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Nanotoxicological Effects of SiO_2 Nanoparticles on *Spodoptera frugiperda* Sf9 Cells

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Abstract: The application of silica nanoparticles (NPs) in the biomedical field experienced a great development. The driving forces for these and future developments are the possibility to design NPs with homogeneous size and structure amenable to specific grafting. Moreover, it is possible to tune the characteristics of the NPs to meet the requirements of each specific cell and desired application. Herein, we analyzed the effect of silica NPs of various sizes and surface charge on the viability of *Spodoptera frugiperda* cells (Sf9 cell line) with the aim of extending the knowledge of possible toxicity of the NPs in the environment and development of new tools for insect control. Moreover, these results will also contribute to develop more effective systems for gene vectors delivery and recombinant proteins expression. Bare silica NPs of 14 nm, 380 nm and 1430 nm as well as amine-modified silica NPs of 131 nm and 448 nm were obtained by the Stöber method. The NPs were characterized by DLS and zeta potential measurements. The cell viability was assessed by the MTT test. It was observed that the 14 nm NPs possess the highest toxic effect. Indeed, after 24h, the viability of the cells exposed to the lower concentration of NPs (0.12 mg/ml) was about 40% of the value obtained for the control cells not exposed to NPs. Moreover, the exposure to other negative charged NPs also causes a lower activity when compared with the control. Alternatively, lower concentrations of positive charged NPs (i.e.: 0.12 or 0.6 mg/ml) demonstrated to stimulate the proliferation of the cells and higher concentrations (i.e.: 7.2 mg/ml) did not present significant differences with the control. In conclusion, we have demonstrated that the NPs possess an effect that is highly influenced by the size, charge and concentration. Although, silica NPs are being used in the biomedical field, these results contribute to further understanding the risk that could be associated to nanoparticles and how these can be modified in order to meet the requirements of each desired application.

Keywords: Nanoparticles, silica, *Spodoptera frugiperda*, nanotoxicology.

INTRODUCTION

Nowadays the widespread and sustained use of conventional chemical insecticides (i.e.: pyrethroids, organophosphorus) has led to the development of resistance in different species of insect vectors of human and veterinary diseases [1]. In addition, insecticide resistance is a very important issue in agricultural pests which produces high economic losses in crop production and stored products [2]. For these reasons the use of new alternative compounds with insecticidal activity is required.

The application of silica nanoparticles (NPs) in the biomedical field experienced a great development [3]. The driving forces for these and future developments are the possibility to design NPs with homogeneous size and structure amenable to specific grafting [4]. Moreover, it is possible to tune the characteristics of the NPs to meet the requirements of each specific cell and desired application [5]. In this sense, silica nanoparticles are being extensively employed as delivery systems for several molecules, including antibiotics, medicinal drugs and DNA among others [6-8]. Alternative uses of nanoparticles are bioremediation of contaminated environments, controlled release of fragrances, biocides, and antifungals on textiles [9]. In particular, the ability to synthesize highly uniform nanomaterials with different sizes, shapes and with various incorporated molecules has been successfully employed as insecticides. Indeed, silver and cobalt nanoparticles synthesized together with extracts from

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plants and fungi were effective as mosquito larvicide [10-13]. In addition, Stadler *et al*., reported the insecticide activity of nanostructured alumina over *Sitophilus oryzae* and *Rhyzopertha dominica*, which are major insect pests in stored food supplies throughout the world [14]. Moreover, studies with silicon nanoparticles on vectors of Malaria, Yellow Fever, Dengue and Chickungunya such as Aedes, Anopheles and Culex mosquitoes demonstrated its effectiveness as an insecticide to control these insects. However, further research is needed to identify their mode of action and non-target toxicity, in order to determine the potential of other nanostructured materials as pest control options for insects [15].

The order Lepidoptera (butterflies and moths) is the second largest order within the class Insecta. Indeed it is a group of insects which includes more than 100,000 described species. Many of them, are considered destructive plagues during their larval stage, affecting economically important crops such as soy and corn. *Spodoptera frugiperda*, *Spodoptera littoralis*, *Trichoplusia ni*, *Helicoverpa zea*, *Heliothis virescens* and *Rachiplusia nu* are some of the most abundant and widely distributed lepidopteran species in the world [16].

