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Acute toxicity of silicon dioxide nanowires (SiO_2NWs) in saltwater microcrustacean, *Artemia franciscana*

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Abstract: Silicon dioxide nanowires (SiO_2NWs) have been taken into account as a novel nanocarrier to improve bioavailability of therapeutic compounds. However, despite the ever-increasing utilization of these nanocarriers, much less attention has been paid to determine their *in vivo* toxicity. Indeed, this study was designed to evaluate acute toxicity of SiO_2NWs in *Artemia franciscana* nauplii, as a biological model. To this end, the sensitivity range of *A. franciscana* nauplii was estimated at different concentrations of 0 (control), 1, 10, 50, 100, 150, and 200 mg L^{-1} SiO_2NWs for 96 h based on ISO TS 20787. The outcomes exhibited that 96-h EC_{50} value of SiO_2NWs was 122.167 mg L^{-1} for *A. franciscana*. Moreover, SiO_2NWs at high concentrations increased the immobilization rate of *A. franciscana* nauplii. Therefore, SiO_2NWs were not acutely toxic to *A. franciscana* nauplii according to the information provided by European Union legislation and European Union Council Directive 67/548/EEC of 27 June 1967. Given that this study just assessed the acute toxicity of SiO_2NWs, however, further studies must be conducted to evaluate intra- and extra-cellular effects of such nanowires.

Keywords: Nanowires; Silica; Brine shrimp; Acute toxicity; Nanocarriers.

Introduction

In recent decades, nanotechnology, as a multidisciplinary field, is being extensively and optimistically applied in the various technologies and industries. Among nano-materials, silica or silicon dioxide nanowires (SiO_2NWs) have attracted researcher's attention because of their electrical, mechanical, and optical properties. In particular, SiO_2NWs have been taken into consideration as an effective drug nanocarrier because of their high drug-loading capacity, surface-to-volume ratio, compatibility and biodegradability, large-area porous structures, and low toxicity [1]. Although nanomedicine-based studies have reported the low toxicity of these high-performance nanocarriers, the critical toxic ranges of these novel nanovectors should be assessed because few information is available about *in vivo* toxicity of SiO_2NWs. Accordingly, Nelson et al. (2010) stated that hardly any attempts have been conducted to determine the *in vivo* toxicity of silica-based nanomaterials [2].

Brine shrimp, *Artemia sp.*, as a ubiquitous organism in saline lakes worldwide, has been widely employed in toxicological studies due to its filter-feeding strategy, high offspring production, rapid hatching and easy accessibility of nauplii, and simplicity of culture [3]. In addition, this euryhaline zooplanktonic organism has been applied as a biological model to assess the toxicity of manufactured nanomaterials as far as the ISO TS 20787 has recently been published to standardize nanotoxicity experiment with this creature [4]. In view of this scenario, this study was designed to assess acute toxicity of SiO_2NWs in saltwater microcrustacean, *Artemia franciscana*.

Materials and Method

SiO_2NWs characteristics

SiO_2NWs were provided by The Korea University. To characterize these nanowires, Dynamic Light Scattering (DLS; Nanophox (Sympatec, Clausthal-Zellerfeld, Germany)) was used to determine the size, volume mean diameter (VMD), and surface mean diameter (SMD) of SiO_2NWs. Moreover, Transmission Electron Microscopy (TEM) was employed to evaluate SiO_2NWs morphology.

Test organism

Artemia franciscana cysts were obtained from INVE Aquaculture N.V./S.A., Belgium. To carry out the toxicity experiment, dried cysts were first hatched in transparent

"V"–bottomed glass incubators equipped with continues aeration. The incubators containing 1 L of sterilized artificial seawater (30 g L^{-1} synthetic seawater salt (13045 Process®, Aqua Craft®, Inc., USA)) were also exposed to continuous light illumination of 1500 lx. Finally, the nauplii hatched during 24 h and were transferred into fresh seawater medium.

Acute toxicity test

Acute immobilization test was conducted according to ISO TS 20787 [4]. To assess the sensitivity range of newly hatched (Instar I) *A. franciscana* nauplii, the organisms were exposed to SiO_2NWs at different concentrations of 0 (control), 1, 10, 50, 100, 150, and 200 mg L^{-1} for 96 h. For each treatment, three replicates were applied and the experiment was performed in 100 ml glass vessels containing 100 ml of the artificial seawater. This experiment was also carried out under the following conditions: light/dark, 12 h/12 h; salinity, 30 g L^{-1}; and water temperature, 29 °C. After 96-h exposure, the immobilized and dead nauplii were distinguished microscopically. Finally, EPA probit analysis software (Version 1.5) was used to calculate the effective concentrations (EC_s) of SiO_2NWs.

