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Toxicity evaluation of nanocrystalline silver-impregnated coated dressing on the life cycle of worm *Caenorhabditis elegans*

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ABSTRACT

In recent times, however, due to the emergence of bacterial strains with resistance to conventional antibiotics, silver has again gained attention as an alternative for developing new efficient bactericides, including the use of silver nanoparticles (AgNPs). However, the improper disposal of these items after use may cause toxicological effects on organisms in the environment. To evaluate the potential environmental hazard of nanosilver-coated dressings, the nematode *Caenorhabditis elegans* was chosen as a test organism. The assays were conducted in 24-well plates that contain four different sizes of coated dressing to obtain different concentrations. L1 and L4 *C. elegans* larval stages were exposed to these nanosilver concentrations. Dressing cutouts were arranged between two layers of agar for 3 days and *Escherichia coli* (OP 50 strain) was added as food source for the worms. After the exposure period, growth, reproduction, fertility, silver concentration in the medium and the concentration of reactive oxygen species (ROS) in the worms were evaluated. Scanning and transmission electron microscopy analyses were performed on the coated dressings, as well as analyses of zeta potential, ionic release and antibacterial power in two bacterial strains (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). It was verified the antibacterial power of the coated dressing, in both bacteria strains tested. Characterization of the coated dressing indicated heterogeneous nanoparticles, as well as distinct zeta potentials for the medium in water and saline medium (0.9% NaCl). L1 larval worms exposed to nanosilver-coated dressing showed a high ROS concentration and reductions in growth, fertility and reproduction. Worms exposed to the coated dressing during the L4 stage showed almost no response. Overall, the obtained results indicate the potential environmental hazard of nanosilver-coated dressings.

1. Introduction

The antimicrobial properties of silver are well-known, indeed its use in wound disinfection has been documented since the 18th century (Klasen, 2000). Silver nitrate ($AgNO_3$) has been widely used in the treatment of ulcers but, with the discovery of penicillin, its application as bactericide has declined (Klasen, 2000; Chopra, 2007). In recent times, however, due to the emergence of bacterial strains with resistance to conventional antibiotics, silver has again gained attention as an alternative for developing new efficient bactericides (Chopra, 2007; Gurunathan et al., 2014). Based on the same evidence, the development of new nanotechnologies has led to an increase in the use of silver nanoparticles (AgNPs). There is a range of materials that use nanosilver as a raw material, including wound coated dressings, surgical instruments and many other goods and products (Bosetti et al., 2002; Cohen et al., 2007; Chen and Schluesener, 2008; Paladini and

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Pollini, 2019; Kalantari et al., 2020). The currently available coated dressings contain varying silver concentration and include products like Acticoat (Smith and Nephew) and Actisorb (Johnson and Johnson) (Silver et al., 2006). Silver, as a antibacterial, is used in various products; the AgNPs impregnate the surfaces of them, like coated dressing, leading to the release of silver ions (Ag^+). This release is associated with the antimicrobial efficacy (Wijnhoven et al., 2009).

Smith and Nephew manufactures the Acticoat Flex coated dressing. It markets ACTICOAT® FLEX 3 (registered in Brazil by national agency ANVISA through number 80804050025) and ACTICOAT® FLEX 7, with differences in the maximum silver concentration (1.64 mg/cm^2 and 2.0 mg/cm^2, respectively). ACTICOAT® FLEX 3 and ACTICOAT® FLEX 7 act as active antimicrobial barrier for at least 3 and 7 days, respectively (Smith & Nephew Medical Ltd.)

Like other nanoparticles, AgNPs exhibit toxicity related to the size/surface relationship, where the smaller the particle, the greater its toxicity. Smaller particles more easily cross cell membranes and, because of their high relative surface, interaction with organelles is easier (Liu et al., 2010; Wang et al., 2012; Gliga et al., 2014). The entry of nanoparticles into cells occurs either through the plasma membrane (diffusion or membrane channels) or endocytosis (AshaRani et al., 2009; Singh et al., 2009). Once inside, molecules accumulate outside the mitochondria, and promote reactive oxygen species (ROS) generation. AgNPs can also interact with organelles to cause oxidative damage to proteins, membranes, and DNA. Another possible mechanism involves the interaction of nanoparticles and silver ions in Ca^{2+} release in the cytoplasm. This release triggers cell signalling cascades that activate catabolic enzymes including phospholipases, proteases, and endonucleases, all of which damage the mitochondrial membranes and cytoskeleton that can culminate in programmed cell death (Orrenius et al., 1992, 2015).

The namatode *Caenorhabditis elegans* is widely applied in experimental studies due to its short life cycle, small size and easy cultivation. It is also as it is very sensitive to environmental changes (Hope, 1999; Leung et al., 2008; Rodriguez et al., 2013). The worm feeds on bacteria, especially living or dead *Escherichia coli* (Liu et al., 2012). This species was, in fact, one of the selected test organisms in the NanoReg initiative (NanoReg, 2016). Based on data from the scientific literature cited above, this study aimed to evaluate the potential toxicity of the ACTICOAT FLEX 3 coated dressing on the model organism *C. elegans*.

2. Materials and methods

2.1. Coated dressings acquisition

Acticoat Flex 3 coated dressings from Smith and Nephew were purchased via the internet. To perform the tests, there were used the coated dressings of lots 1715, 1647, 1616, and 1819.

