DOSE DEPENDENT EFFECTS OF SILVER NANOPARTICLES ON REPRODUCTION AND DEVELOPMENT OF DIFFERENT BIOLOGICAL MODELS

EFFETS DOSE-DEPENDANTS DE NANOPARTICULES DE ARGENT SUR LA REPRODUCTION ET LE DEVELOPPEMENT DES DIFFERENTS MODELES BIOLOGIQUES

EFFETTI DOSE-DIPENDENTI DELLE NANOPARTICELLE DI ARGENTO SULLA RIPRODUZIONE E SULLO SVILUPPO DI DIVERSI MODELLI BIOLOGICI

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Abstract

The aim of this study was to investigate the possible impact of silver nanoparticles (Ag-NPs) on reproduction and development of different model organisms, such as sea urchins, barnacles, brine shrimps and zebrafish. *In vivo* toxicological tests were performed; the biological models were exposed to serial dilutions of commercial Ag-NPs. Our findings revealed that: i) all tests were suitable to verify the impact of Ag-NPs on models in term of exposure; ii) all tests can provide an idea of the role of NPs on environmental health; iii) an integrated interdisciplinary approach, using several model organisms, is recommended in order to have an overview of the effects of NPs on water environment.

Keywords: *nanosilver; nanoparticles; Paracentrotus lividus; Amphibalanus Amphitrite; Artemia salina; Danio rerio; toxicity.*

Résumé

Le but de cette étude était d'étudier le possible impact des nanoparticules d'argent (Ag-NP) sur la reproduction et le développement des différents modèles des organismes, tels que les oursins, les balanes, les crevettes de saumure et le poisson zèbre. Des tests toxicologiques ont été réalisées *in vivo*, les modèles biologiques ont été exposés à des dilutions successives de Ag- NP commerciale. Nos résultats ont révélé que: i) tous les tests ont permis de vérifier l'impact de l'Ag-IP sur des modèles en terme d'exposition, ii) tous les tests ont donné une idée du rôle des IP sur la santé de l'environnement; iii) une approche intégrée et interdisciplinaire, utilisant plusieurs des organismes modèles, est recommandé afin d'avoir un aperçu des effets des NP sur l'environnement aquatique.

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Mots-clés: *nanoparticules d'argent; les nanoparticules ; Paracentrotus lividus; Amphibalanus amphitrite; Artemia salina; Danio rerio; toxicité.*

Riassunto
Lo scopo di questo lavoro è stato valutare l'impatto delle nanoparticelle di argento (Ag-NPs) sulla riproduzione e sullo sviluppo di vari organismi modello, quali *Paracentrotus lividus*, *Amphibalanus amphitrite*, *Artemia salina* e *Danio rerio*. Sono stati eseguiti test tossicologici mediante esposizione *in vivo*; i modelli sono stati esposti a diluizioni seriali di Ag-NPs commerciali. I risultati hanno dimostrato che: i) i test utilizzati sono adatti a verificare l'impatto delle Ag-NPs sui modelli a seconda delle esposizioni; ii) l'impatto sugli organismi e, quindi sulla salute ambientale, è dose e tempo-dipendente; iii) l'approccio integrato tra diversi modelli permette una valutazione predittiva dell'impatto delle nanoparticelle sull'ambiente acquatico.
Parole chiave: *nano silver; nano particelle; Paracentrotus lividus; Amphibalanus amphitrite; Artemia salin;, Danio rerio; tossicità.*

Introduction

Silver nanoparticles (Ag-NPs) are among the most common NPs due to their antimicrobial potential (Asharani et al. 2009). The wide and intense use of Ag-NPs in cosmetics, therapeutic agents, textile and household products raised a public concern regarding their safety associated to possible human and environmental exposure (Matranga and Corsi 2012). No safety regulations are already delivered by organizations for standards (ISO, OECD, etc.) for the use and disposal at the end of the life cycle. The interactions of nanomaterials with cells, uptake mechanisms, distribution, excretion, toxicological endpoints and mechanism of action are controversial. No validated tests are up to date available to check either exposure and risk biomarkers on human and environmental health.
Aim of this study was to set up toxicity tests considering the REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances, EU law June 1st 2007) regulation to demonstrate a possible impact of Ag-NPs on reproduction and development of model organisms.

