities in zebrafish, *Danio rerio*, (Bar-Ilan, Albrecht, Fako, & Furgeson, 2009) and reduced growth rate and reproductive capacity in soil nematode *Caenorhabditis elegans* (Roh et al., 2009). The AgNPs as contaminants effect a decline in proliferation of mouse spermatogonial stem cell (C18-4) (Braydich-Stolle et al., 2010) and cause mitochondrial dysfunction and induction of ROS which in turn set off DNA damage and chromosomal aberrations resulting in cell cycle arrest (Asharani, Lian Wu, Gong, & Valiyaveettil, 2008). The accumulation of silver and its toxicity vary according to the stability, agglomeration, aggregation, dispersion, sedimentation, dissolution, and mobility of AgNPs in the aquatic environment (Handy et al., 2008; Navarro et al., 2008; Fabrega, Luoma, Tyler, Galloway, & Lead, 2011). These processes are dependent on physicochemical properties of the particle that are in turn influenced by environmental parameters such as pH, temperature, ionic strength, and presence of ligands or natural organic matter. The

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oxidation of AgNp in the cell or the medium to Ag^+ probably enhances toxicity (Dibrov, Dzioba, Gosink, & Hase, 2002). There has been a long debate on whether AgNP-associated toxicity is mediated by the release of Ag+ or by the particle itself. On the one hand, some studies support the theory that the toxic effect of AgNPs, whether coated or not, is derived from the release of Ag+ (Fabrega et al., 2011; Yang et al., 2012; Buffet et al., 2013), which causes cell damage via an increase in ROS production and an induction of apoptotic pathways (Hwang et al., 2008). On the other hand, some have concluded that the AgNP effect is related to the particle itself (Siller et al., 2013; Choi & Hu, 2008; Griffitt et al., 2012). Nevertheless, it is established that the toxicity of AgNPs is influenced by how well the particles are dispersed within the medium (Kim, Truong, Wehmas, & Tanguay, 2013).

In the marine aquatic system, the contaminants of sea water have a potent effect on survivability and reproductive efficiency of the animals (Rand, Wells, & Mcarty, 1995, His, Beiras, & Seaman, 1999). The sessile organisms are dependent on food, and dispersal of reproductive cells and development of egg on the surrounding medium and the appraisal on the toxic effect of contaminants are required to reduce the alterations in aquatic system. The sessile marine polychaete worm, *Hydroides elegans*, are found attached to hard substrates (Udhayakumar & Karande, 1996), and the present study attempted to demonstrate the effect of AgNPs as a contaminant on its reproductive efficiency. The AgNPs were found to impact survivability of germ cells, fertilization, and development of fertilized egg of *H. elegans*. The cytotoxicity of AgNPs in germ cells and developing eggs was directly related to the increasing concentration of AgNPs in the medium suggesting bioaccumulation of the contaminant.

Methods

Chemical

The silver nanoparticles (AgNPs) 10 nM, synthesized by the chemical reduction method (Turkevich, Stevenson, & Hiller 1951), were obtained from the National Center for Nanoscience and Nanotechnology, University of Madras, Maraimalai Campus, Chennai, India.

Characterization of silver nanoparticles

The reduction method was used to monitor the Ag+ relics from nanoparticles and Ag+ ions by diluting in distilled water, and a sharp peak was observed by UV-visible spectrometry at 410 nm. The morphology and size of nanoparticles were measured by field emission scanning electron microscope (FESEM-HITACHI SU6600). A minute drop of nanoparticle solution was cast on aluminum foil and subsequently dried in air before transferring to the microscope.

Collection of test animals

The marine polychaete worm *H. elegans* was collected from the bottom of the ships or boats anchored in the harbor at Royapuram, Chennai, India, and in the laboratory which was kept in filtered sea water at room temperature.

Germ cells

The polychaete worms were carefully teased out from the tube with the help of forceps, and the sex of the worm was identified by observing the lateral sides of the abdomen; the females were orange whereas the males were dull white in color. The males spawned within 1 min of its removal from the calcareous tube, and the females shed the eggs continually in the surrounding sea water (Arumugam, 2012).

Developmental assay

The aim of this study is to examine the toxicity in the developmental stages of *H. elegans* using the method of developmental assay according to Gopalakrishnan (2002) and Gopalakrishnan, Thilagam, and Vivek Raja (2008). The stock solution of 10 mM AgNPs was used for preparation of medium with AgNP concentration of 1, 3, 5, 7, and 9 nM. The EC_{50} value of the germ cells, fertilized egg, and developmental stages up to blastula was determined in different concentrations of AgNPs with a control of filtered sea water.

Spawned sperms (200 μl) were added to 2 ml of the experimental medium each containing AgNPs at different concentrations (1, 2, 3, 4, and 5 nM) and kept for 30 min; 500 μl of this treated sperms from each concentration was pipetted into 2 ml of filtered seawater containing untreated eggs (50 eggs), mixed, and observed at intervals of 10 min under microscope. Similarly, sea water containing 50 eggs (50 to 100 μl) in media containing AgNPs at various concentrations (1, 2, 3, 4, and 5 nM) were kept for 30 min. Sperm suspension (2 ml) was added to 500 μl of AgNP-treated eggs in sea water, mixed well, incubated, and observed for fertilization and developmental stages until the control attained 2–3-cell stages. The cumulative time and percentage of survival in egg and developmental stages were recorded and calculated as follows.

Cumulative time: The time required to develop into a stage after external fertilization.

$$\text{Percentage of successful development of a particular stage} = \frac{\text{Number of eggs reached the particular stage successfully without any deformities}}{\text{Total number of eggs observed [Successful, unsuccessful and deformed]}} \times 100$$

Single cell gel electrophoresis (comet assay)

The sperm cells exposed to AgNPs at concentrations of 3 and 5 nM, and the control of non-exposed sperm was

washed with phosphate-buffered saline (PBS) and investigated for DNA damage using single cell gel electrophoresis by comet assay (Cabrita, Robles, Rebordinos, Sarasquete, & Herraez, 2005).