2.2. Observation of silver crystals by scanning electron microscopy

Small cutouts of the coated dressing were dried by the Autosamdri-815 (Sample Drying at the critical point) and then visualized by a High-Low Low-Vacuum Scanning Electron Microscope (SEM), Jeol, JSM - 6610LV, with EDS probe to analyze the presence of silver on the surface of the coated dressing fibers. With the aid of the free image editing software ImageJ, the crystals were measured and obtained the frequency size distribution.

2.3. Observation of silver crystals by transmission electron microscopy

Pieces of the coated dressing were placed in 2 different solutions, one with MilliQ water and the other in 0.9% NaCl saline solution. After 3 days, drops of these solutions were transferred to carbon grids for electron microscopy for 10 min and then were placed on filter paper to dry for 24 h. The analyses were performed with a Jeol JEM-1400 120 keV Transmission Electron Microscope, coupled with an EDS probe.

2.4. Zeta potential measurement

Pieces of the coated dressing were inserted into deionised water and 0.9% saline over 3 d, and the solutions were sonicated at 10% of the total 400 W power of the Bonitech Branson sonicator and then analyzed on the equipment (Litesizer 500).

2.5. Ionic release of silver from nanoparticles

To perform the release of Ag^+ ions from silver dressing, four pieces (each one of 1 cm^2) were included in 15 mL of distilled water. An aliquot of 0.5 mL of media was replaced at each time (1, 2, 6, 12, 24, 36, 48, 60, 72 h). Aliquots were evaluated using a VGP 210 atomic absorption spectrophotometer (BuckScientific, East Norwalk, CT, USA) by electrothermal atomization using pyrolytic graphite furnace.

2.6. Silver concentration in the coated dressing

To obtain the total silver concentration contained in the coated dressings at cm^2 of the coated dressing, four pieces of coated dressing (1 cm^2) were mineralized using 9 mL of nitric acid (96%) and 1 mL of hydrogen peroxide (100%). The mixture was then heated using a micro digester (mls 1200 mega). The silver content was measured by atomic absorption spectroscopy as described above.

2.7. Silver in the NGM medium

The Nematode Growth Medium (NGM) that was used to wrap the coated dressing was removed after 3 days and placed in an eppendorf tube and stored in a freezer. After that, samples were melted at 90 °C and analyzed by atomic absorption spectroscopy, as described previously.

2.8. Antibacterial power of coated dressing

To evaluate the antimicrobial potential of Acticoat flex 3 against *Pseudomonas aeruginosa* (ATCC 15442) and *Staphylococcus aureus* (ATCC 12598), an agar diffusion-based method was used as follows: 15 ml of Mueller Hinton agar medium was added to a Petri dish (90 × 15 mm); after solidification of the medium, a MacFarland 0.5 bacterial suspension (1.5 × 10^8 UCF/mL) of each strain was prepared and plated onto the entire surface of the medium using a swab. Then a portion of the circular impregnated bandage (50 mm in diameter, 1.64 mg/cm^2) was placed centrally on the surface of the culture medium containing the seeded bacteria. The plates were incubated at 37 °C for 24 h and, after the diameter of the inhibition zone was measured. The experiment was performed in triplicate.

For the determination of the susceptibility profile of the evaluated strains, a second batch of experiments was carried out with antibiotics, where *P. aeruginosa* was exposed to Ciprofloxacin (5 μg), Ceftazidime (30 μg), Gentamicin (10 μg), Piperacycline-Tazobactam (100mcg-10mcg), and Meropenem (10 μg), and *S. aureus* to Ciprofloxacin (5 μg), Penicillin (10U), and Tetracycline (30 μg). The tests and the interpretation of the results were performed following the Performance Standards for Antimicrobial Susceptibility Testing protocol (CLSI M100, 2017).

2.9. Animal model and maintenance

Animals of strain N2, Bristol (wild strain) were cultured in Petri cell culture (medium and large) with nematode-appropriate NGM culture medium (NGM: nematode growth media) (3.0 g of NaCl/L; 5.0 g of peptone/L; 5.0 mg of cholesterol/L, 1 mmol of $CaCl_2$/L; 1 mmol of

$MgSO_4$/L; 25 mmol of KH_2PO_4/L; and 17 g of agar/L diluted in 1 L of autoclaved Milli Q water at pH 6.0 after preparation) and kept under temperature-controlled by incubator set at 20 °C. The plates were seeded with *Escherichia coli* bacteria, non-pathogenic strain OP50, with optical density 1.0 at 600 nm, as a food source for *C. elegans*, according to the traditional maintenance procedures described by Brenner (1974).

2.10. Preparation of animals for experiments

To develop the experiment, it was necessary to obtain a population of synchronized animals with the same larval stage. Ovate animals were removed by washing the culture plates using M9 buffer (3.0 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl and 1 mL 1 M $MgSO_4$ diluted in 1 L of sterilized Milli Q water, pH 6) using Pasteur pipettes and then accommodated in 50 mL Falcon tubes for centrifugation 1000*g* for 10 min, at 20 °C and pellet formation of animals and disposal of supernatant containing NGM and *E. coli* residues. Then, the bleaching procedure was performed using a caustic solution (NaOH 10 M and 2.5% sodium hypochlorite) in which the ovated animals are lysed, releasing their fertilized eggs. At the end of this procedure only the eggs remain alive, being separated by centrifugation (150 *g* for 15 min at 20 °C with a 30% sucrose solution). Finally, the eggs were transferred to Petri dishes containing M9 medium and incubated at 20 °C for 14 h until the animals hatched and reached their first stage of larval development (L1). The entire synchronization procedure follows the protocol proposed by Stiernagle (2006), which was originally formulated to remove fungal and bacterial contamination from *C. elegans* cultures.

