Effects of Silver Nanoparticles on DNA Damage in Gills of the *Ruditapes Philippinarum*

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Abstract: With the rapid development of nanotechnology, a certain number of nanoparticles will inevitably be released into the marine ecosystem. In this work, the effects of silver nanoparticles (Ag NPs) on DNA damage of marine bivalve *Ruditapes Philippinarum* were evaluated. The clams were exposed to 0, 10 and 40 μg/L of Ag NPs for 14 days respectively, and samples are performed at the 0, 3, 7, 14 days. The results showed that that Ag NPs were considered to cause genotoxic effect on clam gills, and induced a time-dependent increase of DNA damage. The gills are more sensitive to high Ag NPs concentration. The genotoxicity developed in a dose- and time-dependent manner.

1 INTRODUCTION

Silver nanoparticles (Ag NPs) are the most commonly used due to their unique physico-chemical properties, such as electrical and thermal conductivity, catalytic activity, and antibacterial activity (Chernousova, 2013; McGillicuddy, 2017). This fast expansion will inevitably drive the release of nanoparticles into marine ecosystems. It is estimated that the concentration of Ag NPs in water is in the range of ng/L, and in the mg/kg range in the soil and sediment (Blaser, 2008). Because aquatic organisms are in constant contact with pollutants through swallowing, gill entry, skin absorption, etc., they are more susceptible to the toxic effects of nanoparticles than terrestrial organisms (Moore, 2006). A marine mesoderm study showed that Ag NPs induced DNA damage and oxidative damage to *Scrobicularia plana (*Buffet, 2014). Ag NPs not only have antibacterial effects, but also have ROS-derived oxidative stress, biofilm damage and DNA damage (Zuykov, 2011). Therefore, the toxic effects and mechanisms of Ag NPs on aquatic organisms have attracted more and more attention to assess their impact on the ecological environment and human health.

As the most important component of cells, DNA is critical in maintaining cell homeostasis and genetic information transmission, and impact analysis of aquatic DNA has proven to be a very suitable method for assessing the genotoxicity of environmental pollutants, enabling the detection of toxic effects of low concentrations of pollutants in a variety of species. Comet experiments have been applied in previous studies to study levels of DNA damage in marine and freshwater bivalves exposed to pollutants (Dhawan, 2009). Comet experiments, also known as single-cell gel electrophoresis assays (SCGE), are a fast and sensitive technique that requires only a small number of cells to provide information on DNA damage and repair (Collins, 2008). Single-cell samples are fused in a low melting point agarose gel for lysis, followed by electrophoresis under alkaline conditions, and after the end of the electrophoresis, it can be observed under a fluorescence microscope that the stained intact DNA fragments can only remain in situ due to large molecular weights and are spherical in shape, while smaller fragments of broken DNA migrate to the positive electrode, forming a comet shape (Canesi, 2012).

Ruditapes Philippinarum is often used as a sentinel species in ecotoxicological studies due to their ability to filter large volumes of water, leading to contaminant (Faggio, 2018). Study found bivalve in its organization (mainly the gills and digestive gland) accumulation of metals and other pollutants, and that direct contact between the gills and the external environment can cause more serious damage (Tice, 2000). The gills can be used as the most

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sensitive tissue sites for detecting genotoxicity, and DNA damage in gill tissues can be measured by comet experiments to be able to assess the potential effects of Ag NPs on the genetic aspects of clams. Therefore, the aim of this study was to expose *Ruditapes Philippinarum* to different concentrations of Ag NPs, identify the toxic effects of Ag NPs, and assess the effect from the perspective of genotoxicity by determining the degree of DNA damage in the gills of clams.

2 MATERIAL AND METHODS

2.1 Preparation of Silver Nanoparticles

Ag NPs (purity>99.7%) were purchased from Sigma-Aldrich with the particle size specified as <100 nm, a stock solution of 50 mg/L was prepared in ultrapure water, sonicated for 1 h and kept in constant shaking to breakdown particles aggregates before adding to the exposure tanks.