Statistical analysis

The assays were performed in triplicates and the mean with the standard deviation was determined which was subjected to variation analysis by using statistical package for social sciences (SPSS, PASW version 18 for Windows, SPSS Inc., Chicago, USA) to find out the variation between the control and experimental groups. The statistical analysis clearly indicated that the percentage of development at different cell stages varied significantly ($P < 0.05$).

Results

UV-visible spectroscopy and FESEM analysis

The silver nanoparticles prepared by reduction method appeared pale yellow in color in UV-visible spectroscopy showed the absorption peak of maximum 400–450 nm (Fig. 1). The AgNPs obtained with the sodium citrate reduction method were predominantly spherical with diameters ranging from a few nanometers to 27.7–39.5 nm (Fig. 2).

Fertilization and early developmental stages of *H. elegans*

The spherical shaped unfertilized eggs (42 μm) released from the female tubules were allowed to mix with the sperms collected from the male tubules, and the elevation of fertilization membrane marked fertilization, which was observed within 5.33 ± 0.33 min. The first cleavage plane observed was meridional and occurred within 30.53 ± 0.20 min after fertilization, whereas the second cleavage plane was also meridional but at right angles to the first cleavage and occurred within 40.68 ± 0.45 min. The third cleavage was horizontal, dividing the four blastomeres into eight blastomeres, arranged in two tiers taking about 50.94 ± 0.42 min. The cleavage that followed converted the 8-cell stage to 64-cell stage in 110.33 ± 1.52 min and became difficult to trace till the blastula stage in about 125.66 ± 1.24 min followed by blastula rotation in 1263.66 ± 1.52 min. Finally, the larvae were released after 1328.33 ± 7.63 min from the time of fertilization (Fig. 3).

Assessment of median effective concentration of silver nanoparticles on fertilization and early developmental stages of *H. elegans*

The toxicity of silver nanoparticles on sex cells, fertilization, and early developmental stages (2-, 4-, and 8-cell stages, blastula, and larvae) of *H. elegans* were tested at concentrations of 1, 2, 3, 4, and 5 nM. The EC_{50} value was evaluated as 3 nM for germ cell 2-, 4-, and 8-cell developmental stages and 2 nM for blastula stage up to larval release.

Toxicity effect of silver nanoparticle on sperm and egg of *H. elegans*

The fertilization rate in control (88%) gradually decreased with a difference of 15, 25, 37, 49, and 63% in 1, 2, 3, 4, and 5 nM concentrations of AgNPs, respectively, after 30 min of exposure with the variations were significant in ANOVA ($P < 0.05$). The increase in the difference of fertility rate was 2.5-fold between 1 nM concentration and the EC_{50} value ($P < 0.05$) (Fig. 4). The fertilization rate significantly decreased with the increased concentration of AgNPs as 69, 58, 47, 36, and 22% at 1, 2, 3, 4, and 5 nM, respectively, when compared to control (86%).The EC_{50} value 47% was observed in 3 nM concentration.

Effect of silver nanoparticles on early developmental stages of *H. elegans*

Fertilization membrane

The fertilization was effected by 98.60 ± 0.2% in 4.56 ± 0.35 min in sea water devoid of silver nanoparticles taken as control. At 1–5 nM concentrations of AgNPs, the cumulative time increased from 5.31 ± 0.07

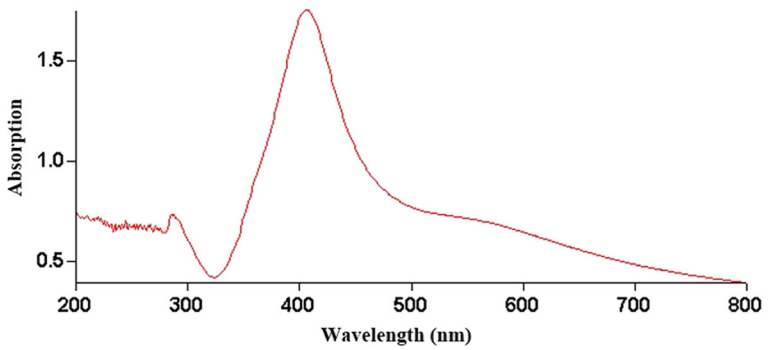

Fig. 1 UV-visible spectrum of synthesized silver nanoparticles

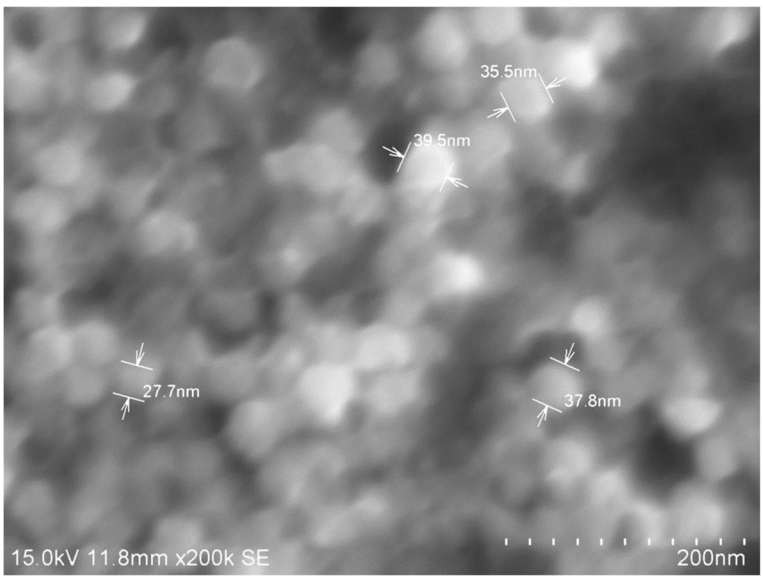

Fig. 2 Field emission scanning electron microscope (FESEM) images of silver nanoparticles showing the particles of spherical shape

to 7.55 ± 0.48 and the variation was significant in ANOVA ($P < 0.05$). The rate of effective fertilization decreased from (96%) at 1, 2, and 3 nM concentrations; however, the variation of 20 and 24.1% at 4 and 5 nM concentrations were statistically significant ($P < 0.05$). This clearly indicated that the rate and success of fertilization were altered at concentrations of AgNPs above the EC_{50} value (Figs. 5a and 6 and Table 1).

