Toxicity of silver nanoparticles in biological systems: Does the complexity of biological systems matter?

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ABSTRACT

Currently, nanomaterials are more frequently in our daily life, specifically in biomedicine, electronics, food, textiles and catalysis just to name a few. Although nanomaterials provide many benefits, recently their toxicity profiles have begun to be explored. In this work, the toxic effects of silver nanoparticles (35 nm-average diameter and Polyvinyl-Pyrrolidone-coated) on biological systems of different levels of complexity was assessed in a comprehensive and comparatively way, through a variety of viability and toxicological assays. The studied organisms included viruses, bacteria, microalgae, fungi, animal and human cells (including cancer cell lines). It was found that biological systems of different taxonomical groups are inhibited at concentrations of silver nanoparticles within the same order of magnitude. Thus, the toxicity of nanomaterials on biological/living systems, constrained by their complexity, *e.g.* taxonomic groups, resulted contrary to the expected. The fact that cells and virus are inhibited with a concentration of silver nanoparticles within the same order of magnitude could be explained considering that silver nanoparticles affects very primitive cellular mechanisms by interacting with fundamental structures for cells and virus alike.

1. Introduction

Silver nanoparticles (AgNPs) are among the most studied nanomaterials due to their broad palette of applications. In the medical field, AgNPs display potent antiviral and antimicrobial activity (Dizaj et al., 2014; Franci et al., 2015; Huh and Kwon, 2011; Lara et al., 2011). They also exhibit anti-inflammatory properties (Nadworny et al., 2010) and may be used for the treatment of burns and wounds, among others (Klasen, 2000).

Although AgNPs provide many benefits, little is known about their overall toxicity. The few published studies of toxic effects of AgNPs on biological systems, *i.e.* viruses, bacteria or human cells, report different and even contradictory results. Doubtlessly, their non-specific inhibitory properties represent a hazard for the environment, particularly to microbial communities, when not properly disposed of (Holden et al., 2014).

The development of new strategies to study the toxicity of nanomaterials and comparative evaluation of their effects on different biological systems have become priority tasks. An important challenge lies in that nanomaterial toxicity is not easy to predict, because the properties of nanomaterials are linked to their structural characteristics. Indeed, although in bulk some materials are non-toxic, they might be in the nanoscale ("Nat Nano," 2011).

It is generally assumed that the toxicity of nanomaterials decreases as the complexity of biological systems rises (Franci et al., 2015; Krishnaraj et al., 2016; Kwak and An, 2016; Martinez-Gutierrez et al., 2010; Panáček et al., 2009; Rai et al., 2009; Seitz et al., 2015). Thus, the more complex the cell/organism is, the less sensitive it is thought to be to the toxic effects of AgNPs. However, some studies suggest there is no such a difference (Greulich et al., 2012; Matveeva et al., 2006; You et al., 2011). Still, comparative analysis of the toxic effects of a single silver nanoparticle on biological systems of different level of complexity are scarce.

In addition, the few available studies are not exhaustively comparative for several reasons: first, they contrast the effect of AgNPs on only two or three different biological groups (Ghosh et al., 2010; Panáček

et al., 2009); second, they use different culture media, culture conditions and methodology; third, different AgNPs are used, with different shapes, sizes, capping agent and so on. Due to the absence of accurate comparative analysis, the toxic effect of AgNPs on different taxa cannot be determined yet.

This work shows a comparative analysis of the toxic effects of a single specific nanomaterial on biological systems of different complexity levels. The evaluated nanomaterial consisted of Polyvinyl Pyrrolidone (PVP)-capped AgNPs. The studied organisms in this work included bacteria (Gram-positive and Gram-negative), protists, fungi, animal and human cancer cell lines, covering 4 of the 5 natural taxa, and additionally, we examined the toxic effects of PVP-capped AgNPs on viruses.

1.1. Background

While the number of publications in the nanotoxicology field has increased by 600% over the last decade, no standardized protocols for the systematic analysis of toxicology of nanomaterials have been published yet ("Nat Nano," 2012). AgNPs characterization, cell viability assays, culture conditions and toxicity assays are not standardized, and thus lead to differences in result interpretation. Besides, the effective concentrations corresponding to metallic silver content is often missing. Only few reports give a complete characterization of the AgNPs used, which must include their morphology and size distribution, stability and functionalizing agent, among others (Chen et al., 2013; Manno et al., 2013; Mehrbod et al., 2009). In this regard, recently we reported a complete characterization and evaluation of an Ag-based nanomaterial, including clinically relevant parameters such as the minimum bactericidal concentration (MBC), minimum inhibitory concentration (MIC), median lethal dose (LD50), bactericidal action time and the time of exposure for a given bacterial load, among other properties (Jaime-Acuña et al., 2016).

