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# A novel method for assessing the toxicity of silver nanoparticles in *Caenorhabditis elegans*

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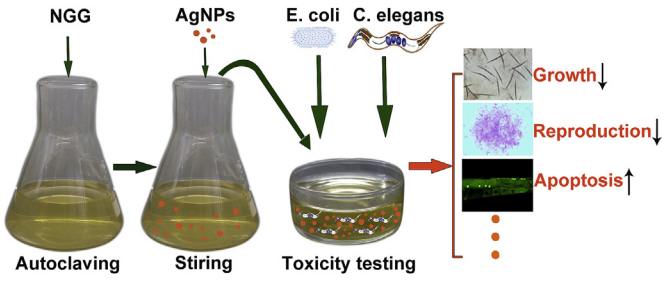
## HIGHLIGHTS

- A semi-fluid NGG method for assessing toxicity of AgNPs using *C. elegans* is proposed.
- AgNPs affect germ cell apoptosis, reproductive ability and lifespan of *C. elegans*.
- The semi-fluid NGG has more advantages and sensitivity for evaluating the toxicity of AgNPs using *C. elegans*.
- For semi-fluid NGG, the population growth assay is firstly applied to detect ecological toxicity of AgNPs.

## GRAPHICAL ABSTRACT

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## ABSTRACT

At present, nanotechnology has been producing nanoscale materials with unprecedented speed. Nanomaterials could be inevitably released into the environment owing to their widespread use, and their potential toxicity has caused a great concern. With regard to assessment of nanomaterial toxicity, many studies probably don't truly reflect their toxicity, because the nanoparticles were not stable and uniformly dispersed in the medium. In the present study, the semi-fluid nematode growth gelrite medium (NGG) was used to achieve better distribution of silver nanoparticles (AgNPs). We aimed to evaluate the toxicity of AgNPs in three different culture methods, such as the NGG, nematode growth medium (NGM) and K-medium (KM). Our transmission electron microscopy, hydrodynamic diameter, and inductively coupled plasma-atomic emission spectrometry results demonstrated that AgNPs homogeneously and stably dispersed in NGG compared to that in liquid KM. Furthermore, the conventional toxicity end points, such as body length, fecundity, lifespan, population growth, germline cell apoptosis, reactive oxygen species, and mitochondrial membrane potential were used to assess the toxicity of AgNPs to *Caenorhabditis elegans* (*C. elegans*) in NGG, NGM and KM. Our results showed that the toxicity of AgNPs obtained in the NGG test medium was much higher than that in the standard NGM and KM. In addition to the improved dispersion of nanoparticles, NGG also offered advantages for long-term studies and

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likely provided a convenient nematode toxicity testing method. These results revealed that the NGG test medium was a suitable and sensitive culture method for the evaluation of AgNPs toxicity using *C. elegans*.


## 1. Introduction

With the increased production and use of nanomaterials in various fields, nanomaterial toxicity is becoming an increasingly important issue in nanotechnology. Currently, AgNPs are one of the most widely used commercial nanomaterials owing to their excellent antimicrobial activity (Duran et al., 2016). For the past decades, the toxicity of AgNPs has been demonstrated in many models, including in bacteria (Xiu et al., 2012), cell culture systems (Wang et al., 2013), zebrafish (Asharani et al., 2008), *C. elegans* (Roh et al., 2009; Maurer et al., 2016), and mice (Rahman et al., 2009). In these models, *C. elegans* is emerging as one of the most useful model systems for assessing potential toxicity of nanoparticles because of its particular advantages.