2.2 Laboratory Assay

The clams, *Ruditapes Philippinarum* were purchased from Zhangcun Seafood Market in Qingdao and domesticated for 3 days under laboratory conditions (pH=8.1; Temperature=16.3±0.5 ℃; Salinity=31; Dissolved Oxygen=8.3). The clams with no damage, sensitive response and similar size were divided into three groups: control and 10 μg/L or 40 μg/L of Ag NPs. Set three parallel for each group, and each group has a volume of 5 L seawater. During the experiment, the clams were not fed satisfactorily and the test water was changed every 24 h. On the 0, 3,7, 14 days of exposure, clams (n=2) were randomly selected from each group and dissected immediately, cut it with scissors to make the tissue as small as possible, add 3 mL trypsin, mix well, pipette the cell suspension in a water bath at 37 ℃ to resolve the tissue cells into a single cell suspension. Centrifuge at 500 $r \cdot min^{-1}$ and take the supernatant. Then centrifuge at 1500 $r \cdot min^{-1}$, remove the supernatant, and add 1 mL of PBS (0.1 mol/L) to the centrifuge tube, a single-cell suspension can be obtained, followed by single-cell electrophoresis experiments. All of the above experimental steps are performed under dark conditions to prevent UV rays from affecting the results. Determination of clam gill cell DNA Olive tail moment (OTM) by CASP comet image analysis software, OTM = (Tail Center of Gravity Position - Head Center of Gravity Position) × tail DNA content.

2.3 Statistical Analysis

Data of DNA damage was shown as mean ± standard of deviation. Significant differences between exposure groups were detected using one-way analysis of variance (ANOVA) and only $P<0.05$ was accepted as significant. Then Origin 9.2 was used for plotting.

3 TEST RESULTS AND DISCUSSIONS

Fig. 1 shows that the control group with a normal nucleus with a complete head and no migration of the tail. On the 3 days, the nucleus DNA of clam gills showed that a gradual increase in tail length and migration in parallel with a slight decrease in head size, indicating the beginning of DNA damage. On the 7 days, it can be seen from these figures that more DNA-strand breaks of clam gills, the length of the tail gradually increased, and the fluorescence intensity of the tail gradually increased, indicating that DNA damage increases with the increase of dose and duration of Ag NPs exposure. The highest doses (40 μg/L) of Ag NPs showed a statistically significant increase in tail intensity compared with the control group on the 14 days. The results of the genotoxic effect of Ag NPs varies with the doses and duration of exposure.