Herein, we analyzed the effect of silica NPs of various sizes and surface charge on the viability of *Spodoptera frugiperda* cells (Sf9 cell line) with the aim to extend the knowledge of their possible environmental toxicity and potentiality for the development of new tools for insect control. Moreover, these results would also contribute to develop more effective systems for gene vectors delivery and recombinant proteins expression.

MATERIALS AND METHODS

Nanoparticle Synthesis and Characterization

Different types of silica nanoparticles were synthesized. Solid silica nanoparticles were obtained by the Stöber method [17]. Briefly, tetraethyl orthosilicate (TEOS) was added to a stirred solution of ammonium hydroxyde (30 %) in a water/ethanol mixture to obtain silica nanoparticles. Solutions were stirred for 24 hours at room temperature and the resulting nanoparticles were recovered by centrifugation and washed with water until neutral pH was reached. Silica nanoparticle concentration was determined by weighting the residual mass of an aliquot dried at 80°C. Amino-modified silica nanoparticles were obtained using the Stöber method described above modified only by the simultaneously addition of aminopropyltriethoxysilane (APTES) and TEOS which were incorporated in a 1:4 ratio to the ammonium hydroxyde solution.

Particle size and zeta potential analysis was performed by the light diffraction method. The samples were suspended in filtered 10 mM KCl, sonicated for 30 seconds, and subsequently analyzed. Triplicate analyses were performed for each sample of nanoparticles.

Cell Culture and *in vitro* Toxicity Experiments

Sf9 cell (derivate from the Fall Armyworm; *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) suspension cultures (Invitrogen Life Technologies) were grown in sterile Erlenmeyer flasks under continuous shaking at 100 rpm in Sf900II medium supplemented with 1% (v/v) fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (Invitrogen Life Technologies) at 27 °C.

Sf9 cells were seeded in 24 cell culture well plates at a density of 1 x 10^6 cells. mL^{-1}. Sf900II medium supplemented with 1% (v/v) fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution was added to the cells along with different types and amounts of the nanoparticles under study. After 24hs incubation, mitochondrial redox activity was assessed via reduction of the MTT reagent as an indicator of cell viability. This colorimetric assay is based on the ability of the mitochondrial dehydrogenase enzymes of living cells to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) into an insoluble formazan. At the above mentioned culture time, 100 µl of MTT solution (5 $mg.mL^{-1}$ in PBS) was added to each sample and incubated for 4 h at 27ºC. Medium was withdrawn through aspiration and cultured cells were treated with 1 mL of ethanol 99.5 % for 15 minutes. The absorbance at 570 nm was measured using a UV-Visible spectrophotometer. In all cases, results are expressed as mean ± SD from triplicates experiments.

Statistical Analysis

Mortality data were processed with POLO Plus (LeOra Software 1987). Data were pooled and analyzed based on probit analysis to estimate the lethal dose (LD50) (milligrams of SiO_2 nanoparticles per milliliter) that killed 50% of treated *Spodoptera frugiperda* Sf9 cells [18-19].

RESULTS AND DISCUSSION

Silica NPs with various diameters were obtained in a one step synthesis by the Stöber method (Fig. **1**: Scheme). DLS studies performed in 10 mM KCl showed a good dispersion for all particles, independently of their size and surface. The mean particle diameters of the SiNPs obtained were 14 nm, 380 nm and 1430 nm, as well as 131 nm and 448 nm for amine modified nanoparticles. The zeta potential (ζ) of all the particles ranged between ca. -30 mV and -50 mV, sug-