AgNPs can inactivate viruses of different type, such as HIV-1, monkey pox virus, hepatitis B, distemper and arenaviruses, among others (Bogdanchikova et al., 2016; Chen et al., 2013; Elechiguerra et al., 2005; Rogers et al., 2008; Speshock et al., 2010). It has been proposed that AgNPs inhibit HIV-1 virus binding to host cells by interacting with gp120 glycoprotein, thus blocking the viral entry (Elechiguerra et al., 2005). Other studies also showed that AgNPs inhibit at the early phases of Tacaribe virus replication (Speshock et al., 2010). AgNPs also inhibit influenza virus interfering with the fusion of the viral membrane, inhibiting viral penetration into the host cell (Mehrbod et al., 2009). On the other hand, AgNPs have also the capacity to kill cells with different levels of complexity, both prokaryotic and eukaryotic. Regarding bacteria, it has been suggested that toxicity depends upon both the molecular structure of the microbial cell wall and the bacterial metabolism, so the effects could be different in Gram-negative and Gram-positive bacteria (Li et al., 2010b; Shrivastava et al., 2007; Yoon et al., 2007). Protists microorganisms, both protozoa and microalgae, have been barely studied (Kvitek et al., 2009; Oukarroum et al., 2012). No report has been established about the comparative toxicity among protists. In the case of fungi, several works showed that AgNPs are able to inhibit both yeast cells and filamentous fungi, (Kim et al., 2012; Martinez-Gutierrez et al., 2010; Vazquez-Muñoz et al., 2014) but there are no reports that proposes a different toxicity among the different groups of fungi, despite their biological and structural differences (yeast-like vs hyphal shapes). Only a handful of recent nanotoxicological studies suggest that biological systems of different taxa are inhibited by similar concentrations of AgNPs (Greulich et al., 2012; You et al., 2011). In addition, AgNPs seem to be toxic for more complex biological systems, such as plants and animals (Ahn et al., 2014; Lee et al., 2012; Li et al., 2010a).

In this work, we present a systematic analysis of toxicity of AgNPs in biological systems from different taxonomical groups. Implications of such analysis are discussed.

2. Materials and methods

2.1. Virus, microorganisms, cells and culture conditions

For systematic analysis, the virus used was the attenuated MP12 strain of the Rift Valley Fever Virus (RVFV) (ssRNA, Bunyaviridae) from a virus stock grown at the BSL-3 labs of the Centro de Investigación en Sanidad Animal–National Research Institute for Agricultural and Food Technology (CISA-INIA), Valdeolmos, Spain (Lorenzo et al., 2010).

The bacterial strains used were *Escherichia coli* DH5α (Gram-negative) and a clinical isolate of *Staphylococcus aureus* (Gram-positive), obtained from the collection kept at the Centro de Nanociencias y Nanotecnología–Universidad Nacional Autónoma de México. Bacteria were grown in Muller-Hinton broth. For plates, 1.5% of agar was added.

Protist organism used was microalgae *Rhodomonas* sp., obtained from the Algae Collection of the Faculty of Marine Sciences of the Universidad Autónoma de Baja California. The algae were grown in F2 media.

Fungal strains were *Candida albicans* ATCC SC5614 (dimorphic, pathogenic yeast) and *Fusarium oxysporum*, Race III, (filamentous, phytopathogenic), obtained from Microbiology Department–Centro de Investigación Científica y Educación Superior de Ensenada. Fungi were grown in YPD broth. For plates, 1.5% of agar was added.

The mammalian cells were Murine Antigen-presenting Dendritic Cells derived from a bone-marrow culture of 6-week-old male BALB/c mice. Bone marrow extraction and dendritic cell differentiation with the use of granulocyte/macrophage colony-stimulating factor (GM-CSF) were conducted according to the Inaba protocol (Inaba et al., 1992). Vero cells (ATCC Cat. No. CCL-81) were obtained from cell stock at the CISA-INIA. The cell lines were grown in DMEM culture medium. Human cancer cells were HeLa and MDA-MB-231 cell lines, purchased from American Type Culture Collection (ATCC). The cell lines were cultured in RPMI or DMEM media.

2.2. AgNPs preparation and characterization

AgNPs were obtained from Vector Vita Ltd® (Novosibirsk, Russia). The total concentration of silver in AgNPs is 12 mg/ml, and they are stabilized with PVP. In a previous report, AgNPs were subjected to optical analysis, with a UV–vis spectrophotometer (Multiskan Go, Thermo Scientific); the scanned spectra ranged from 200 to 800 nm. The lyophilized samples were analyzed by FT-IR in a range from 4000 to 400 cm^{-1}, in a Nicolet 6700, Thermo Scientific FT-IR spectrometer. The morphology of the AgNPs was examined by Transmission Electron Microscopy in a TEM Jeol JEM 2100. Argoviís AgNPs mean hydrodynamic diameter and stability (zeta potential) were measured by DLS in a Zetasizer Nano NS (DTS 1060), in deionized water, at 25 °C (Juarez-Moreno et al., 2016). AgNPs dilutions were prepared in a range from 0.001 to 100 μg/ml of silver, in order to determine the lethal concentration of AgNPs, according to previous data (Juarez-Moreno et al., 2016; Vazquez-Muñoz et al., 2014).