2.11. Plate preparation

All the experiments were performed in triplicate using true independent replicates. To assemble the plates, the coated dressings were cut in circles with a 1 cm-diameter and placed in a 24-well plate. To obtain different coated dressing concentrations, it was assumed that the silver concentration was uniform over the entire coated dressing area. The following silver concentrations were selected: 1.64 mg/cm^2 (whole puddle equivalent = 1/1 or 100%), 1.23 mg/cm^2 (three-quarter puddle equivalent = 3/4 or 75%), 0.82 mg/cm^2 (half-puddle equivalent = 1/2 or 50%), and 0.41 mg/cm^2 (quarter-puddle equivalent = 1/4 or 25%) and CTRL for control group with animals exposed only to NGM and *E. coli* OP50 (Figs. 1 and 2 of Supplementary Material).

CTRL for animals exposed to coated dressings that were released for 6 days, 3 days releasing on the agar, after the agar is removed and the coated dressing washed with autoclaved Milli Q water, after they were put back in the agar for 3 more days. The mounting of the exposure plate was performed in a sterile environment by applying a first NGM film (500 μL). Afterward, the coated dressing cutouts were introduced into each one of the 24-well plates and covered with another layer of NGM (500 μL), thus forming two films around the coated dressing (Fig. 1 of Supplementary Material). Then the plate was incubated at 37 °C, simulating human body temperature for 3 days, another group of plates was incubated at 20 °C, also for 3 days. This time was selected as this is indicated by the manufacturer for material release, with the bottom of the plate facing up so that the silver released by the coated dressing could be available to the animals on the surface of the NGM during the exposure period. It was observed a darker coloration in the NGM that was in contact with the cuttings that had a larger size (higher concentrations). At the end of the third day of incubation, the exposure plates were removed from the 37 °C incubator and cooled to 20 °C (exposure temperature). Immediately after, 50 μL of *E. coli* OP50 was pipetted as a food source. Following, *C. elegans* in larval stage L1 (an average of 20 animals per puddle) were introduced into the puddles.

The average number of animals introduced into each pool was reached by estimating 10 aliquots with a volume of 10 μL of a *C. elegans* suspension in the liquid medium, which was performed by counting. The total number of animals among all aliquots was divided by the estimated aliquots to determine the volume to be pipetted into each pool at the time of exposure (Solis and Petrascheck, 2011).

After incubation, the plates were kept in an incubator at 20 °C for 96 h, the necessary time for the animals to complete their developmental cycle through the larval stages L1, L2, L3, and L4 and reach the adult stage, so they are able to grow and reproduce, giving rise to the first progeny (Hope, 1999). Then, their evaluation of growth, fertility rate, and reproduction was performed.

In the case of animal exposure in L4, the plate assembly procedures were repeated, with incubation modifications, where it occurred only at 20 °C (both during the dressing release period and during the 3-day exposure), using only a pool with a nominal concentration of 1.64 mg/cm^2 (100%).

All the analyses performed in this work were based on the international standard ISO 10872: 2010 adopted as reference for the toxicity tests evaluated by NANoREG. For the analysis, photos were taken of the puddles, using a magnifying glass (80×) coupled with a camera (Leica, model S8 APO). All analyses were performed with the help of free image editing software ImageJ. For better visualization of the animals in the evaluation of growth, reproduction, and fertility, the photo acquisition procedures were performed after staining of the animals with Rose Bengal dye.

2.12. Growth assessment

After taking the pictures of the adult animals stained with Rose Bengal, the subjects were measured with the ImageJ software. The growth rate (in mm) was measured by subtracting adult animal size from L1 larval stage animal size.

2.13. Fertility assessment

In order to calculate the fertility percentage, the presence or absence of eggs within the body of adult animals was observed individually. An animal was considered pregnant (fertile) if the number of eggs within its body is ≥ 1. After counting the number of pregnant hermaphrodite worms and subtracting the number of male worms from the total adult animals, the fertility percentage was calculated by dividing the number of fertile animals by the number of adult animals and multiplying by 100.

2.14. Reproduction assessment

To proceed with the reproduction calculation, the number of L1 larval stage animals counted in each pool was divided by the number of adult fertile animals in the respective pool. Results were expressed as the number of larvae per exposed adult.

2.15. Reactive oxygen species concentration (ROS) dosage

It was performed via fluorescence microscopy using the whole worm exposed to the probe H_2DCF-DA (SIGMA), according to Büchter et al. (2013). After 1 h-incubation, photos were taken with the Dino-Lite microscope (AM4115T-GFBW) at 200× of magnification, using 480 and 510 nm for excitation and emission, respectively. Subsequently, using the ImageJ software, the area of the animals was measured, together with fluorescence intensity and, around the animals, the background was also measured and the following expression calculated: ROS = (FI-BG)/A, where FI stands for worm fluorescence intensity, BG for background and A for the animal area.