A. **2-cell stage**
   At the 2-cell stage, the difference in the cumulative time between the control (94.53 ± 0.20%) and sea water containing AgNPs at increasing concentrations (1 to 5 nM) showed an increase of 2.8, 5.84, 9.80, 39.4, and 49.22%, and at EC_{50}, the increase was statistically significant ($P < 0.05$). However, effective success in development decreased with increase of concentration of AgNPs from about 89.46 ± 0.25% at 1 nM concentration to 17.60 ± 0.30% at 5 nM concentration, and the decrease was statistically significant ($P < 0.05$) (Figs. 5b and 6 and Table 1).
B. **4-cell stage**
   Observation of the cumulative time taken to reach the 4-cell stage between the control (89.30 ± 0.20%) and increasing concentration from 1 to 5 nM was 2.0 to 7.35 min which was statistically significant ($P < 0.05$) and the percentage of effective development declined with increasing concentration of AgNPs from 78.53 ± 0.30% at 1 nM concentration to 50.53 ± 0.30% at 4 nM concentration with eventual arrest of development at 5 nM concentration and the variation at EC_{50} was statistically significant ($P < 0.05$) (Figs. 5c and 6 and Table 1).
C. **8-cell stage**
   The development of embryo to 8-cell stage was also observed to show an increase in cumulative time from 59.53 to 62.26 min at 4 nM concentration with the difference of 0.86 to 5.98% when compared to control ($P < 0.05$). The percentage of successful development decreased to 40.23 ± 0.25% at 4 nM concentration, and at EC_{50}, the variation was significant ($P < 0.05$) (Figs. 5d and 6 and Table 1).
D. **16-cell stage**
   The development of 16-cell stage in control was assessed as 59.51 ± 0.51 min, and the cumulative time taken for the 16-cell stage at 1, 2, 3, and 4 nM concentrations of AgNPs increased with the increasing concentrations 60.10 ± 0.05, 60.17 ± 0.07, 73.13 ± 0.58, and 80.53 ± 0.54 min, respectively. The concentration of 5 nM arrested 16-cell developmental stage. However, the percentage of successful development in control 77.43 ± 0.23% decreased to 68.43 ± 0.45%, 66.50 ± 0.26%, 49.13 ± 0.15%, and 34.53 ± 0.20% in 1, 2, 3, and 4 nM concentrations of AgNPs, respectively (Figs. 5e and 6 and Table 1). The variation at EC_{50} concentration for cumulative time and effective development was significant ($P < 0.05$).
E. **64-cell stage**
   The cumulative time of 64-cell stage steadily increased from 90.46 ± 0.90, 96.46 ± 1.05, 100.54 ± 1.63, and 115.53 ± 0.54 min at 1, 2, 3, and 4 nM concentrations, respectively, when compared to control of 83.31 ± 0.75 min. The percentage of successful development in control 66.60 ± 0.20% decreased to 61.33 ± 0.30%, and 22.66 ± 0.20%, at 1 and 4 nM concentrations of AgNPs, respectively.

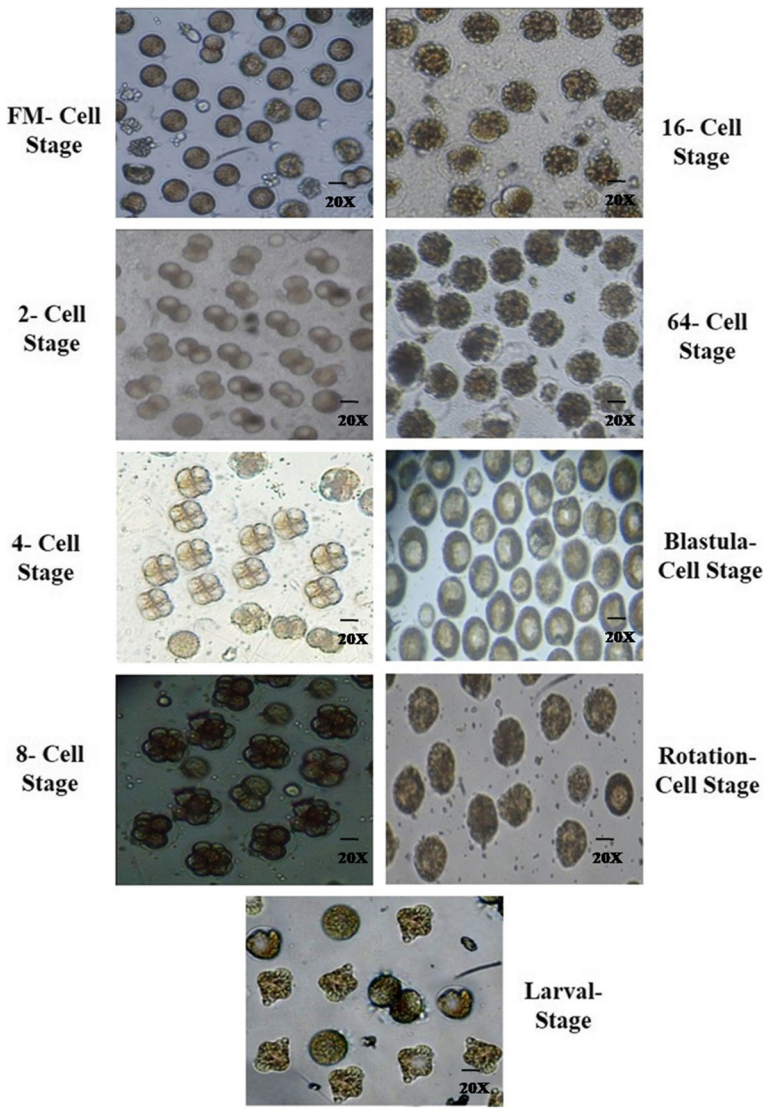

Fig. 3 Fertilization and early developmental stages of *Hydroides elegans* in control

The variations in cumulative time and success development at EC_{50} were statistically significant ($P < 0.05$) (Figs. 5f and 6 and Table 1).