2.3. Ag$^+$ ions release in different culture media

1-ml sample was collected from each culture media after 0 and 24 h of AgNPs addition and centrifuged at $13,000 \times g$ for 20 min. The supernatant solution, free of cells and particulate biomaterial, was then subjected to UV–vis spectroscopy and ICP analysis. UV–vis spectroscopy analysis was performed to evaluate the silver plasmonic activity of centrifuged and no-centrifuged media samples (AvaSpec ULS2048-UA-50 spectrophotometer). On the other hand, the total Ag content of the same samples was evaluated by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) using a Variant Liberty 110 Spectrometer.

2.4. Toxicological assays

For the virus infection assays, 3×10^1–3×10^2 plaque-forming units (PFU) of RVFV were incubated in DMEM for 1 h at 4 °C with serial dilutions of the AgNPs and an identical input of virus was pre-incubated in the same conditions with medium alone. After incubation, each mix of virus and AgNPs were inoculated onto Vero cells grown in MW6 plates. After 1 h of adsorption the inoculum was removed, the cells were washed and semi-solid medium with agar was added. Plates were incubated until infection plaques were clearly developed, then fixed and crystal-violet stained. Plaques were counted and the percentage of viral inhibition for each AgNPs dilution was calculated as follows:

$$\% \text{ Viral inhibition} = \left(\frac{\text{No. of plaques with medium alone} - \text{average No. of plaques in each treatment}}{\text{No. of plaques with medium alone}} \right) \times 100$$

For bacteria, the M09-A3 microdilution assays from the Clinical Laboratory Standard Institute (CLSI, 2012) was used, with some modifications. Bacteria were inoculated in Muller-Hinton broth. All bacterial strains were adjusted at 1×10^4 cells per milliliter and incubated at 180 rpm and 37 °C for 24 h. Bacterial cultures were exposed to different AgNPs concentrations. Then, plates were read in a UV–vis spectrophotometer (Multiskan Go, Thermo Scientific), and the percentage of inhibition was determined in comparison with bacterial culture without AgNPs exposure.

Microalgae initial inoculum was adjusted to an optical density (OD) of 0.065 of absorbance at $\lambda = 670$ nm in a Jenway 6505 UV–vis spectrophotometer. *Rhodomonas* sp. were cultured in F2 media, and exposed to different concentrations of AgNPs, from 1 to 100 µg/ml for 24 h, at room temperature and under continuous light conditions.

Yeast and filamentous fungi were prepared according to microdilution assays. Fungi were inoculated in YPD broth adjusted at 1×10^4 cells per milliliter and incubated with different concentrations of AgNPs, at 37 °C for 24 h. The plates were read in a UV–vis spectrophotometer, and then the percentage of inhibition was determined.

Murine bone marrow derived dendritic cells were grown in RPMI culture media, supplemented with 10% Fetal Bovine Serum (FBS), 1% of the Biowest Antibiotic-Antimycotic (streptomycin and penicillin G), at 37 °C with 5% CO_2 atmosphere. Cytotoxicity was evaluated with the dual fluorescein-diacetate (FDA)/ethidium bromide (Et-Br) test after 24 h of cell incubation with different AgNPs concentrations. The FDA/Et-Br staining can discriminate between live metabolically active cells and not viable cells. Vero cells were seeded in 96-multiwell plates with DMEM media, and 24 h later, the AgNPs were added to the medium. Cells were exposed to AgNP-containing medium for 24 h. Cells were incubated at 37 °C and checked daily with the microscope. Viability of Vero cells after the different treatments at the times indicated was evaluated by the MTS Cell Proliferation Assay (Promega). Viability (%) was calculated as follows:

$$\% \text{Viability}: \left(\frac{\text{OD 450 nm of treated cell wells}}{\text{OD 450 nm of control AgNPs} - \text{free cell wells}} \right) \times 100$$

HeLa and MDA-MB-231 cancer cell lines were cultured in RPMI-1640 or DMEM media supplemented with 10% FBS (BenchMark, Gemini Bio Products), 1% penicillin streptomycin (Sigma-Aldrich), 1% L-glutamine and 1.5 g/l sodium bicarbonate. Cells were maintained at 37 °C and 5% CO_2. Cell viability was assessed by the MTT method described by Mosmann (Mosmann, 1983). Briefly, cells were adjusted to an initial count of 10,000 cells per well, in a 96-well plate. Cells were exposed to different concentrations of AgNPs for 24 h at 37 °C, in an atmosphere of 5% CO_2, in RPMI-1640 or DMEM media. Afterwards, cells were rinsed with PBS and cell viability was assessed with the

toxicology assay kit TOX-1 (Sigma Aldrich) based on the reduction of MTT reagent. Dimethyl sulfoxide (DMSO) was used as cell viability control. Cell cultures in absence of AgNPs were used as a control. The background absorbance of cell viability test was performed by measuring the absorbance of multiwell plates at 690 nm and subtracted it from the absorbance values obtained at 570 nm, according to manufacturer instructions. Absorbance measurement at 570 and 60 nm with a plate reader (Multiskan Go, Thermo Scientific) allowed to compare cell viability controls with cells showing damage induced by different concentrations of AgNPs. All data obtained from AgNPs treated cells were normalized to data from three positive control wells with no treatment in three independent experiments with their internal triplicates.