2.16. Statistics

Data were expressed as mean ± 1 standard error of the mean (SEM). L1 worm growth, reproduction, fertility, and ROS concentration data were analyzed by a mixed model analysis of variance (ANOVA),

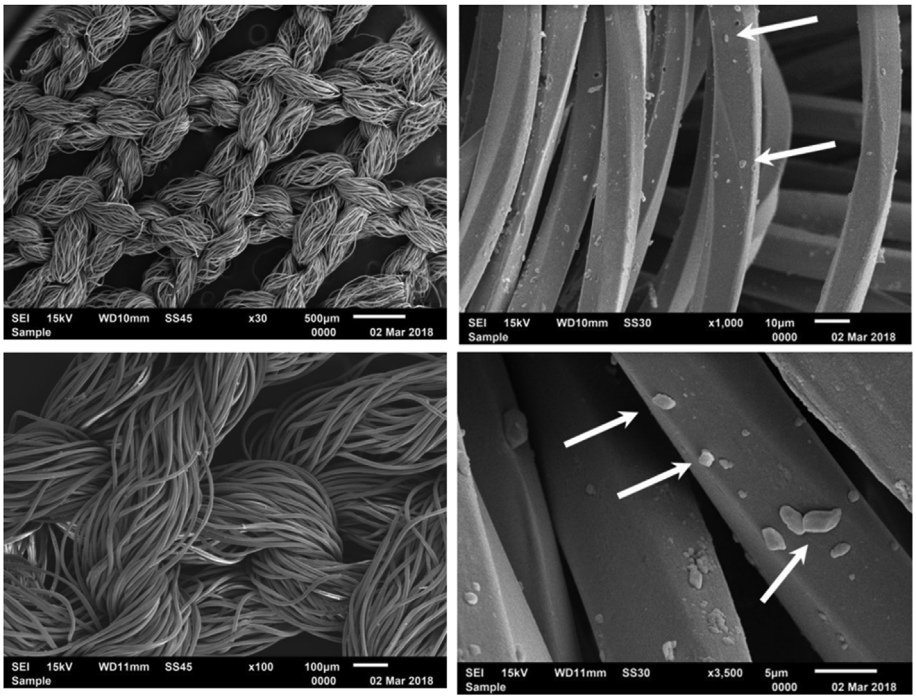

Fig. 1. Scanning electron microscopy (SEM) images of the nanosilver-coated dressing. White arrows indicate the silver particles impregnated in the coated dressing fibers.

being the random factor, the different plates employed in the independent experiments and fixed the factor was nanosilver concentration (Searle et al., 2006). Previously, normality and homoscedasticity assumptions were evaluated through Shapiro-Wilks and Levene tests, respectively, and mathematical transformations applied if at least one of these was violated (Zar, 1984). The same procedure was employed to analyze L4 worms data. Statistical differences among means treatment were performed using the post-hoc test of Newman-Keuls. In all cases, a significance level (α) of 0.05 was adopted.

3. Results and discussion

3.1. Physico-chemical results

3.1.1. Characterization of silver-coated dressing

Through SEM analysis, it was possible to observe the structure of the dressing with the entangled polyester fibers (Fig. 1a and b) and particles randomly dispersed and impregnated in the dressing fibers (Fig. 1c and d). To perform a size distribution analysis, silver nanoparticles from the coated dressing samples were analyzed through TEM, after immersion in saline solution (0.9% NaCl; Fig. 2a and b) or MilliQ water (Fig. 2c and d). From the EDS analysis performed on the ACTICOAT FLEX 3 coated dressing, it was possible to detect the following compounds: silver (75.4%), oxygen (18.5%) and carbon (5.78%) (Fig. 3 of Supplementary Material).

The coated dressings immersed in the 0.9% saline solution showed greater agglomeration than the ones immersed in Mili Q water. Measurements performed with ImageJ software demonstrated that the particles immersed in the 0.9% saline solution had a larger size (mean of *c.a.* 80 nm) than the particles that were immersed in MilliQ water (mean of *c.a.* 40 nm), which were much less crowded (Figs. 3 and 4). Reports including Doty et al. (2005) and Michaels et al. (2000) revealed that high NaCl concentrations cause AgNPs to aggregate. The zeta potential of the nanoparticles immersed in 0.9% saline solution averaged −17.8 mV, whereas those immersed in deionised water averaged −25 mV. Some guidelines show that zeta potential values between ± 20–30 mV are moderately stable (Patel and Agrawal, 2011; Bhattacharjee, 2016). Thus, the obtained results indicated that the nanoparticles released by the coated dressing in saline solution have a tendency to be less stable than those in deionised water. Interestingly, the manufacturer's use instructions state that the coated dressing must be moistened in potable water and not in saline, an advisory that once again corroborates our results.