G. **Blastula to blastula rotation**

The cumulative time of blastula stage in control was 113.04 ± 0.73 min. This apparently increased to 126.46 ± 0.56, 146.59 ± 0.60, and 168.06 ± 0.50 min at 1, 2, and 3 nM concentrations of AgNPs, respectively. Also, the percentage of successful development in control was recorded as 58.73 ± 0.15%, and AgNP concentration of 1, 2, and 3 nM steadily decreased to 54.30 ± 0.30%, 53.30 ± 0.30%, and 22.40 ± 0.20% (Figs. 5g and 6 and Table 1). The variations observed between EC_{50} and control to increase in cumulative time and decrease in effective development was significant at $P < 0.05$.

The cumulative time for blastula rotation was determined to control as 139.28 ± 1.20 and 155.02 ± 0.64, 180.33 ± 1.52, and 208.56 ± 0.44 min in 1, 2, and 3 nM concentrations, respectively, and the percentage of successful development in control was 49.30 ± 0.20% and 46.60 ± 0.20%, 45.5 ± 0.26%, and 6.63 ± 0.15% at 1, 2, and 3 nM concentrations, respectively (Figs. 5h and 6 and Table 1).

H. **Larval release**

The cumulative time taken for larval release in control was 1328.33 ± 7.63 min and at 1 and 2 nM concentrations of AgNPs was 1445.19 ± 2.71 and 1518.48 ± 5.43 min, respectively, with 3, 4, and 5 nM concentrations causing arrest in the development. The percentage of successful larval release in control was 36.26 ± 0.30%; however, it was lowered at

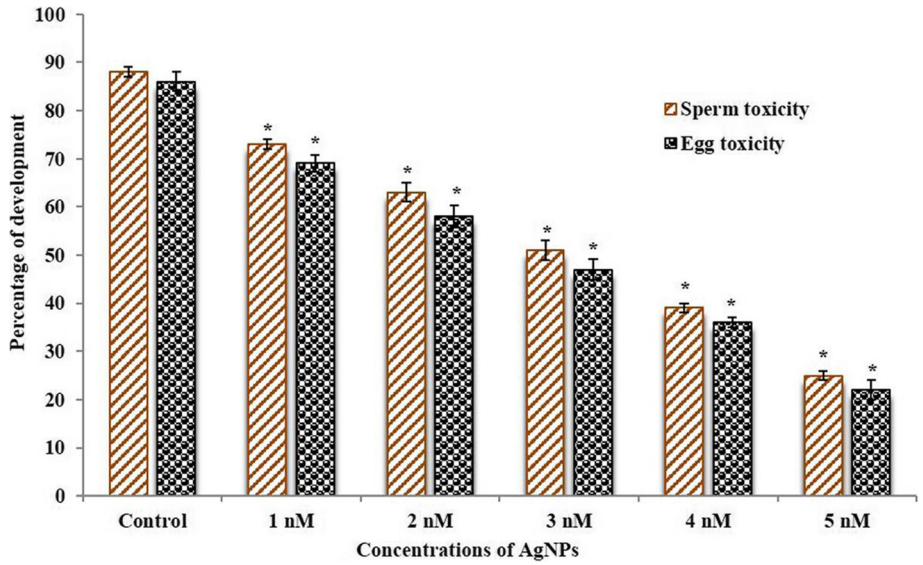

Fig. 4 Effect of silver nanoparticles on sperm and egg of *Hydroides elegans*. The mean difference is significant when compared to control (*$P < 0.05$ level)

concentration of 1 and 2 nM AgNPs to 30.53 ± 0.20% and 11.00 ± 1.00%, respectively (Figs. 5i and 6 and Table 1). The variations observed between the EC_{50} and control in cumulative time and success of development were statistically significant ($P < 0.05$) respectively.

Comet assay

In this present study, the result of untreated cells showed comets consisting of a compact head with or without a very short tail, indicating double-stranded DNA. However, the comets of AgNP-treated cells with 3 nM EC_{50} concentration have a distinct head with a tail. The comet assay is viewed with the distribution of cells according to the percentage of DNA in tail moment. The percentages of tail DNA of maximum concentration (5 nM) and EC_{50} concentration (3 nM) results were compared with appropriate control (Fig. 7, Additional file 1: Figure S1A, B and C, Table S1). The cells in control evoked about 3.70% of tail DNA damage that showed no significant effect; however, developmental stages in EC_{50} concentration of 3 nM showed tail DNA damage of about 21.35% and maximum concentration of 5 nM evoked 26.40% damage in tail DNA which was compared to control. Therefore, in this regard, the result of our study clearly indicates that silver nanoparticles used, by above 5 nM concentration, will cause high level of DNA damage to the *H. elegans* due to the toxicological effect.

Discussion

Polychaetes are ecologically important marine organisms, comprising about 30 to 80% of the total benthic fauna regardless of the ocean depth (Hutchings, 1998). They are important fouling communities, often forming dense aggregates on submerged surfaces in coastal area and sand and are common receivers of environmental contaminants including heavy metals and other compounds.