Results and Discussion

The hydrodynamic size and morphology of SiO_2NWs are depicted in Fig. 1. DLS analysis demonstrates that the hydrodynamic size, VMD, and SMD of SiO_2NWs were 1688.23 nm (X99), 1287.98 nm, and 1263.89 nm, respectively. Moreover, TEM morphograph displayed long rod-shaped SiO_2NWs with relatively uniform in size distribution.

The results of the immobilization rate of *A. franciscana* nauplii after 96-h exposure to different concentrations of SiO_2NWs are shown in Fig.2. After 48-h exposure duration, no immobilization was observed in the control and exposure groups. After 96-h exposure period, the immobilization rate was measured between 26.6-66.6 % at different concentrations. The control group showed 6.7 % immobilization rate, and the minimum and maximum immobilization rates were recorded at concentrations of 10 and 150 mg L^{-1} SiO_2NWs, respectively. The 96-h EC_{50} value of SiO_2NWs was found to be 122.167 mg L^{-1} (with 95% confidence limits of 65.93-296.64 mg L^{-1}). On the basis of the European Union legislation [5] and European Union Council Directive 67/548/EEC of 27 June 1967 [6], any substance with a 96-h EC_{50} value of more than 100 mg L^{-1} must be categorized as a non-toxic substance to aquatic organisms. Therefore, the findings of this study showed no toxicity of SiO_2NWs against *A. franciscana* nauplii. On the contrary, Nelson et al. (2010) demonstrated that LD_{50} of pressure-injected silica nanowires into the yolk of embryonic zebrafish was about 110 pg g^{-1} embryo; consequently, these authors interpreted that toxic doses of such nanowires were extremely low [2]. In reviewing the literature, much less information is available concerning harmful and toxic effects of SiO_2NWs. Therefore, further studies should be carried out to evaluate different aspects of SiO_2NWs toxicity and determine whether SiO_2NWs can be used as a safe nanocarrier or not.

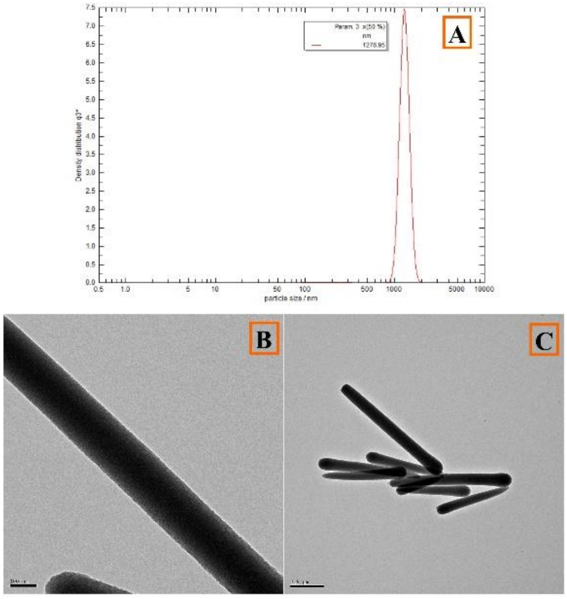

Fig. 1. The hydrodynamic size based on DLS (A) and morphology based on TEM (B and C) of silicon dioxide nanowires.

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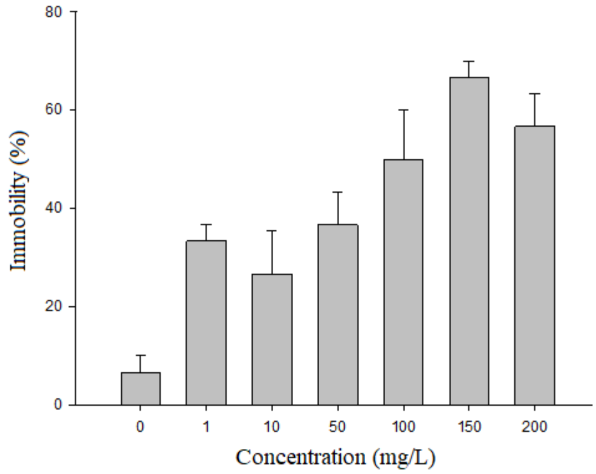

Fig. 2. Immobilization rates of *Artemia franciscana* nauplii after 96-h exposure to different concentrations of silicon dioxide nanowires

Conclusions

The findings of the current research add a growing body of literature concerning acute toxicity of SiO_2NWs in *A. franciscana*. That is, this study demonstrated that SiO_2NWs were not acutely toxic to *A. franciscana* with 96-h EC_{50} value of 122.167 mg L^{-1}. Moreover, the immobilization rate of *A. franciscana* nauplii increased following exposure to high concentrations of SiO_2NWs. In conclusion, the data suggest that SiO_2NWs can be applied as a safe nanocarrier, yet more studies need to be performed to be sure that our findings were not just aberrations.

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[6] EC. "Annex VI of Directive 1999/45/EC to consolidated version of directive 67/548/EEC. General classification and labelling requirements for dangerous substances and preparations."