AgNP toxicity towards bacteria was reported in several studies (Lok et al., 2006; Choi et al., 2008). Pal et al. (2007) evaluated the effects of bactericidal activity in different exposure media. The authors observed that exposure in agar presents greater toxicity compared to liquid medium. The use of silver is advantageous when compared to other classes of antibiotics because microorganisms, in general, do not show resistance to this compound (Romero-Urbina et al., 2015; Sangappa and Thiagarajan, 2015). Baker et al. (2005) and Panácek et al. (2006) presented data on the antimicrobial capacity of silver against *E. coli* and *Staphylococcus aureus*. Baker et al. (2005) found that particles with a higher surface-to-volume ratio provide a more efficient means for antibacterial activity against *E. coli*, and Panácek et al. (2006) found similar results, where 25 nm sized-AgNPs show greater antimicrobial and bactericidal activities compared to larger particles including against highly multidrug-resistant strains such as methicillin-resistant *S. aureus*. Mohan et al. (2014) also reported a high antimicrobial activity of AgNPs against *Pseudomonas aeruginosa*. The AgNP antibacterial capacity was also observed in the present work, is that there is evidence that silver diffuses in the medium and that there is an antimicrobial action against these two microorganisms that we evaluated, because there is a halo of inhibition (Tables 1 and 2 and Figs. 4–6 of Supplementary Material).

3.1.2. Ionic release of silver

The total silver content of the dressing was 1.44 mg/cm^2. The coated dressing analyzed in this study presented a controlled silver release over time. Indeed, 34% of the total silver content of the dressing was released after 72 h (Fig. 4). It is worth to mention that the release of the bactericide agent is required to achieve antimicrobial activity in wound dressings (Mebert et al., 2016). AgNP have the ability to release silver ions, and this property contributes to the bactericidal effect

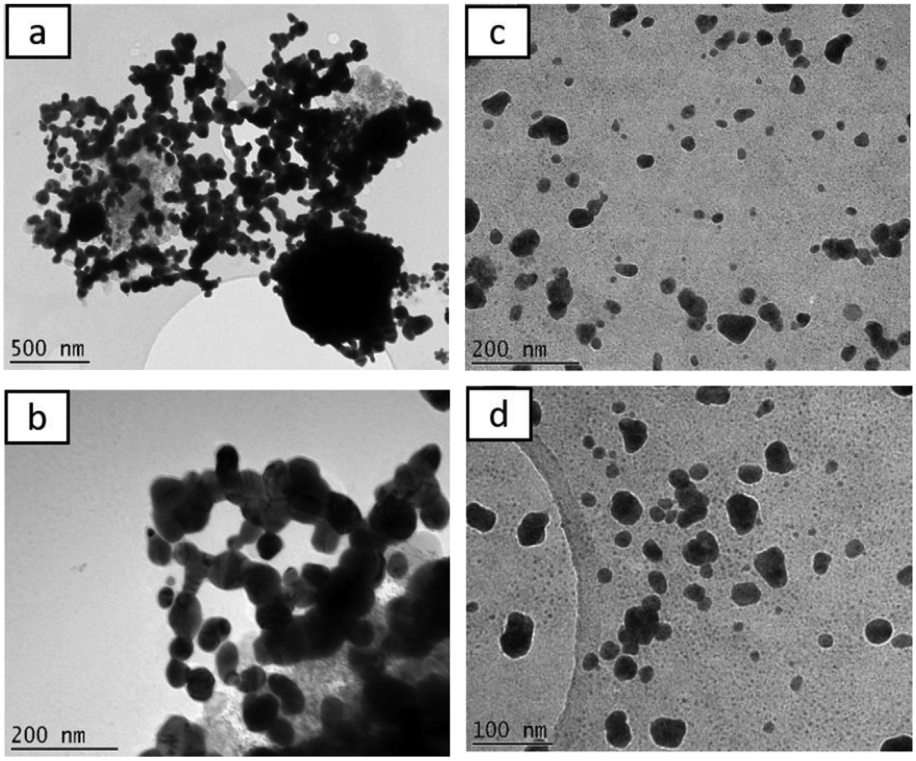

Fig. 2. Transmission electron microscopy (TEM) images of the nanosilver-coated dressing immersed in saline solution (0.9% NaCl; a and b) or MilliQ water (c and d).

already provided by silver (Morones et al., 2005). In recent work using *C. elegans*, it was found that also the shape of AgNP (nanoparticles, nanowires and nanoplates) influences toxicity (Moon et al., 2019).

3.2. Toxicity evaluation in C. elegans

3.2.1. Reactive oxygen species concentration (ROS) dosage

The Fenton reation, ion release and ROS generation occur on the surface of nanoparticles, thus very small molecules (< 30 nm) have a relatively large surface area and then, also have higher toxic effects than the larger ones (Auffan et al., 2009). With a highly variable particle distribution, as seen in Figs. 2 and 3, it can be inferred that at a concentration of 100% there should provide enough small nanoparticles to induce higher ROS concentration in worms, as shown in Fig. 5. The obtained results agree with Yang et al. (2018), who found high ROS concentration in *C. elegans* exposed to commercial AgNPs and explained that oxidative stress is the main toxic mechanism generated by these particles. The augmented ROS concentrations could affect the metabolism of energy in *C. elegans*, since oxidative chemicals as in this case results to be the coated dressing, generate mitochondrial malfunction, and consequently, the oxidative stress in the cytoplasm may increase inducing cell apoptosis (Luo et al., 2017).