Nanotechnology has extensive applications of metal conjugated nanoparticles, and silver nanoparticles with its cytotoxicity have evoked a study on its disposal in aquatic bodies as a pollutant (Saba et al., 2012). Investigations on human cell lines have demonstrated AgNPs with inhibitory effect on metabolic (Park, Neigh, & Vermeulen, 2011) and activating mutagenesis in DNA (Asharani et al., 2008). The present study has attempted to give a brief account on the pollutant effect of AgNPs on the germ cells, fertilization, and embryonic development of *H. elegans*.

AgNPs in its colloidal form was prepared and identified by UV-vis spectroscopy as a peak at 408 nM in agreement with the finding of Maribel, Guzman, Dille, and Godet (2009). Since the animal spawns in the sea, the sea water devoid of AgNPs was taken a control and the experimental sea water contained increasing concentrations of AgNPs (1, 3, 5, 7, and 9 nM) to compare and explain the influence of AgNPs on the germ cells and the developmental stages.

The EC_{50} of developmental stages of *H. elegans* was determined which showed the tolerance range of AgNPs in embryo development, and based on EC_{50} value, the variations in development were assessed in *H. elegans*. The germ cells, the sperm, and unfertilized egg showed high tolerance to AgNPs and with a survival rate reduced by 37 and 39% at EC_{50}. In control, 98.60 ± 0.20%

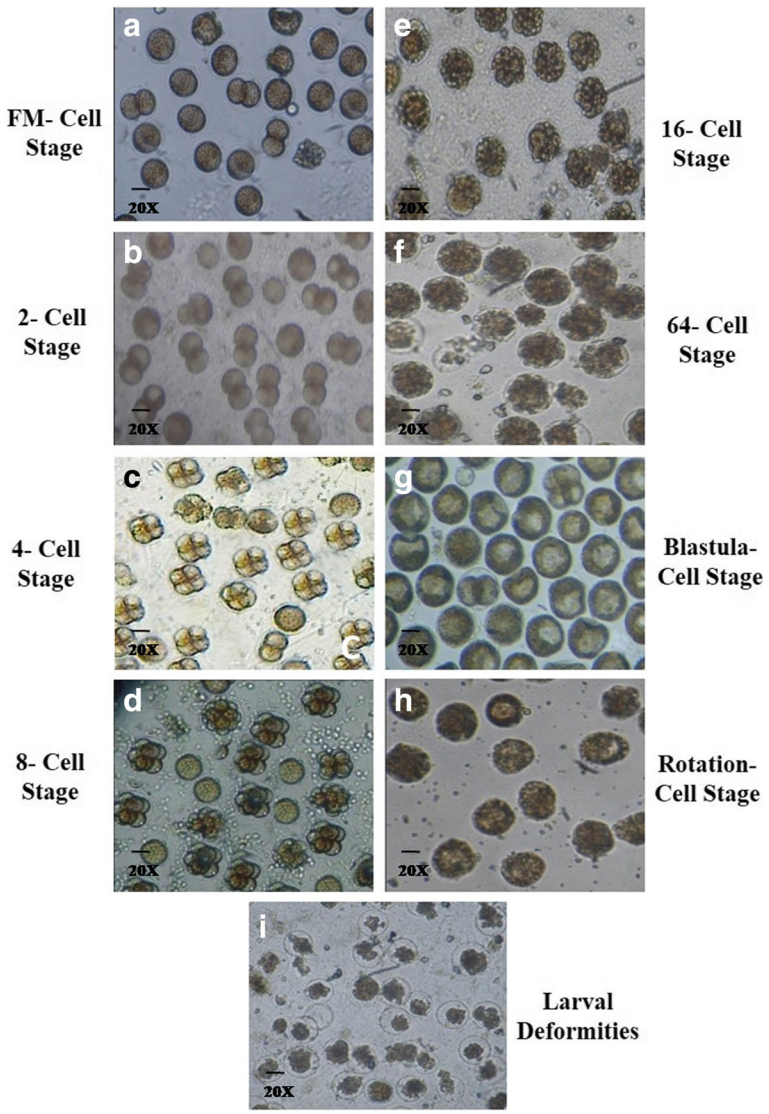

Fig. 5 a–i Effect of silver nanoparticles (EC_{50}—3 nM) on fertilization and early developmental stages of *Hydroides elegans*

of egg developed fertilization membrane, out of which only 36.20 ± 0.30% developed into larva at 3 nM concentration. To further increase in concentration, even fertilization membrane formation was reduced significantly. At higher concentration of AgNPs in seawater, the embryo did not develop normally and showed deformities and stopped development. Earlier studies on toxicity of *H. elegans* showed the EC_{50} values for Pb, Ni, and Zn for the first cell division and for the formation of fertilization membrane (FM stage) were 30.37, 38.30, and 44.22 mg l^{-1}, respectively, when the gametes were mixed together in the toxicant (Gopalakrishnan, Thilagam, & Raja, 2007). Moreover, exposure of gametes before fertilization decrease the fertilization rate in *H. elegans*, and thus, indicating the fertilization membrane formed may prevent the toxicants entering into the oocytes (Gopalakrishnan et al., 2008). In the present finding, sperm at EC_{50} showed decrease of 51% when compared to control (88%). The decreased fertilization rate with increasing concentration AgNPs suggested tolerance at concentration below EC_{50} and sensitivity above it. Similarly, Gopalakrishnan et al. (2008) have reported a decrease in fertilization rate by 31% at 80 μg Hg and 42.3, 71.2, 78.9, and 78.2% at 100 μg Cd, Pb, Ni, and Zn, respectively, compared to the controls after a 20-min exposure of sperm to control seawater and to heavy metals. Vashchenko, Zhadan, Malakhov, and Medvedeva (1995) detected that mercury ions affected motility and lowered the fertilizing capacity of *Strongylocentrotus intermedius* sperm, although they observed a stimulating effect at 5.6 mgHg l^{-1}. To evaluate from the reported investigations, the toxicity of AgNPs was evident in sperm