*C. elegans* is a broadly distributed nematode species in the soil ecosystem which plays important roles in decomposition and nutrient cycling. It has a standardizable developmental stage and short lifespan with a completely sequenced genome. Recently, *C. elegans* has been successfully used in environmental and toxicological studies of toxicants from the whole-animal level down to the single-cell level (Leung et al., 2008; Wu et al., 2013, 2014). Moreover, *C. elegans* has a translucent body that can be exploited to observe the distribution of nanomaterials in the organism. Furthermore, *C. elegans* has been widely used as a well-known biological model to evaluate the toxicity of engineering nanomaterials (Cha et al., 2012; Rui et al., 2013; Yang et al., 2014). In general, three methods have been adopted to expose *C. elegans* to nanomaterials. In the first method, *C. elegans* is exposed to liquid medium, normally K-medium (KM) and S-medium (Ma et al., 2009; Wang et al., 2009; Maurer et al., 2016). However, the nanomaterials easily form aggregates and sediments using this method. The size of the aggregates increase more than 100 times approximately the pristine size of nanoparticles. Additionally, some metal nanoparticles exposed in liquid media could be partially dissolved or they could release ions under the effect of hydration kinetics. The aggregates and dissolution of nanoparticles may reduce its actual exposure dose to experimental organisms, which ultimately affects the actual toxicity effect test. In the second method, nanomaterials are suspended in solid NGM agar plates (Kim et al., 2012). Worms have close contact only with the surface of solid NGM, affecting the contact between worms and nanomaterials. Therefore, nanoparticles exposed to solid NGM influence the effective exposure dose. Lastly, nanomaterials mixed with the *Escherichia coli* (*E. coli*) *OP50*, which is used as the daily food for nematodes, have been utilized (Daewlaetsina et al., 2013; Jung et al., 2015). Concentrated *E. coli* and its secretion accelerate the transformation of nanoparticles and influence nanomaterials toxicity evaluation. As a whole, the media, namely liquid, solid NGM, and concentrated bacteria liquid media used for exposure of *C. elegans* to nanoparticles, could significantly influence the actual concentration of nanomaterial handling worms, and thus affect the precise evaluation of toxicity of nanoparticles. Until now, several challenges are encountered due to the lack of standardized protocols. In order to improve the experimental conditions for nanotoxicity, serious consideration is critical to obtain reliable and realistic data.

Here, the semi-fluid nematode growth gelrite medium (NGG), as a novel culture method for *C. elegans*, was used as the test medium to evaluate the toxicity of AgNPs which were the most commonly used engineered nanomaterial. We optimized the NGG for *C. elegans* as a better model to evaluate nanomaterial toxicity (Brinke et al., 2011). Our data demonstrated that the modified method greatly increased the nanomaterial distribution. The toxicity evaluation, including longevity, fecundity, body length, population growth, gonad apoptosis, ROS and mitochondrial membrane potential, revealed that the NGG test medium was a reliable and sensitive culture method for the evaluation of AgNPs toxicity using *C. elegans*.

## 2. Methods

### 2.1. C. elegans culture conditions

Stock *C. elegans* Bristol strain (N2) was obtained from the Caenorhabditis Genetics Center (Minneapolis, USA) and maintained on NGM agar (17 g agar, 2.5 g peptone, and 3 g NaCl in 975 mL deionized water, with 1 mL 1 M $CaCl_2$, 1 mL 1 M $MgSO_4$, 25 mL 1 M $KPO_4$ buffer (pH 6), and 1 mL cholesterol solution (5 mg/mL in ethanol) added after autoclaving), and seeded with *E. coli OP50* as a food source.

The synchronized worms were transferred to the NGG (modified NGM with agar replaced by 1.5 g gellan gum), and seeded with *E. coli* (Muschiol et al., 2009; Brinke et al., 2011). The growth temperature was set at 20 °C.

### 2.2. Exposure and characterization of AgNPs in the culture medium

AgNPs (diameter size: <100 nm) coated with PVP (Sigma-Aldrich, China) were dissolved in deionized water by using ultrasound and then different concentrations of AgNPs were added to 200 mL NGG. The mixture was continuously stirred for 60 min at 60 °C with a magnetic stirrer until AgNPs were uniformly distributed in the NGG. The mixture was stored at room temperature in the dark. AgNPs in the NGM and KM (30 mM KCl, 50 mM NaCl, 3 mM $CaCl_2$) were prepared as described above using the NGM and KM as culture medium. Worms were treated using standard procedures as described previously (Leung et al., 2008). Briefly, subsequent solutions of AgNPs were prepared in the NGG, NGM and KM. According to the needs of different experimental measurements, synchronized young adult hermaphrodites of various amounts and different periods obtained from the same culture plate were exposed to AgNPs in the test medium and cultured at 20 °C.

To investigate the distribution of AgNPs in NGG, the top, middle, bottom of NGG were collected for Ag content measurement by using ICP-AES (Agilent 7500, Japan). To detect the stability of AgNPs in NGG medium, AgNPs in NGG with 1, 5, 10 or 25 d were collected by centrifugation at 100,000 rpm for 30 min. The Ag element content of the supernatant and precipitate was measured by ICP-AES from three independent experiments. The morphology of AgNPs was observed using TEM (JEM-2011, Japan). The hydrodynamic diameter of AgNPs was characterized using a Zetasizer (Malvern Nano series, Malvern Instruments Ltd., U.K.).