2.5. Statistical analysis

The results were expressed as mean ± standard deviation of three independent experiments. Next, analysis of variance was performed, followed by Tukey's multiple comparison test. The results were considered statistically significant when $p < 0.05$.

3. Results

3.1. AgNPs characterization

AgNPs used in this work consist in spheroids of 35 ± 15 nm in diameter. UV–vis analysis shows a peak at 410 nm, which is typical for metallic silver nanoparticles. ATR FT-IR measurements of lyophilized PVP-AgNPs presented a profile similar to the PVP standard and the mean hydrodynamic diameter and zeta potential of the AgNPs, measured by DLS, are 78 nm and −15 mV respectively (Juarez-Moreno et al., 2016).

3.2. Toxicological assays

Vero cells infection assays was performed to evaluate whether RVFV viral infectivity was reduced by AgNPs. Little amounts of AgNPs reduce infectivity of RVFV. Plaque-forming units were reduced 82% with 6 µg/ml and by 98% at a concentration of 12 µg/ml of AgNPs (Fig. 1).

The minimum inhibitory concentration (MIC) for the bacterial strains *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) was 12 µg/ml of AgNPs. On the other hand, effective Lethal Concentration (EC_{100}) in the Rhodophyte microalgae *Rhodomonas* sp. was 4 µg/ml of AgNPs. In fungi, the MIC of the yeast *C. albicans* was 42 µg/ml of AgNPs, while for filamentous fungus *F. oxysporum*, was 20 µg/ml of AgNPs. Finally, the lethal dose in all animal cells and cancer cells evaluated, after 24 h, was found in the range of 10 to 12 µg/ml of AgNPs (Fig. 2). In summary, AgNPs exerted inhibitory effects in all biological models tested *in vitro*. Table 1 shows the Effective inhibitory concentrations of AgNPs in the biological systems evaluated.

EC (Effective concentration); MIC (Minimal Inhibitory Concentration); LD (Lethal dose). Values are the average of at least three determinations. The standard deviation did not exceed 5% in each case.

3.3. Effect of culture media on the release of silver ions

In order to evaluate whether culture media exerts an important role on the release of Ag^+ ions, either by facilitating or by limiting their release, we carried out the evaluation of total silver (AgNPs and Ag^+ ions) in the different culture media containing AgNPs; silver ions released were measured in supernatants of the same culture media subjected to centrifugation (Supplementary Fig. 1). ICP-AES analysis revealed differential release of Ag^+ ions on different culture media. Moreover, after 24 h, Ag^+ ions released in Muller-Hinton (MH) and RPMI media was reduced in comparison to Ag^+ ions initial release
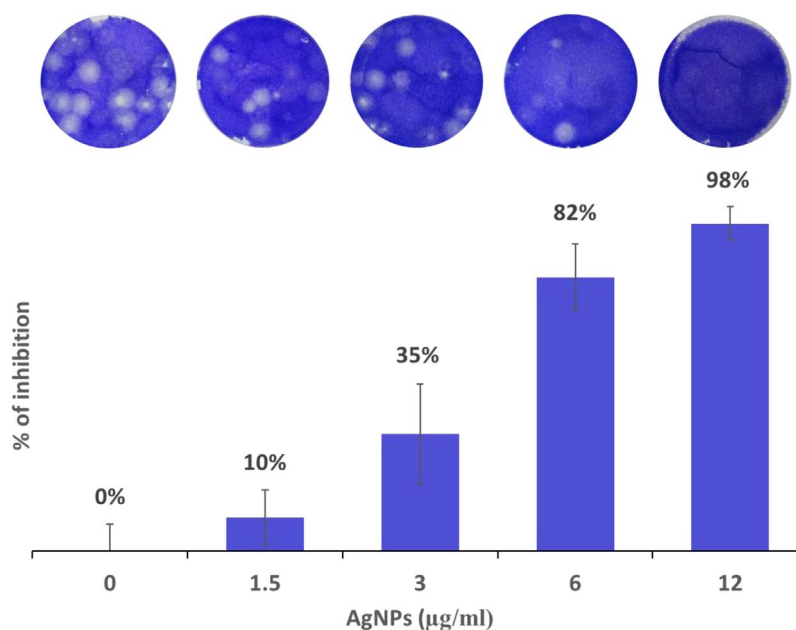
Fig. 1. Effect of the AgNPs on RVFV Vero cells infection. Plaque-forming inhibition plates (upper circles) with different AgNPs concentration treatments are shown. Percentage of inhibition of three different assays is presented (bars represent standard deviation. ** $p < 0.05$).

showed after AgNPs addition (Suppl. Fig. 1A). It is noteworthy that an increment of total Ag content was found on YPD media supernatant after 24 h of AgNPs addition. We hypothesized that this Ag increment could be ascribed to Ag^+ ions reduction forming AgNPs again. In that sense, UV–vis spectra analysis of YPD culture media supernatant revealed an absorbance peak around 400 nm corresponding to AgNPs plasmon activity (Suppl. Fig. 1C). RPMI and MH media UV–vis spectra showed absorbance peaks around 290 nm corresponding to Ag^+ ionic form (Bellantone et al., 2002).