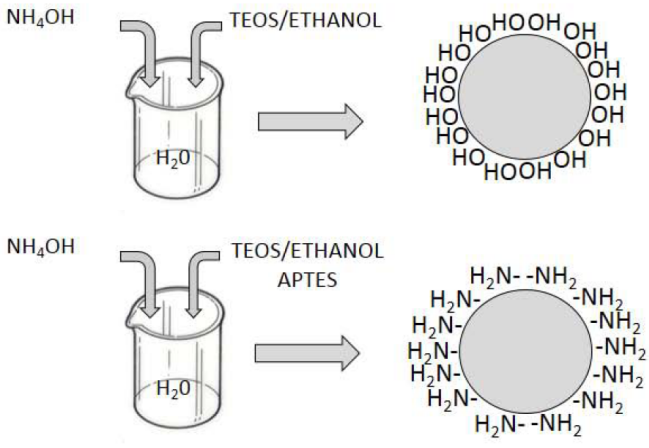

Fig. (1). Scheme of the synthesis of silica nanoparticles.

gesting that the colloidal stability is given via inter-particle electrostatic repulsion. The addition of APTES during the synthesis of the particles significantly modified their zeta potential. Indeed, for amine-bearing particles ζ value was +20 ± 5 mV independently of their size. These results confirm the successful incorporation of the amine moieties within the SiNPs (Table **1**).

The viability of the cells was assessed by the MTT test. It was observed that the 14 nm NPs possess the highest toxic effect. Indeed, after 24h the viability of the cells exposed to the lower concentration of NPs (0.12 $mg.mL^{-1}$) was about 40% of the value obtained for the control cells not exposed to NPs and there were 17% viable cells after an equal time exposure to 0.6 $mg.mL^{-1}$ of 14nm SiNPs. Moreover, the exposure to other SiNPs also causes a decrease in cell viability when compared to the control. In fact, the viability of the cells after exposure to 0.12 $mg.mL^{-1}$ SiNPs of 380 nm and 1430 nm was 45% and 71%, respectively. Overall, these data are in good agreement with the literature showing a decrease in cytotoxicity of silica nanoparticles with increasing particle size [20]. Alternatively, the lowest concentrations of amine functionalized SiNPs (i.e.: 0.12 or 0.6 $mg.mL^{-1}$) demonstrated to stimulate the proliferation of the cells and at the highest concentration evaluated (i.e.: 7. $mg.mL^{-1}$) the viability was 68% and 56% for the cells exposed to amine functionalized SiNPs of 131 nm and 448 nm, respectively (Fig. **2**). In addition, for intermediate SiNPs concentrations, the viability of the cells exposed to 448 nm SiNPs was higher than the ones exposed to 131 nm. Thus, in this case a decrease in cytotoxicity of amine functionalized SiNPs with increasing particle size was observed. As a general trend, the toxicity of the amine functionalized SiNPs was lower than the non functionalized ones. It is worth to mention that the viability of the cells exposed to 2.4 $mg.mL^{-1}$ of all the SiNPs under study was higher than 60% in most cases, except for the 14 nm SiNPs in close agreement with reported results obtained with other cell types, further confirming that the lethal concentration of SiNPs for this cells is over that value [21]. The toxicity of the nanoparticles against *Spodoptera frugiperda* Sf9 cells evaluated by the estimation of the lethal dose (LD_{50}) demonstrated that the LD_{50} decreases with the reduction in particle size. Indeed, the LD_{50} increased from 0.133 mg/ml to 4.709 mg/ml for the 14 nm and 1430 nm nanoparticles, respectively (Table **2**). Alternatively, modification of the nanoparticles with amine moieties significantly increased the LD_{50}, further confirming the lower toxic effect of these

Table 1. Size and zeta potential of silica particles.