3.2.2. Physiologic parameters

Kim et al. (2017) evaluated the effect of AgNPs on *C. elegans* growth and reported that concentrations of 0.01 mg/L already induces significant differences, reducing size. Data from Contreras et al. (2014) showed that concentrations between 1 and 10 mg AgNPs/L significantly reduce in *C. elegans* growth, however, after continuous exposure through several generations, growth return to normal. Then, a pattern of acclimatisation was evidence when worms are exposed to 100 mg AgNP/L. The *C. elegans* fertility is also affected by AgNPs, where smaller particles induce the greatest toxicity effects. Roh et al. (2009) provided data where concentrations of 0.1 mg AgNPs/L impairs *C. elegans* reproduction, and reduces the number of offspring by up to 70% after a 24 h-exposure. In this study, we verified that all evaluated physiological parameters were compromised when the organisms were exposed to the coated dressing (Fig. 5). The data from in this study corroborate those already found in the literature, where exposure to silver nanoparticles also reduced the size of adults and reduced fertility and reproduction of worm *C. elegans*.

Stage L4 was less prone to show toxic effects after nanosilver-coated dressing exposure than the L1 (Table 1). The obtained results may indicate that stage L4 is more resistant than the initial L1 larval stage. Chaweeborisuit et al. (2016) exposed all *C. elegans* larval stages to plumbagin (a nematicide). These authors reported that L4 is least sensitive, while L1 shows the highest sensitivity. Chu and Chow (2002) also observed that L1 animals are more susceptible when exposed to different cadmium concentrations than animals at more advanced stages. Overall, the use of the L1 stage offers greater sensitivity to observe potential deleterious effects of chemical stressors. *C. elegans* has a cuticle that is a layered internal structure with surface specializations and is known to undergo changes in its composition and structure during the life stages of the worm (Kramer, 1997). Probably the permeability in L4 is lower due to its thicker cuticle (Chaweeborisuit et al., 2016).

As previously mentioned, to obtain different exposure concentrations, the dressings were cut into different sizes. The data showed that there was high variability among same-sized dressings with regards to the silver concentrations released into the NGM, this can be easily observed by the high standard deviation of the samples (Fig. 7 of Supplementary Material), especially in the 100% cut (1.64 mg silver/cm^2). This finding could indicate that the silver is not homogenously distributed in the coated dressing.

The dressings that released their contents for 3 days in the NGM, were washed and added to a new NGM medium, and they still released silver, as verified by atomic absorption spectroscopy (211.33 ± 33.64 μg/g). However, no toxic effects were observed in worms exposed to these dressing (See Table 3 of Supplementary Material).

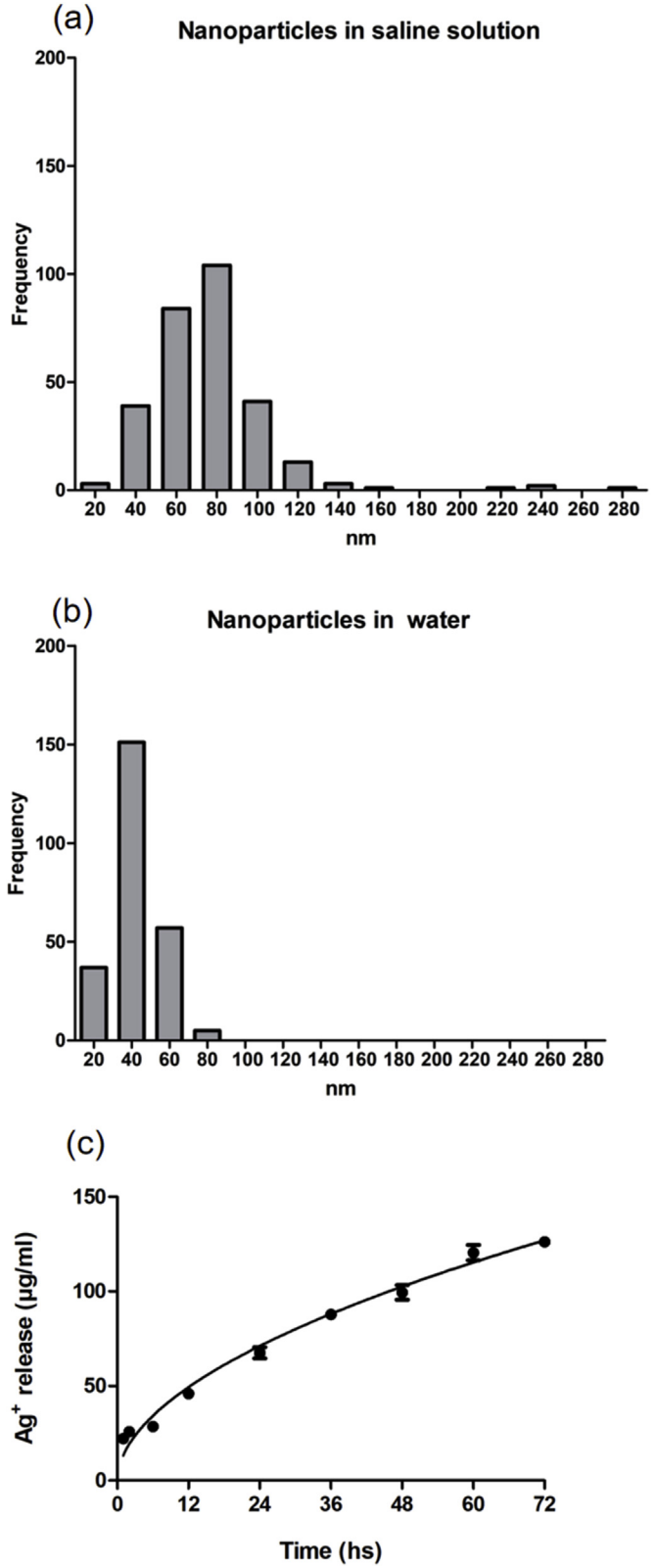

Fig. 3. The frequency size distribution of nanosilver-coated dressing immersed in 0.9% NaCl saline solution (a) or in MilliQ water (b). Silver (Ag) release versus time, expressed in concentration units (mg/ml) (c).