3.4. AgNPs MIC's reported in the literature. A meta-analysis

In addition, we performed an exhaustive search in the literature regarding the AgNPs MIC of organisms from different taxonomic levels. Not all reports show the characterization data of the AgNPs used nor the effective concentration of silver. For this work, 20 reviews (published from year 2000 to 2016) and around 150 research papers (from 2005 to 2016) were analyzed. From the later, only 70 were considered for further analysis, because they presented enough information related to the characterization of the AgNPs, such as the total
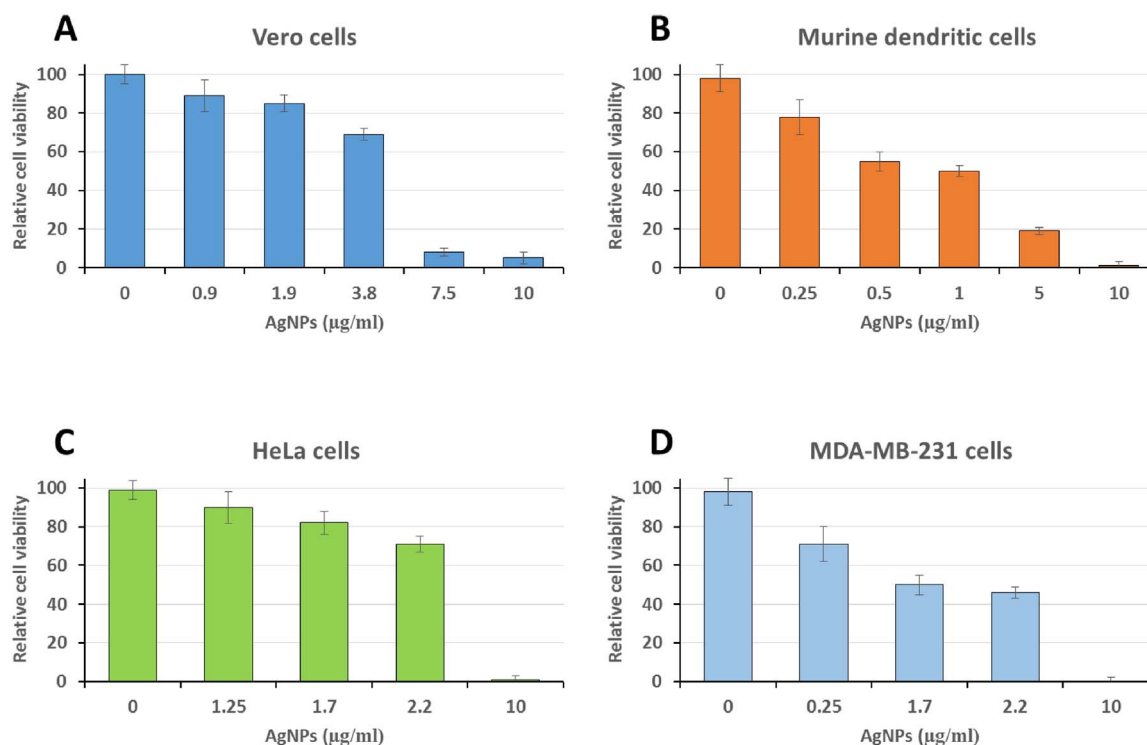

Fig. 2. Cell viability of animal and human cancer cell lines after 24-h treatment with AgNPs. Vero cells (A) and murine dendritic cells (B); HeLa cancer cell line (C) and the MDA-MB-231 cancer cell line (D). Relative cell viability of three different assays is presented (bars represent standard deviation. ** $p < 0.05$).

Table 1
Effective inhibitory concentrations of AgNPs in the biological systems.

Group	Organism/Cell line	AgNPs inhibitory concentration (μg/ml)
Virus	RVFV	$EC_{98} = 12$
Bacteria	*E. coli* (Gram negative)	$MIC = 12$
	S. aureus (Gram positive)	$MIC = 12$
Fungi	*C. albicans* (yeast)	$MIC = 45$
	F. oxysporum (filamentous)	$MIC = 20$
Protist	*Rhodomonas* sp. (microalgae)	$MIC = 4$
Animal cell lines	Vero	$LD_{90} = 7.5$
	Dendritic (murine)	$LD_{100} = 10$
Human cancer cell lines	HeLa	$LD_{100} = 10$
	MDA-MB-231	$LD_{100} = 10$

silver concentration used, and the lethal effective concentration (Table 2). The most common form of reported NPs is spheroid and the overall size distribution ranged from 1.1 to 230 nm, although most fell in the range of 10 to 30 nm. Some AgNPs were uncoated; among those reported to be functionalized, the most common capping agents were PVP, sodium citrate, and polyvinyl alcohol (PVA). AgNPs produced by "green synthesis" (synthesized used natural extracts as reducing agent) were also considered in the analysis (Fayaz et al., 2010; Gajbhiye et al., 2009; Noorbakhsh et al., 2011; Shahverdi et al., 2007).