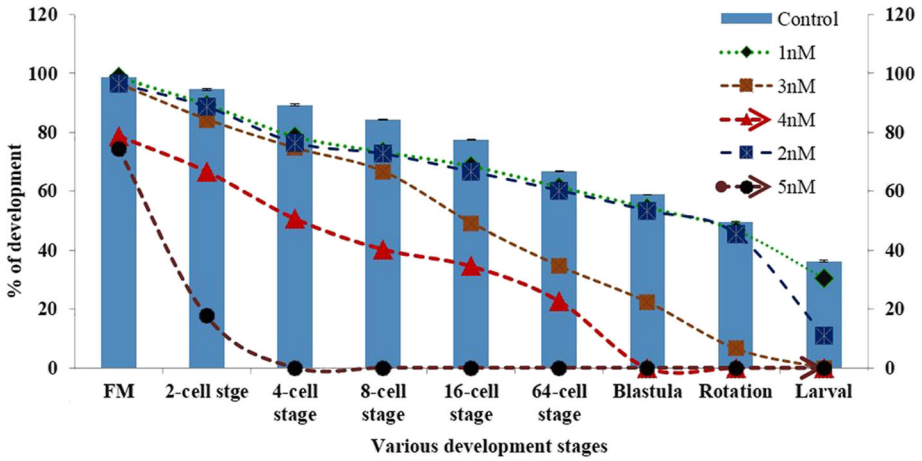

Fig. 6 Percentage of *Hydroides elegans* in early developmental stages with different concentrations of silver nanoparticles. The mean difference is significant when compared to control (*$P < 0.05$ level)

and egg viability, effective fertilization, and delayed fertilization showing that the metal toxicity effected gametes fertilization either by arresting energy producing process that slowed down fertilization. The formation of fertilization membrane and the first division showed retardation in development and time. At lower concentrations of AgNPs, the influence was less, but at concentration above EC_{50}, the effect was evidently clear. A study on in vitro toxicity in the germline C18-4 showed greater sensitivity to AgNPs than that in the BRL 3A liver cells, which are widely used in toxicity studies and comparable to that of cadmium (Braydich-Stolle, Hussain, Schlager, & Hofmann, 2005). This explains the sensitivity of the germ cells to contaminants in the form of nanoparticles.

In 2-cell stage, survivability was possible up to 5 nM of AgNPs, with EC_{50} at 3 nM of 84.26 ± 0.3% and the cumulative time increased by 2.9 min. However, in 4-, 8-, and 16-cell stages, the development was arrested at 4 nM stage and at the blastula stage at 3 nM concentration. This sufficiently explains the bioaccumulation influence through the increasing developmental stages. The EC_{50} at each successive stage decreased due to the accumulated AgNPs at each stage that fail to disintegrate or metabolize at each stage. Our observations are supported by earlier work done in sea urchin embryos for uptake of nickel chloride by Timourian and Watchmaker (1972).

Interestingly, the observation of cumulative time showed that the difference to the control increased with the development. The difference of cumulative time was 2.9 min for a fertilization membrane formation which increased progressively before an arrest of development, 14.98 min (2 nM) to 55.02 min at blastula stage.

The concordant study of DNA damage by comet assay revealed the increase in DNA damage with the developmental stages. This indicated impairment of division and

Table 1 Effect of silver nanoparticles on fertilization cumulative time of *Hydroides elegans*

Developmental stages	Control in sea water (min)	Mean time in minutes				
		Concentration of silver nanoparticles expressed in nanomolar (nM)				
		1	2	3	4	5
Fertilization membrane	4.56 ± 0.35	5.31 ± 0.07	5.69 ± 0.31	6.65 ± 0.34*	7.04 ± 0.40*	7.55 ± 0.48*
2 cell	29.58 ± 0.61	30.41 ± 1.03	31.31 ± 0.83	32.48 ± 0.48*	41.25 ± 0.87*	44.56 ± 0.58*
4 cell	49.46 ± 1.01	50.45 ± 1.10	51.28 ± 0.81	51.57 ± 0.44*	53.1 ± 0.05*	–
8 cell	59.02 ± 1.03	59.53 ± 0.90	60.02 ± 0.61	61.06 ± 0.63*	62.26 ± 0.17*	–
16 cell	59.51 ± 0.51	60.10 ± 0.05	60.17 ± 0.07	73.13 ± 0.58*	80.53 ± 0.54*	–
64 cell	83.31 ± 0.75	90.46 ± 0.90*	96.46 ± 1.05*	100.54 ± 1.63*	115.53 ± 0.54*	–
Blastula	113.04 ± 0.73	126.46 ± 0.56*	146.59 ± .60*	168.06 ± 0.50*	–	–
Rotation stage	139.28 ± 1.20	155.02 ± 0.64*	180.33 ± 0.52*	208.56 ± 0.44*	–	–
Larval	1328.33 ± 7.63	1445.19 ± 2.71*	1518.48 ± 5.43*	–	–	–

The mean difference is significant when compared to control
*$P < 0.05$ level

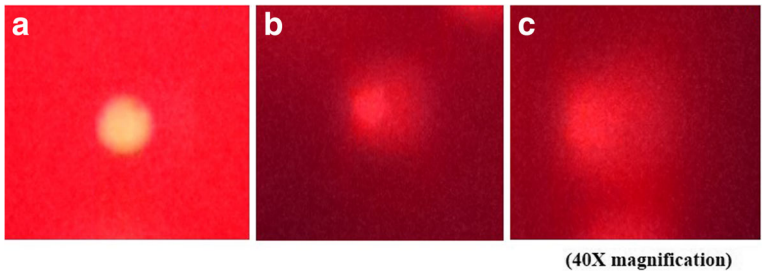

Fig. 7 Comet assay showing DNA damage in sperms of *Hydroides elegans* incubated in medium containing silver nanoparticles. Control untreated sperm (**a**), 3 nM (**b**), and 5 nM silver nanoparticle-treated sperm (**c**)

transcription that explains the extended time of development and finally arrest in development. The mutagenic potential of silver nanoparticles was evaluated in embryonic fibroblasts (MEF-LacZ) and supports our findings (Park et al., 2011). The influence of AgNPs in the energy metabolizing process is evident from the fact that after EC_{50} concentration at each developmental stage, the period of cumulative time relatively increased in comparison to the control and also time of development of the previous stage. The cumulative time at each developmental stage increased due to accumulation of nanoparticles that reduced its ability to resist the transformations in the cell.