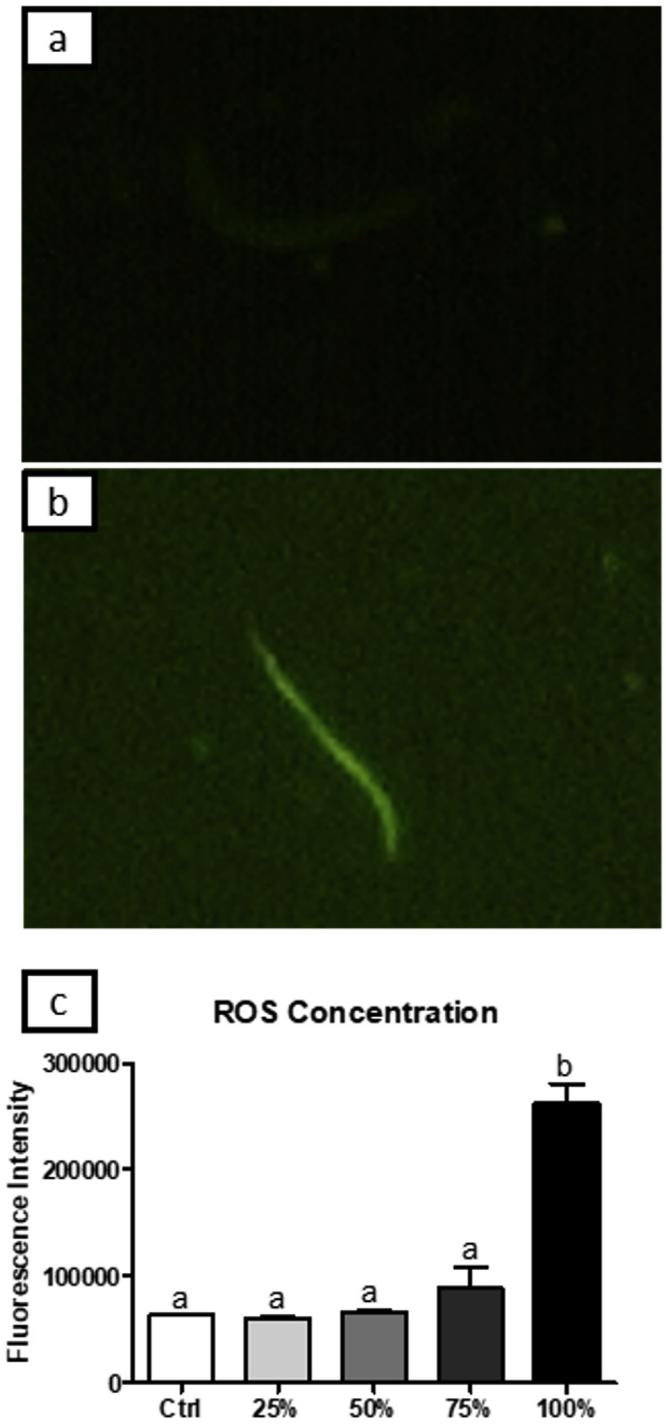

Fig. 4. Reactive oxygen species (ROS) concentration measured through fluorescence emission using the H_2DCF-DA probe, where greater fluorescence intensity indicates a higher ROS concentration. (a) *Caenorhabditis elegans* exposed to a coated dressing with 1.64 mg silver/cm^2 at stage L1. (b) *C. elegans* from control group. (c) Fluorescence intensity in *C. elegans* stage L1 for the different treatments: 1.64 mg silver/cm^2 (whole puddle equivalent = 1/1 or 100%), 1.23 mg silver/cm^2 (three-quarter puddle equivalent = 3/4 or 75%), 0.82 mg silver/cm^2 (half-puddle equivalent = 1/2 or 50%), 0.41 mg silver/cm^2 (quarter-puddle equivalent = 1/4 or 25%) and the control group (Ctrl). Values are expressed as mean + 1 standard error. Equal letters indicate the absence of significant differences ($p > 0.05$).