4. Discussion

It is commonly accepted that the toxicity of nanomaterials could be related to the complexity of biological systems, particularly when comparing the toxicity between microorganisms and animal cells (Chen et al., 2013; Martinez-Gutierrez et al., 2010; Panáček et al., 2009). However, published results of the AgNP's MICs in different cells do not show such trend (Table 2). According to both the meta-analysis performed and our experimental data, silver nanoparticles exert an inhibitory effect in all biological systems tested, regardless of their structural or physiological characteristics, at the cellular level. Literature shows that AgNPs have the capacity to inhibit different types of virus infections, and affect both prokaryotic and eukaryotic cells (Table 2). In the present work, the same AgNPs were used to evaluate their effective lethal concentration in a comparative way, at a cellular level *in vitro*. Our results give new insights for a broader comparison of the toxicity of a single nanomaterial. In all cases, it was found that the inhibitory effect is similar for all biological systems tested; the inhibition range was around 10 μg/ml of silver for virus infections and most organisms, as shown in Table 1. However, toxicity may be affected by different parameters, such as nanomaterial and cell concentration, culture media and growth conditions. Culture media components such as peptone and/or dextrose can affect the release or availability of Ag^+ ions, as it was found in this comparative study of the Ag+ release to the RPMI, MH and YPD media (Suppl. Fig. 1). It is necessary to carry out detailed studies in this sense (*manuscript in preparation*).

Our results differ from what has been previously reported. For instance, only partial comparative analysis are available. The difference in the inhibitory concentrations reported seems to be different between organisms of the same taxonomical group (*i.e.* bacteria of different species), as well as in organisms of very different groups (bacteria vs human cells, for example) (Martinez-Gutierrez et al., 2010; Panáček et al., 2009). Only few works show data similar to ours, but by comparing a smaller set of organism groups (Greulich et al., 2012; You et al., 2011).

In the analysis of the published literature, we found that not all

studies present the characterization of the evaluated AgNPs, while some did not report the effective concentration of silver at all. In order to perform a better comparative analysis, it is important to present a comprehensive characterization of the nanomaterials, such as morphology, size distribution, surface chemistry, metallic silver content, and stabilizers, among others. On the other hand, we consider that alongside the differences between the AgNPs used in the different works, it is also important to analyze other criteria, such as the size of the initial inoculum of cells or the use of different culture media. Actually, when testing toxicity, the culture medium plays a role as important as the nanomaterial itself, because it can modify the inhibitory concentrations of the nanomaterial by up to 3 orders of magnitude (*manuscript in preparation*). It is also important to consider the cell division rate, which in turn impacts both the overall population growth and the interaction time of single cells with the nanomaterials.

4.1. AgNPs vs virus infection

The AgNPs have been tested in different families of viruses, with several structures, with or without envelope and with variations in their genetic information content (DNA or RNA, single or double stranded) (Elechiguerra et al., 2005; Rogers et al., 2008; Speshock et al., 2010; Trefry and Wooley, 2012; Xiang et al., 2011; You et al., 2011). For all of them, the effective viral infection inhibition concentrations reported are above 5 μg/ml of silver, usually ranging between the 10^1–10^2 μg/ml of silver. In most works, the viral infection inhibition concentration is around 10^1 μg/ml of silver.

4.2. AgNPs vs bacteria

It has been reported that AgNPs MICs may differ among bacterial species, especially between Gram-positive and Gram-negative bacteria (Li et al., 2010b; Shrivastava et al., 2007; Yoon et al., 2007). AgNPs MICs reported range from 10^{-1} to 10^2 μg/ml of silver. In most reported works, the MIC is in the same order of magnitude around 10^1 μg/ml of silver.

AgNPs vs protists. Several works show that microalgae may be slightly more susceptible to AgNPs than protozoa. Even so, the inhibitory concentration of silver is within one order of magnitude in both, algae and protozoa (Kvitek et al., 2009; Oukarroum et al., 2012; Taylor et al., 2016). Most of the reports show an effective concentration near 10^1 μg/ml of silver.

4.3. AgNPs vs fungi

AgNPs inhibit both yeast and filamentous fungi. The MIC reported varies between different reports by up to five orders of magnitude, from 10^{-2} to 10^2 μg/ml of silver. Most of them was found around 10^1 μg/ml of silver, for both yeast and filamentous fungi (De Souza et al., 2006; Gutarowska et al., 2012; Noorbakhsh et al., 2011; Vazquez-Muñoz et al., 2014).

4.4. AgNPs vs animal cells and cancer cell lines

In animal cells the reported toxicity greatly varies depending on the type of study *e.g. in vitro* with cell cultures or *in vivo*, with animal models. There is no significant difference between the lethality in full microscopic animals like nematodes (Ahn et al., 2014) and *Daphnia* (Blinova et al., 2013; Li et al., 2010a) *versus* mammalian cell cultures (Greulich et al., 2012; Martinez-Gutierrez et al., 2010; Panáček et al., 2009; Park et al., 2010) or cancer cell lines (Ghosh et al., 2010). The lethal concentrations range from 10^0 to 10^2 μg/ml of silver. In cell lines cultures most of the works report a LD_{100} close to 10^1 μg ml^{-1}.