Diameter (nm)	Zeta potential (mV)	Volume (μm^3) ($v = 4\pi r^3/3$)	Surface area (μm^2) ($A = 4\pi r^2$)
14 ± 3	-31 ± 6	1.4×10^{-6}	0.001
131 ± 6	+18 ± 3	1.18×10^{-3}	0.054
240 ± 8	-37 ± 5	7.24×10^{-3}	0.181
380 ± 6	-41 ± 6	28.73×10^{-3}	0.454
448 ± 8	+21 ± 4	47.08×10^{-3}	0.631
1430 ± 18	-41 ± 8	1.53	6.424

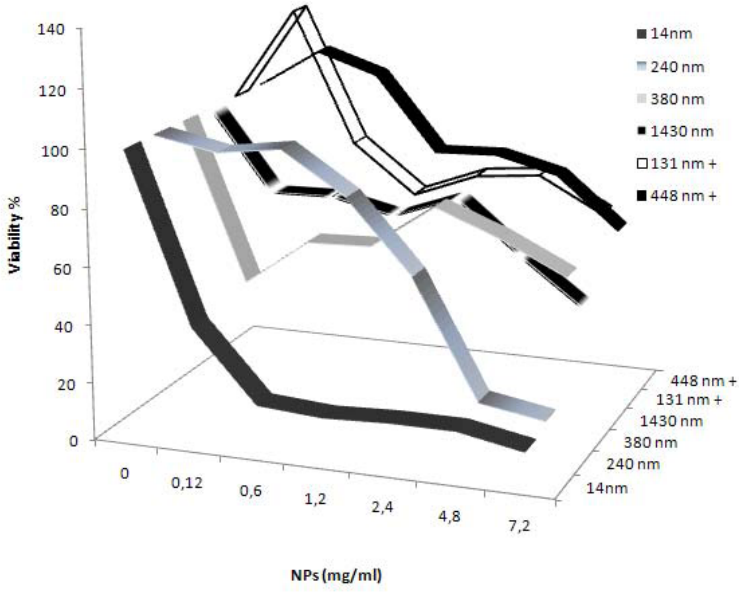

Fig. (2). Viability of Sf9 cells exposed to silica nanoparticles.