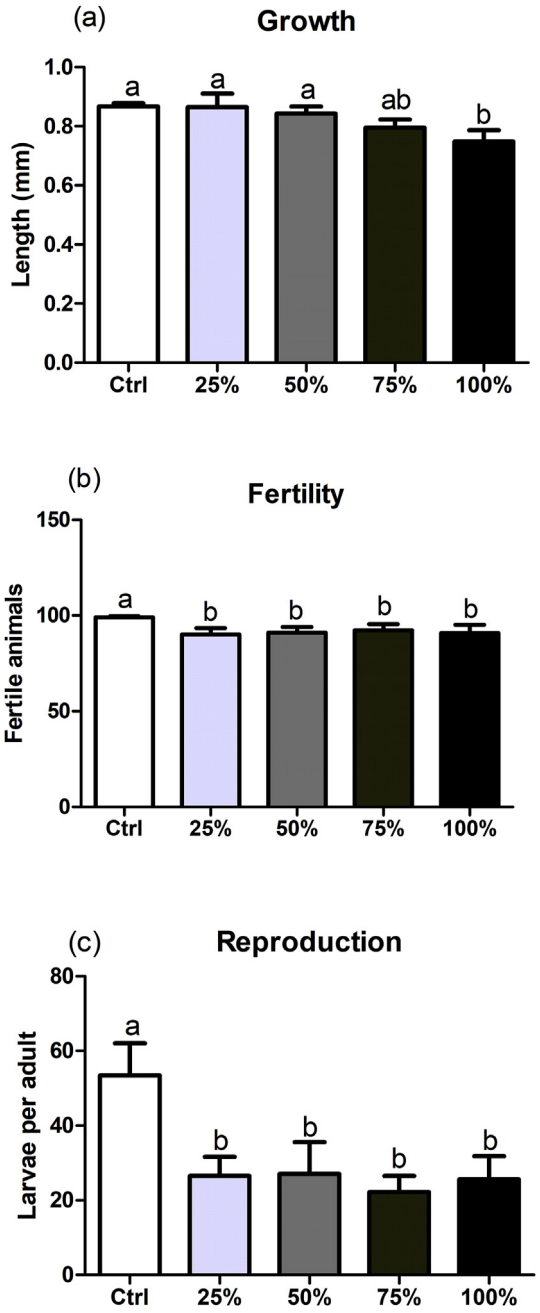

Fig. 5. Physiological responses in *Caenorhabditis elegans* after 3-day exposure to the following treatments: 1.64 mg silver/cm^2 (whole puddle equivalent = 1/1 or 100%), 1.23 mg silver/cm^2 (three-quarter puddle equivalent = 3/4 or 75%), 0.82 mg silver/cm^2 (half-puddle equivalent = 1/2 or 50%), 0.41 mg silver/cm^2 (quarter-puddle equivalent = 1/4 or 25%) or control (Ctrl). Values are expressed as mean + 1 standard error for growth (a), fertility (b), and (c) number of larvae generated (n = 4–5 for each treatment). Equal letters indicate the absence of significant differences ($p > 0.05$).

4. Conclusions

It was observed that in water the particles were less agglomerated and more stable as verified in zeta potential, unlike the saline medium (0.9% NaCl), where the particles were more agglomerated, which is expected situation under those physiological conditions. Results indicated that Acticoat Flex 3 has an antibacterial effect as do antibiotics in the strains tested (*P. aeruginosa* and *S. aureus*). Nanosilver-coated dressing impaired reduced reproduction, growth and fertility and induced higher ROS concentration in L1 larval stage animals, unlike L4 stage animals, which showed lower susceptibility or no changes in the measured parameters Finally, the obtained results suggest that improper disposal of this coated dressing has the potential to cause damage to organisms, including *C. elegans*, and this finding highlights its environmental hazard.

Table 1
Evaluation of the physiological parameters of L4 larval stage worms.

L4 Stage		
Physiological parameters	Mean and Standard Error	
	Control	100%
Growth (mm)	1.05 ± 0.01[a]	1.05 ± 0.02[a]
Fertility (%)	96.67 ± 1.76[a]	98.99 ± 1.01[a]
Reproduction (larvae/adult)	41.52 ± 0.72[a]	36.15 ± 2.30[a]
ROS Concentration	33670.94 ± 1730.08[a]	33882.73 ± 984.47[a]

100% refers to worms exposed to 1.64 mg/cm2 (whole puddle equivalent = 1/1 or 100%) for 3 days. Growth is expressed in millimeters, fertility is expressed as a percentage of fertile animals, reproduction expressed by the ratio of larvae to adult animals. ROS is expressed by the fluorescence intensity using the H_2DCF-DA probe. Values are expressed as mean + 1 standard error. Equal letters indicate no significant differences ($p > 0.05$) for each physiological variable.

CRediT authorship contribution statement

A. Ayech: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **M.E. Josende:** Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization. **J. Ventura-Lima:** Conceptualization, Resources, Writing - original draft, Writing - review & editing. **C. Ruas:** Validation, Writing - review & editing, Visualization. **M.A. Gelesky:** Validation, Resources, Writing - review & editing, Visualization, Funding acquisition. **A. Ale:** Conceptualization, Writing - original draft, Writing - review & editing. **J. Cazenave:** Writing - original draft, Writing - review & editing. **J.M. Galdopórpora:** Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **M.F. Desimone:** Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition. **M. Duarte:** Investigation, Writing - original draft, Writing - review & editing. **P. Halicki:** Validation, Investigation, Writing - original draft, Writing - review & editing. **D. Ramos:** Validation, Resources, Writing - original draft, Writing - review & editing, Funding acquisition. **L.M. Carvalho:** Validation, Writing - original draft, Writing - review & editing, Funding acquisition. **G.C. Leal:** Validation, Investigation, Writing - original draft, Writing - review & editing. **J.M. Monserrat:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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