Taking all, in studies that evaluate the effect of nanomaterials on cultures from viruses to human cells *in vitro*, the lethal concentrations values of AgNPs do not differ by more than two orders of magnitude in

Table 2
AgNPs morphology and their effective antimicrobial concentrations for virus and cells (including bacteria to human cancer cell lines).

Taxa	Morphology	Capping/stabilizer	Synthesis[1]	MIC [Log]	References
Virus	1.1–80 nm poly-shaped, spheroid	Carbon, PVP, BSA, polysaccharides, PVA or uncoated (none)	Chemical Plasma gas-synthesized.	10^0–10^2	Chen et al. (2013), Elechiguerra et al. (2005), Gaikwad et al. (2013), Lu et al. (2008), Rogers et al. (2008), Speshock et al. (2010), Trefry and Wooley, (2013, 2012), Xiang et al. (2011), You et al. (2011)
Bacteria	1.1–230 nm Cubical, Spheroid, Triangular, Irregular	Carbon matrix, sodium citrate, SDS, agar–agar matrix, PVP, PVA, PEG, HEC, Gemini surfactants, Chitosan or uncoated (none)	Chemical Biosynthesis UV photo-reduction, Laser-based reduction, Gamma-irradiation	10^{-1}–10^2	Agnihotri et al. (2014), Alizadeh et al. (2014), Ansari et al. (2011), Brandt et al. (2012), De Souza et al. (2006), Dimkpa et al. (2011), Du et al. (2012), Eugenio et al. (2016), Fayaz et al. (2010), Gao et al. (2013), Ghosh et al. (2010), Goswami et al. (2015), Greulich et al. (2012), Gutarowska et al. (2012), Guzman et al. (2012), Hanh et al. (2016), Hoskote Anand and Mandal, (2015), Jain et al. (2009), Khurana et al. (2013), Kora et al. (2009), Li et al. (2005), Li et al. (2010b), Lok et al. (2006), Martinez-Castanon et al. (2008), Martinez-Gutierrez et al. (2010), Martinez-Gutierrez et al. (2012), Mirzajani et al. (2011), Morones et al. (2005), Negm et al. (2014), Palanisamy et al. (2014), Radzig et al. (2013), Ramalingam et al. (2016), Ruparelia et al. (2008), Shahverdi et al. (2007), Shankar et al. (2014), Suchomel et al. (2015), Tamboli and Lee (2013), Vargas-Reus et al. (2012), You et al. (2011)
Protist	7.7–50 nm Spheroids,	PEG, PVP, SDS, alkane or uncoated (none)	Chemical	10^1–10^2	Allahverdiyev et al. (2011), Kvitek et al. (2009), Oukarroum et al. (2012), Taylor et al. (2016)
Fungi	5–100 nm Spheroids	PVP, PEG, SDS, agar–agar matrix, NH$_3$, PVA, HEC or uncoated (none)	Chemical Biosynthesis	10^{-2}–10^2	(Brandt et al. (2012), Chamakura et al. (2010), Choi et al. (2008), De Souza et al. (2006), Ghosh et al. (2010), Gopinath and Velusamy (2013), Gutarowska et al. (2012), Ishida et al. (2014), Kim et al. (2012), Kumar and Poornachandra, (2015), Martinez-Gutierrez et al. (2010), Monteiro et al. (2013, 2011), Noorbakhsh et al. (2011), Ogar et al. (2015), Pan & ček et al. (2009), Pulit-Prociak et al. (2014), Silva et al. (2014), Suchomel et al. (2015), Vazquez-Muñoz et al. (2014), Xia et al. (2014)
Animal cell lines	5–150 nm Spheroids	Sodium citrate, SDS, Polysaccharide, PVA or uncoated (none)	Chemical Biosynthesis	10^0–10^2	Ali et al. (2016), Allahverdiyev et al. (2011), Arora et al. (2008), Mittal et al. (2014), Park et al. (2010), Speshock et al. (2010), Xiang et al. (2011)
Human cell lines	1.12–90 nm poly-shaped, spheroid	Carbon, PVP, BSA, PEG, SDS, agar–agar matrix or uncoated (none)	Chemical	10^0–10^2	Chen et al. (2013), Elechiguerra et al. (2005), Ghosh et al. (2010), Greulich et al. (2012), Martinez-Gutierrez et al. (2010), Martinez-Gutierrez et al. (2012), Pan & ček et al. (2009)