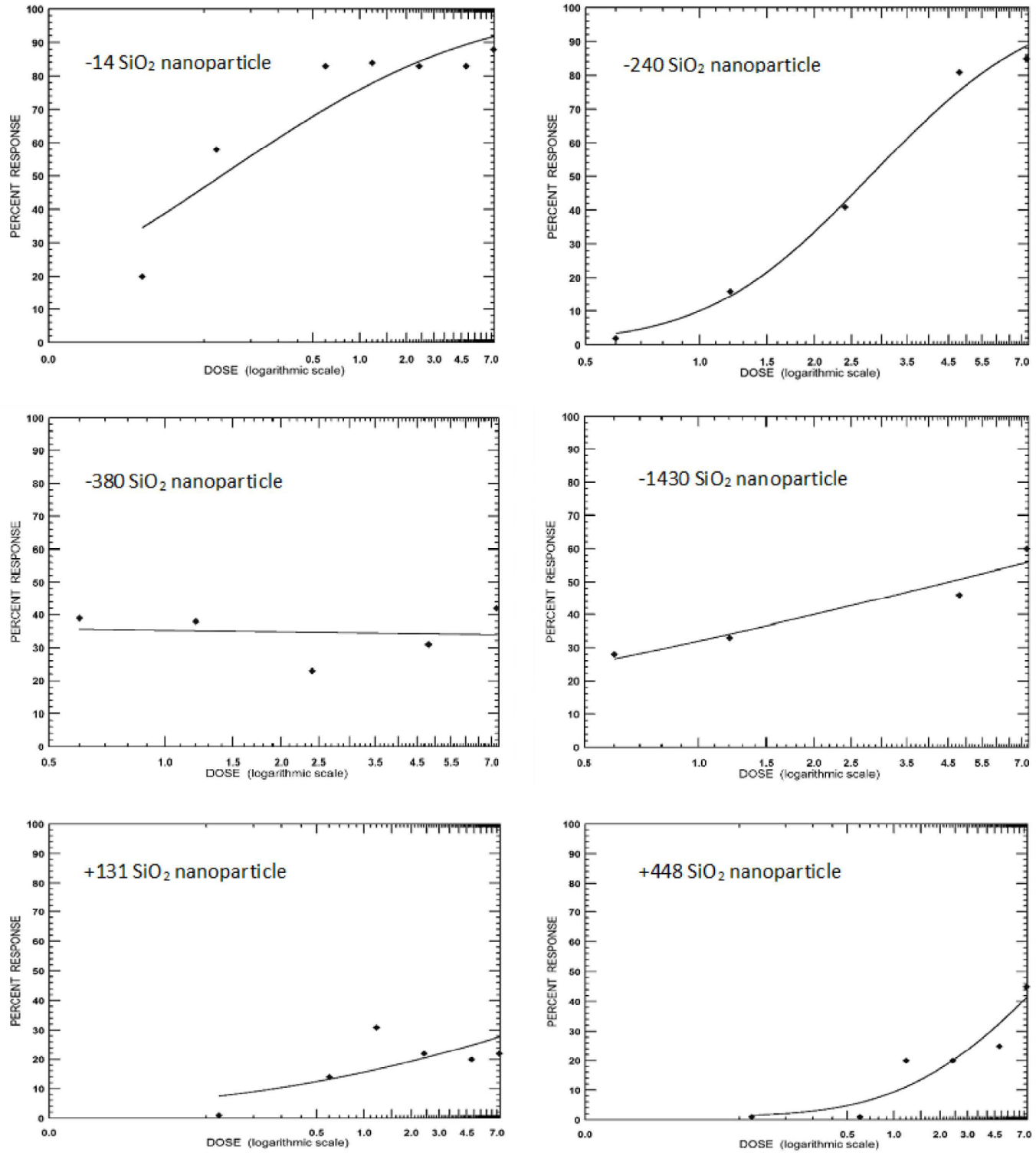

Fig. (3). Graphics show mortality to different sizes and charges SiO_2 nanoparticles of *Spodoptera frugiperda*cells (Sf9 cell line) vs. logarithm of applied doses (milligrams per mililiter).

nanoparticles (Fig. **3**). These results are in close agreement with reported works where amine-functionalization of silica NPs had no impact on cell viability of A549 cell cultures and resulted in significantly reduced inflammatory responses in the lungs of mice treated with these particles versus unmodified silica NPs [22]. In these sense, the literature strongly suggests that reactive oxygen species (ROS) may be responsible for the toxicological effect of nanoparticles [23]. In addition, the size of the nanoparticles is related to the surface, which will ultimately be in contact with the cells. The surface area of each particle is smaller for the particles with lower diameter (Table **1**). Although, considering that the

Table 2. Toxicity of of SiO_2 nanoparticles against *Spodoptera frugiperda* cells (Sf9 cell line).

Nanoparticles	Slope ± SE	$\chi 2$	df	LD_{50} (mg/ml) (95% CI)
SiOH 14 nm	0.798 ± 0.071	28.783	5	0.133 (0.013-0.365)
SiOH 240 nm	2.956 ± 0.228	3.7807	3	2.815 (2.079-3.773)
SiOH 380 nm	-	-	-	>7.2
SiOH 1430 nm	0.726 ± 0.151	1.776	2	4.709 (3.095-9.391)
$SiNH_2$ 131 nm	-	-	-	>7.2
$SiNH_2$ 448 nm	-	-	-	>7.2

volume of each particle is also smaller, the number of smaller particles would be higher than the number of bigger ones for a given mass of particles. Indeed, for particles of 240 nm and 380 nm the particle size increases by a factor of 1.6 which would result in reduction of particle number by 4 times in a unit volume, being this effect more marked for the 14 nm nanoparticles. Thus, smaller particles possess smaller surface area but since there would be more particles, the surface in contact with the cells would be higher. In this sense, it would be expected that a higher interaction will be established with smaller sized particles. Oberdörster *et al*., also reported that particles with greater specific surface areas per mass are more biologically active [24]. Indeed, silica nanoparticles exhibiting the highest specific surface area in contact with cells showed more toxic effects [25].

In conclusion, we have demonstrated that the NPs possess an effect that is highly influenced by the size, charge and concentration. Although, silica NPs are already being used in the biomedical field, these results contribute to further understand the risk that would be associated to nanoparticles and how they can be modified in order to meet the requirements of each desired application.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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