The fertilization success significantly differed among the concentration of AgNPs treated. Previous report on the highest blastula development was recorded in nickel treatment and lower in mercury treatment among the metals studied. The abnormal development of embryo increased as the concentration of the metal increased in seawater and also more abnormal development of the embryo seen in mercury exposure compared to other metals even at low concentration (Gopalakrishnan et al., 2008). Moreover, Chan and Chiu (2015) reported the proof concept study of the effects of coated AgNPs on growth, development, metamorphosis, and settlement of marine larvae as well as silver accumulation and particle biodistribution using three model marine invertebrate species from different phyla.

In the present study, evaluation of AgNPs at various concentrations determined the effect on cumulative time and percentage of developmental stages of *H. elegans*. From the results obtained, it was observed that abnormalities in embryo increases as the concentration of the AgNPs increases. The exposure to nanoparticles in the aquatic environment due to waste disposal of the products consisting of nanoparticles, in various fields such as in medicine, cosmetic, drug delivery, tissue engineering, and therapeutic for treating cancer, probably are detrimental to organisms exposed to it. Thus, the effect of toxicity of the silver nanoparticles depends upon the concentration of the nanoparticles and also the resistance of the exposed species.

Conclusions

The experimental data presented clearly revealed the toxicity of silver nanoparticles to early life stages of a marine polychaete worm *H. elegans*. Moreover, this bioassay is relatively easy to conduct and provides concordant results. Furthermore, the availability of gametes throughout the year and ease of collection makes *H. elegans* a suitable species for routine laboratory toxicity testing. The present investigation evaluates the risk of AgNPs imposed on invertebrate organisms which is exposed to it continuously. Thus, minimized use of such products has to be implemented or alternative and safe methodology adopted while disposing the AgNPs directly or indirectly in the environment to prevent ecotoxicity and its adverse effect on other organisms including human being.

Additional file

Additional file 1: Figure S1. Control analyzed by CASP software (A). Three nanomolar treated concentration analyzed by CASP software (B). Five nanomolar treated concentration analyzed by CASP software (C). **Table S1.** Percentage of tail DNA damage. (DOC 712 kb)

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Authors' contributions
AC designed the work. CD performed the experiment. HG, BS, and PD drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
We declare that we do not need an ethics approval regarding our work on the marine polychaete worm *H. elegans.*

Competing interests
The authors declare that they have no competing interests.

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References

Ahamed, M., Alsalhi, M. S., & Siddiqui, M. K. (2010). Silver nanoparticles applications and human health. *Clinica Chimica Acta, 411*(23-24), 1841–1848.

Arora, S., Jain, J., Rajwade, J. M., & Paknikar, K. M. (2008). Cellular responses induced by silver nanoparticles; in vitro studies. *Toxicology Letters, 179*, 93–100.

Arumugam, S. (2012). Toxic effect of flucloxacillin on the early development of the polychaete *Hyroides elegans*. *Universal Journal of Environmental Research and Technology, 2*(3), 135–142.

Asharani, P. V., Lian Wu, Y., Gong, Z., & Valiyaveettil, S. (2008). Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology, 19*, 1–8.

Bar-Ilan, O., Albrecht, R. M., Fako, V. E., & Furgeson, D. Y. (2009). Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. *Small, 5*(16), 1897–1910.

Benn, T. M., & Westerhoff, P. (2008). Nanoparticle silver released into water from commercially available sock fabrics. *Environmental Science & Technology, 42*(11), 4133–4139.

Braydich-Stolle, L., Hussain, S., Schlager, J. J., & Hofmann, M. C. (2005). *Invitro* cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicological Sciences, 88*, 412–419.

Braydich-Stolle, L. K., Lucas, B., Schrand, A., Murdock, R. C., Lee, T., Schlager, J. J., et al. (2010). Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells. *Toxicological Sciences, 2*, 577–589.

Bryaskova, R., Pencheva, D., Kyulavska, M., Bozukova, D., Debuigne, A., & Detrembleur, C. (2010). Antibacterial activity of poly (vinyl alcohol)-bpoly(acrylonitrile) based micelles loaded with silver nanoparticles. *Journal of Colloid and Interface Science, 344*(2), 424–428.

Buffet, P. E., Pan, J. F., Poirier, L., Amiard-Triquet, C., Amiard, J., Gaudin, P., Risso-de Faverney, C., Guibbolini, M., Gilliland, D., Valsami-Jones, E., & Mouneyrac, C. (2013). Biochemical and behavioural responses of the endobenthic bivalve Scrobicularia plana to silver nanoparticles in seawater and microalgal food. *Ecotoxicology and Environmental Safety, 89*, 117–124.

Cabrita, E., Robles, V., Rebordinos, L., Sarasquete, C., & Herraez, M. P. (2005). Evaluation of DNA damage in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream (*Sparus aurata*) cryopreserved sperm. *Cryobiology, 50*, 144–153.

Chan, Y. S. C., & Chiu, J. M. Y. (2015). Chronic effects of coated silver nanoparticles on marine invertebrate larvae, a proof of concept study. *PloS One, 10*(7), 0132457.

Chen, X., & Schluesener, H. J. (2008). Nanosilver: A nanoproduct in medical application. *Toxicology Letters, 176*(1), 1–12.

Choi, O., & Hu, Z. (2008). Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environmental Science & Technology, 42*, 4583–4588.

Dibrov, P., Dzioba, J., Gosink, K. K., & Hase, C. C. (2002). chemiosmotic mechanism of antimicrobial activity of Ag(−) in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy, 46*, 2668–2670.