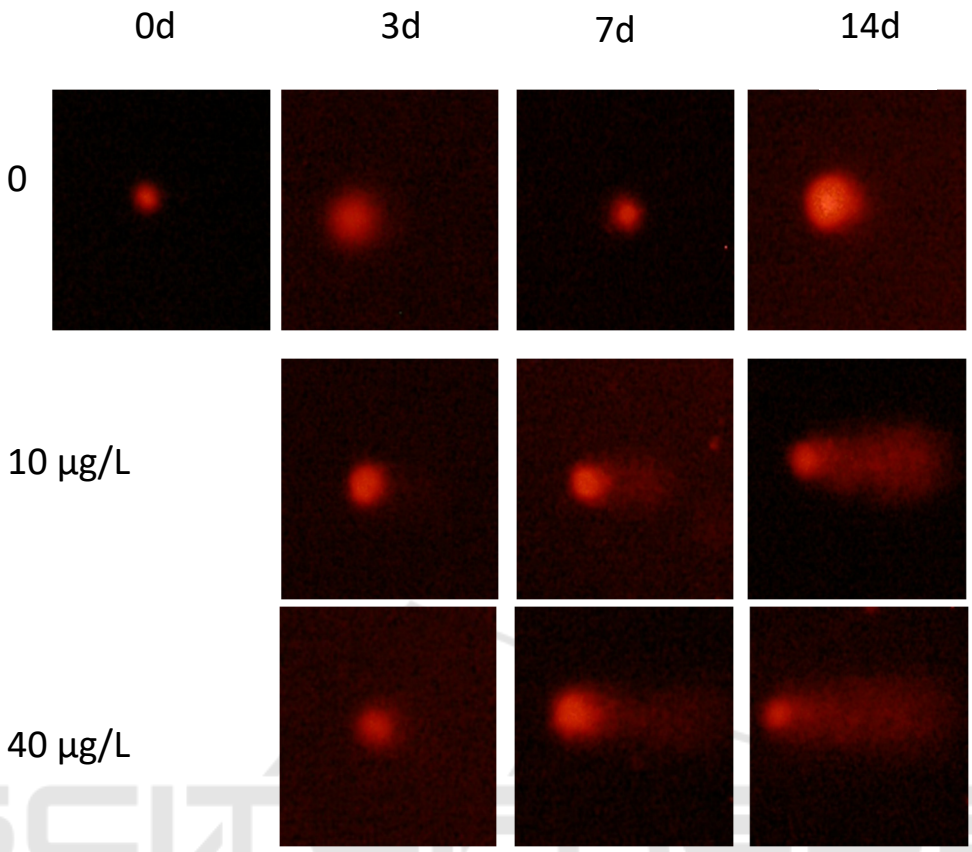

Figure 1: Fluorescence photomicrograph of in gill of clam *Ruditapes philippinarum* exposed to Ag NPs.

As can be seen from Figure 2, the OTM value in the gills was elevated at the 3 days, but there was no significant difference compared with the control group. On the 7th day of the experiment, the OTM value of each treatment group was significantly increased (P<0.05), and the injury was most significant in the treatment group with a concentration of 40 μg/L, and the damage intensity increased with the duration of exposed, and the treatment group reached the maximum value on the 14th day. The above results show that the DNA damage of clam gills is gradually more severe with the increase doses and duration of exposed. The genotoxicity of zebrafish (*Danio rerio*) exposed to $nTiO_2$ (1 and 10 μg/L) for 14, 21 and 28 days was assessed using RAPD-PCR technology, the genomic stability decreased by 37% after 14 days of exposure and increased with duration of exposed, and the highest genotoxic effect was observed at the maximum concentration of $nTiO_2$ (10 μg/L) after exposure 21 days (Rocco, 2015).

The specific mechanism of genotoxicity of nanoparticles to clams cells is unclear, but one study suggests that one possibility is due to their small size (1–100 nm), Ag NPs can penetrate the nucleus through the nuclear pores, and the Ag NPs are highly reactive and surface-charged, interacting directly with DNA or nuclear proteins (Joubert, 2013). Another possibility is the release of metal ions within the cell to induce the production of excess reactive oxygen species, and ROS reacts with DNA molecules to cause damage to purine and pyrimidine bases, as well as the DNA backbone, leading to DNA damage (Rocha, 2014). This indirect damage caused by ROS is the main pathway to DNA damage and can lead to physiological damage, including damage to the reproductive system, inhibition of growth, and damage to various organelles such as lysosomals and mitochondria (Gomes, 2013).

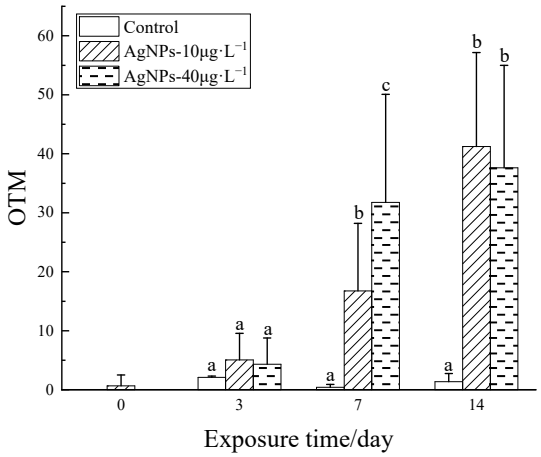

Figure 2: Changes of DNA damage (OTM) in gill of clam *Ruditapes philippinarum* exposed to Ag NPs.

4 CONCLUSION

In this study, the effects of DNA damage in clams exposed to Ag NPs were analyzed by comet experiments, it can be concluded that Ag NPs were considered to cause genotoxic effect on clam gills, and induced a time-dependent increase of DNA damage. The gills are more sensitive to high Ag NPs concentration. The genotoxicity developed in a dose- and time-dependent manner.

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