Materials and Methods

Gametes and early stages of the sea urchin *Paracentrotus lividus*, the barnacle *Amphibalanus amphitrite*, the brine shrimp *Artemia salina* and the zebrafish *Danio rerio* have been exposed to serial dilutions (from 0.0001 g l^{-1} up to 0.1 g l^{-1}) of commercial Ag-NPs (1-10 nm size; Polytech, Germany). Sea urchins and zebrafish are considered by JRC (ECVAM) as promising models, for their high sensitivity, high number of gametes and embryos in transparent envelopes. These models present totipotential stages, where environmental interference may alter the organization of body pattern. On the contrary, brine shrimps and barnacles

represent a rigid scheme of development (mosaic), based on cytoplasmic heterogeneity.
Spermiotoxicity test was performed on sea urchins. Sperms were exposed to Ag-NPs for one hour and used to fertilize eggs maintained in standard sea water. Fertilization and the development of early stages of sea urchins (gastrula and pluteus) were monitored.
Embryotoxicity test was also performed on the zebrafish model. The zygotes were exposed to several concentrations of Ag-NPs for 24 hours (acute test) and for one week (chronic test).
Acute toxicity tests on barnacle nauplii and brine shrimps Instar I larvae were performed for 24 and 48 hours. Mortality analysis was performed after 24 and 48 h of exposure to Ag-NPs and LC_{50} (median lethal concentration) was calculated.

Results

Exposure of these organisms caused different effects, and consequently different exposure bioassays were identified.
Regarding the sea urchin model, no effects were found on fertilization at all the NPs concentrations, but anomalies were identified during the embryonic development, from gastrula to pluteus stage with a dose-dependent severity. Effects on the organization of skeletal rods were observed on the plutei exposed to the lowest concentrations (Figure 1).

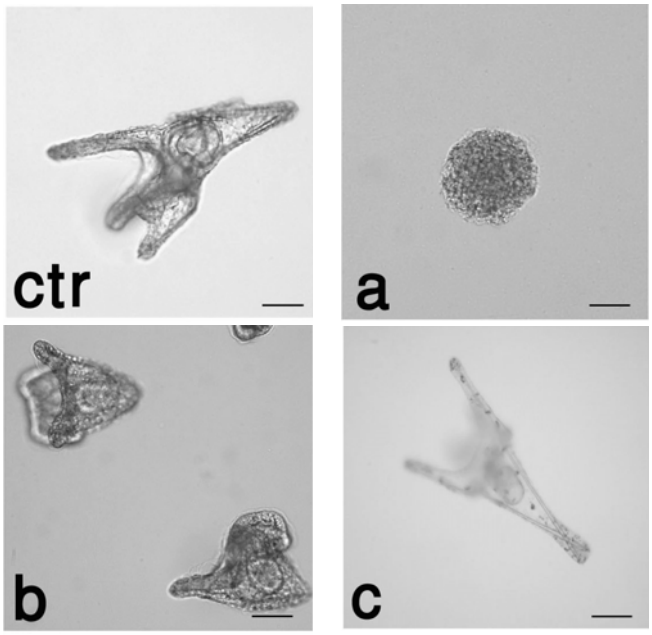

Figure 1

Sea urchin pluteus after Ag-NPs exposure.

a*) apoptosis, 0.1 g* l^{-1} *Ag-NPs;*

b*) anomalies during development, 0.001 g* l^{-1} *Ag-NPs;*

c*) anomalies in skeletal rods, 0.001 g* l^{-1} *Ag-NPs.*

ctr *= control. Bar 100 μm.*

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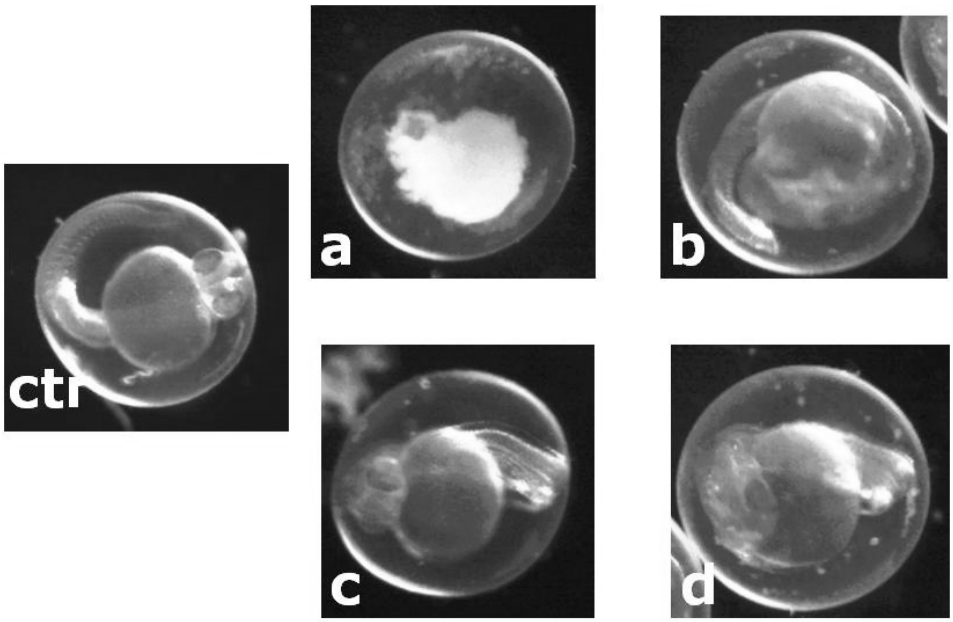
Embryotoxicity test on zebrafish revealed that this model was more sensitive than sea urchin. Acute effects, consisting of an incomplete development, were observed up to 0.001 g $l^{-1}$of Ag-NPs (Figure 2).