PVP = polyvinyl pyrrolidone; BSA = Bovine serum albumin; PVA = polyvinyl alcohol; SDS = Sodium dodecyl sulfate; PEG = polyethylene glycol; HEC = Hydroxyethyl Cellulose; NH$_3$ = ammonia;
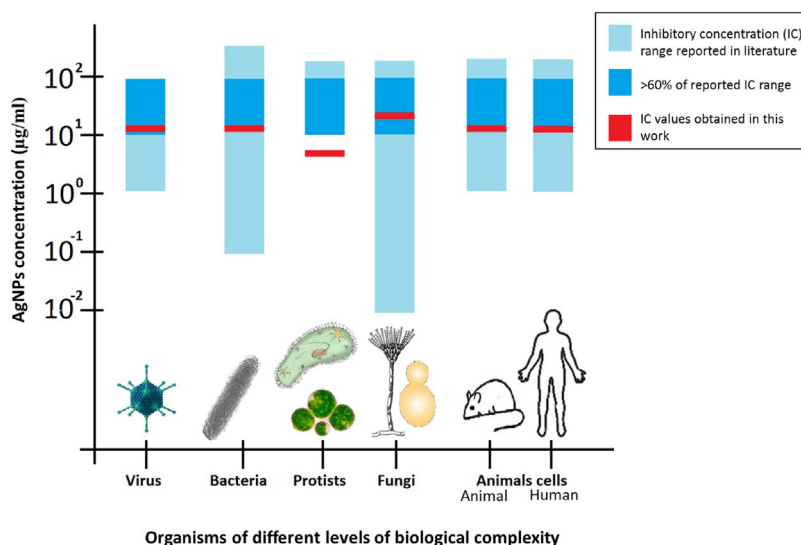
Fig. 3. Inhibition range (light blue bars) of the AgNPs inhibitory concentrations reported in different works, for organisms of different levels of biological complexity. Most studies (about 64%) report an effective inhibitory concentration of 10^1 μg/ml of AgNPs (dark blue bars). The other 36% falls in the range from 10^{-2} to 10^2 μg/ml. The red lines show the concentration range found in our study, using the same nanomaterial (PVP-AgNPs) in all the biological systems tested. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the range of 10^0–10^2 μg/ml of silver. Most studies report an inhibitory concentration range from 10^{-2} to 10^2 μg/ml of silver, and more than 90% inhibition at concentrations close to 10^1 μg/ml of silver, so most results are in the same order of magnitude (Fig. 3). It is important to note that AgNPs of different studies have different structural and chemical characteristics and properties, such as size, shape or stabilizer.

Our analysis shows that, at cellular level, the complexity of biological systems does not matter regarding AgNPs toxicity. In the case of viruses, it has been suggested either that AgNPs block the viral protein receptors which interfere with the attaching capability to the target cell (Elechiguerra et al., 2005) or display a direct effect on the viral particle altering its morphology (Borrego et al., 2016); in both cases, the AgNPs lessen the infective capacity of the viral particles. It was expected that AgNPs would exert a differentiated toxicological effect among cells of different taxa, due to the distinct complexity of the evaluated cells. The fact that cells and viruses were inhibited with a concentration of AgNPs within the same order of magnitude could be explained considering that AgNPs affect very primitive biological mechanisms, *i.e.* by interacting with fundamental structures of cells and viruses alike. It is known that AgNPs species affect cell wall, membrane proteins, membrane permeability, nucleic acids and enzymes in a non-specific way; concomitantly inducing the ROS formation (Chairuangkitti et al., 2012; Hwang et al., 2012; McShan et al., 2014; Oukarroum et al., 2012; Park et al., 2010). Also, all silver species exert some degree of toxicity toward the cells, therefore it was observed a toxic effect on all organisms (Chernousova and Epple, 2013; Foldbjerg et al., 2009; Liau et al., 1997; Zhao and Stevens, 1998).

One major implication of the generalized toxic effect of AgNPs is their potential use as a first-line nano-antibiotic, regardless of the nature of the pathogenic agent to combat, it could be inhibited. Thus, an infectious disease can be immediately treated while the causal agent (virus or microorganisms) is identified, in order to improve the treatment. Nevertheless, toxicity in unicellular organisms–*in vitro*-, and higher multicellular organisms–*in vivo*-, could be very different, due to the structural and physiological differences, such as specialized cellular tissues as the first line of defense (*e.g.* epithelial cells or mucosal tissue). Higher organisms (*i.e.* plants and animals) possess several mechanisms of defense, which allow them to withstand high concentrations of toxic compounds, including heavy metals, such as silver. However, further comparative analysis in this topic is required.

On the other hand, our literature meta-analysis shows that, despite

the differences in the wide spectra of silver nanoparticles reported, most of them have inhibitory properties in the same range of silver concentration. Then, the inhibitory properties may be due to the silver ions release, rather than to the nanoparticle characteristics *per se*. In this regard, in a previous work, we demonstrated that Ag ions release is sufficient to exert a bactericidal activity over *E. coli* cultures (Jaime-Acuña et al., 2016). Furthermore, we also found that the MIC of AgNPs and AgNO₃ were very similar over all organisms tested, pointed out Ag ions as major microbicide agent.

5. Conclusions

To the best of our knowledge, this is the first time that a single nanomaterial is tested in a wide spectra of biological systems of different levels of complexity, ranging from virus to human cell lines (including cancer cell lines). Our experimental analysis showed that viruses and cells of different biological complexity are inhibited *in vitro* at similar concentrations of silver within the order of magnitude near of 10^1 μg/ml of AgNPs.

Additionally, a comprehensive analysis of the literature was conducted to find the effective inhibitory concentration of AgNPs. Despite the differences between the different AgNPs, the lethal concentration in the majority of the studies for both viruses and cells, occurs within narrow concentration range around 10^1 μg/ml of AgNPs.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.toxlet.2017.05.007.

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