Fabrega, J., Luoma, S. N., Tyler, C. R., Galloway, T. S., & Lead, J. R. (2011). Silver nanoparticles behavior and effects in the aquatic environment. *Environment International, 37*(2), 517–531.

Fenech, M. (2006). Cytokinesis-block micronucleus assay evolves into a cytome assay of chromosomal instability; mitotic dysfunction and cell death. *Mutation Research, 600*, 58–66.

Gopalakrishnan, S. (2002). Effects of copper and aluminium on reproductive behaviour, gametes, fertilization and early development of a sedentary polychaete Hydroides elegans (Haswell 1883). Doctoral thesis, University of Madras, pp 170.

Gopalakrishnan, S., Thilagam, H., & Raja, P. V. (2007). Toxicity of heavy metals on embryogenesis and larvae of the marine sedentary polychaete *Hydroides elegans*. *Archives of Environmental Contamination and Toxicology, 52*, 171–178.

Gopalakrishnan, S., Thilagam, H., & Vivek Raja, P. (2008). Comparison of heavy metal toxicity in life stages sperm toxicity, egg toxicity, embryotoxicity and larval toxicity of *Hydroides elegans*. *Chemosphere, 71*, 515–528.

Griffitt, R. J., Brown-Peterson, N. J., Savin, D. A., Manning, C. S., Boube, I., Ryan, R. A., & Brouwer, M. (2012). Effects of chronic nanoparticulate silver exposure to adult and juvenile sheepshead minnows (Cyprinodon variegates). *Environmental Toxicology and Chemistry, 30*, 160–167.

Handy, R. D., Von der Kammer, F., Lead, J. R., Hassellov, M., Owen, R., & Crane, M. (2008). The ecotoxicology and chemistry of manufactured nanoparticles. *Ecotoxicology, 17*(4), 287–314.

His, E., Beiras, R., & Seaman, M. N. L. (1999). The assessment of marine pollution-bioassays with bivalve embryos and larvae. In Advances in Marine Biology; Southward, A.J., Tyler, P.A., Young, C.M., Eds.; Academic Press, San Diego, CA, pp. 1–178.

Hutchings, P. (1998). Biodiversity and functioning of polychaetes in benthic sediments. *Biodiversity and Conservation, 7*, 1133–1145.

Hwang, E. T., Lee, J. H., Chae, Y. J., Kim, Y. S., Kim, B. C., Sang, B. I., & Gu, M. B. (2008). Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. *Small, 4*(6), 765–750.

Kim, K. T., Truong, L., Wehmas, L., & Tanguay, R. L. (2013). Silver nanoparticle toxicity in the embryonic zebrafish is governed by particle dispersion and ionic environment. *Nanotechnology, 24*(11), 115101.

Lara Villegas, H. H., Vanesa Ayala-Nu, V., Carmen Ixtepan Turrent, L. D., & Padilla, C. R. (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. *World Journal of Microbiology and Biotechnology, 26*(4), 615–621.

Maribel, G., Guzman, Dille, J., & Godet, S. (2009). Synthesis of silver nanoparticles by chemicalreduction method and their antibacterial activity. *International Journal of Chemical and Biomolecular Engineering*, 2:3, pp. 104–111.

Navarro, E., Piccapietra, F., Wagner, B., Marconi, F., Kaegi, R., Odzak, N., et al. (2008). Toxicity of silver nanoparticles to *Chlamydomonas reinhardtii*. *Environmental Science & Technology, 42*(23), 8959–8964.

Park, M. V., Neigh, A. M., & Vermeulen, J. P. (2011). The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. *Biomaterials, 32*(36), 9810–9817.

Rand, G. M., Wells, P. G., Mcarty, L. S., (1995). Introduction to aquatic toxicology, pp. 3-67. In: G. M., Rand (ed), *Fundamentals of Aquatic Toxicology -Effects, Environmental Fate, and Risk Assessment*. Washington, D.C., Taylor and Francis Publishers, 1125p.

Roh, J. Y., Sim, S. J., Jongheop, Yi., Park, K., Chung, K. H., & Ryu, D. Y. (2009). Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. *Environmental Science & Technology, 43*(10), 3933–3940.

Saba, A., Seyed Ali, J., Hyun, L., Yong Seok, K., Yong Bae, J., & Hyun Jung, C. (2012). Toxicity of various silver nanoparticles compared to silver ions in *Daphnia magna*. *Journal of Nanobiotechnology, 10*, 1–14.

Siller, L., Lemloh, M. L., Pitricharoenphun, S., Mendis, B. G., Horrocks, B. R., Brümmer, F., & Medaković, D. (2013). Silver nanoparticles toxicity in sea urchin Paracentrotus lividus. *Environmental Pollution, 178*, 498–502.

Timourian, H., & Watchmaker, G. (1972). Nickel uptake by sea urchin embryos and their subsequent development. *The Journal of Experimental Zoology, 182*, 379–388.

Turkevich, J., Stevenson, P. C., & Hiller, J. (1951). A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discussions of the Faraday Society, 11*, 55.

Udhayakumar, M., & Karande, A. A. (1996). Field notes and a fouling serpulid *Hydroides elegans* Haswell polychaeta serpulids present in confined waters of Bombay. *Indian Journal of Marine Science, 25*, 133–136.

Vashchenko, M. A., Zhadan, P. M., Malakhov, V. V., & Medvedeva, L. A. (1995). Toxic effect of mercury chloride on gametes and embryos in the sea urichin Strongylocentrotus intermedius. *Russian Journal of Marine Biology, 21*, 300–307.

Yang, X., Gondikas, A. P., Marinakos, S. M., Auffan, M., Liu, J., Hsu-Kim, H., & Meyer, J. N. (2012). Mechanism of silver nanoparticles toxicity is dependent on dissolved silver and surface coating in Caenorhabditis elegans. *Environmental Science & Technology, 46*, 1119–1127.