Figure 2 - *Zebrafish development after Ag-NPs exposure to 0.1 g l^{-1} (a), 0.01 g l^{-1} (b) 0.001 g l^{-1} (c), 0.0001 g l^{-1} (d) of Ag-NPs for 24 h. ctr = control.*



In addition, chronic exposures induced effects that caused 100% mortality at all dilutions. Toxicity tests on barnacle nauplii revealed 100% of mortality at the three tested dilutions (from 0.1 g l^{-1} up to 0.001 g l^{-1}) after 24 hours. Results on brine shrimps showed that this species was less sensitive to Ag-NPs (100% of mortality only at the highest dilution). Mortality increased after 48 h, but not significantly. LC_{50} values for both organisms at 24 h and 48 h are reported in Table 1.

Table 1 - *LC_{50} with 95% confidence limit (CL) in B. amphitrite and A. salina after 24h and 48h of Ag-NPs exposure.*

	B. amphitrite	***A. salina***
LC_{50} (mg l^{-1}), (CL 95%) – 24 h	0.00032 (n.c.)	0.005 (0.004-0.008)
LC_{50} (mg l^{-1}), (CL 95%) – 48 h	0.0003 (n.c.)	0.0073 (0.0047-0.011)

Discussion

The exposure of Ag-NPs to model organisms induced different damages in reproduction and development. These data are in according to those recently reported by Philbrook and colleagues (2011) on arthropods and mammals exposed to the same NPs.

Ag-NPs exert a negative impact on the normal development of sea urchin and zebrafish, characterized by morphological changes. Similar results have been reported for zebrafish exposed to Ag-NPs (Asharani et al. 2008) and for sea urchins exposed to other NPs (Falugi et al. 2012).

To date no toxicity test on barnacle and brine shrimps exposed to Ag-NPs have been reported. However, our results suggest that both crustaceans are sensitive to Ag-NPs, since acute effects have been observed.

In conclusion, the performed in-vivo toxicological tests are suitable to verify the impact of NPs on models in term of exposure and can give an idea of the role of NPs on environmental health. In addition, an integrated interdisciplinary approach, using several model organisms, is recommended.

Acknowledgments

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References

ASHARANI P.V., WU Y.L., GONG Z., VALIYAVEETTI S. (2008) Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19:255102 - doi:10.1088/0957-4484/19/25/255102

ASHARANI P.V., HANDE M.P., VALIVAVEETTIL S. (2009) Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 10:65.

FALUGI C., ALUIGI M.G., CHIANTORE M.C., PRIVITERA D., RAMOINO P., GATTI M.A., FABRIZI A., PINSINO A., MATRANGA V. (2012) Toxicity of metal oxide nanoparticles in immune cells of the sea urchin. Mar. Environ. Res. 76: 114-121.

MATRANGA V, CORSI I. (2012) TOXIC EFFECTS OF ENGINEERED NANOPARTICLES IN THE MARINE ENVIRONMENT: MODEL ORGANISMS AND MOLECULAR APPROACHES. Mar. Environ. Res. 76: 32-40.

PHILBROOK N.A., WINN L.M., NAIUL AFROOZ A.R.M., SALEH N.B., WALKER V.K. (2011). The effects of TiO_2 and Ag nanoparticles on reproduction and development of *Drosophila melanogaster* and CD-1 mice. Toxicol. Appl. Pharmacol. 257:

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