BioNanoScience (2020) 10:463–472
https://doi.org/10.1007/s12668-020-00738-w

Antibacterial and Antitumoral Activities of the Spider Acylpolyamine Mygalin Silver Nanoparticles

Lilia Coronato Courrol[1] · Abraham Espinoza-Culupú[2] · Pedro Ismael da Silva Jr[3] · Karina de Oliveira Gonçalves[1] · Flávia Rodrigues de Oliveira Silva[4] · Monamaris Marques Borges[2]

Published online: 17 April 2020

Abstract
Mygalin is a synthetic analog of polyamine spermidine isolated from spider hemocytes. Polyamines show potential therapeutic activity against a wide range of human diseases such as cancer and microbial infections. In this work, we analyzed the antibacterial and antitumoral activities of Mygalin silver nanoparticles synthesized by the photoreduction method. The formation and distribution of MygAgNPs were confirmed by UV-visible spectroscopy, zeta potential, and transmission electron microscopy. The obtained nanoparticles were mostly spherical with a particle size distribution in the range of ~ 10–60 nm. We have demonstrated that MygAgNPs increased the effectiveness of the native Mygalin by approximately 6400-fold. Cytotoxicity tests were performed, and it was possible to reach a concentration that was not toxic to healthy cells (NHI-3T3) and at the same time toxic to the tumor cell line (MCF-7). The obtained results suggest that this system shows potential enhanced antibacterial activity against *Escherichia coli*, DH5α and anticancer activity against MCF-7 cell line.

Keywords Antibacterial polyamine · Mygalin · Spider acylpolyamine · Silver nanoparticles · Anticancer

1 Introduction

The global scenario of antibacterial resistance has been of great concern for public health since it can lead patients to death. In the face of continually increasing numbers of bacterial strains that present resistance to current therapies, new treatment strategies have been pursued.

Nature can show us new approaches to fight against antibacterial resistance. For example, arthropods, widespread in several places and found mainly in highly contaminated environments, can defend themselves against microorganisms and parasites by their antibacterial peptides (AMPs). Besides, several AMPs display the ability to modulate the innate immune responses of the host and thereby indirectly promote pathogen clearance [1].

The natural AMPs present in several organisms are not stable and present toxic side effects. For clinical applications, it is necessary to develop the synthetic AMP analogs that overcome these disadvantages [2].

Synthetic Mygalin is a bis- acylpolyamine, N (1),N(8)-bis(2,5-dihydroxybenzoyl) spermine, has 417 Da and was initially isolated from hemocytes of *Acanthoscurria gomesiana* spider [3]. Mygalin exhibits a pronounced effect on splenocyte and macrophage nitric oxide production, implying a potential role in the defense against microorganisms. Mygalin is not cytotoxic to murine cells in vitro and does not affect cellular proliferation or IL-2 production in response to concanavalin A (ConA) [3].

New set-ups to develop novel antibacterial compositions can also be obtained with distinct types of nanoparticles with different sizes and shapes produced by physical, chemical, or biological methods [4–6]

Silver nanoparticles are considered the most effective nanomaterial against bacteria [7–10] The antibacterial effect is not dependent on the size of the nanoparticles, and positive surface charge nanoparticles have higher antibacterial activity [11]. Coupling nanoparticles and natural-based antimicrobials can inhibit the activity of bacterial efflux pumps and the

✉ Lilia Coronato Courrol
lccourrol@gmail.com

[1] Department of Physics, Federal University of São Paulo, Diadema CEP, Sao Paulo 009972-270, Brazil

[2] Laboratory of Bacteriology, Butantan Institute, São Paulo CEP, Sao Paulo 05503-900, Brazil

[3] Laboratory for Applied Toxicology (LETA), Butantan Institute, São Paulo CEP, Sao Paulo 05503-900, Brazil

[4] Center of Science and Materials Technology, Energetic and Nuclear Research Institute, IPEN/CNEN, Sao Paulo, Brazil

formation of biofilms and are one of the strategies to combat multidrug-resistant bacteria [10, 12]. Also, silver nanoparticles used in conjugation with drugs promote better compatibility, lower toxicity, and precise and selective delivery of drugs and can be employed in anticancer therapy [13–15] The anticancer activity of silver nanoparticle-based drug delivery systems is exploited [16].

In this work, Mygalin silver nanoparticles (MygAgNPs) synthesized by the photoreduction method were tested for their microbicidal activity against *Escherichia coli* and compared with the Mygalin in its active form. Furthermore, the efficacy of the MygAgNPs was evaluated against the MCF-7 breast cancer cell line.

2 Materials and Methods

2.1 Mygalin Silver Nanoparticle Synthesis (MyAgNPs)

Mygalin (bis-acylpolyamine N1, N8-bis(2,5-dihydroxybenzoyl)-spermidine) was synthesized according to the classical method of peptide chemistry [17].

Mygalin silver nanoparticles were prepared by irradiating silver nitrate (Sigma-Aldrich) and Mygalin in a deionized water solution with 300 W Xenon lamp (Cermax, Excelitas Technology). The Xenon lamp was positioned 10 cm away from the recipient containing the solution, and the illuminated region covered exactly the recipient diameter, with a 3.6 W/cm^2 estimated intensity. Parameters serve as Mygalin concentration, and the presence of PEG (polyethylene glycol 1000 Sigma). Mygalin and $AgNO_3$ initial concentrations and irradiation time used in the synthesis procedure. (Table 1).

2.2 Characterization of MyAgNPs

Spectrophotometry analyses in the UV-vis region were performed with the UV-vis Shimadzu MultiSpec 1501 spectrophotometer. The measurements were carried out in a 10-mm optical path quartz cuvette in the range 250 and 800 nm.

The particle size, shape, and morphology of the synthesized MygAgNPs were characterized by transmission electron microscopy (LEO 906E, Zeiss, Germany).

The dynamic light scattering method was employed for the zeta potential and particle size analysis of the colloidal MygAgNP using Malvern Zetasizer Nano (size range 0.1–10,000 nm).

2.3 In Vitro Antibacterial Activity

2.3.1 Culture of *E. coli*

E. coli DH5α was grown in Luria-Bertani broth (LB) for 18 h at 37 °C overnight and then transferred into an LB medium and maintained until the exponential growth phase [18]. The bacterial suspensions at 10^8 CFU/mL were then prepared by adjusting the optical density to $OD_{620\ nm} = 0.300 \pm 0.005$.

2.3.2 Antibacterial Activity of *MygAgNPs*

The antibacterial effect of MygAgNPs on *E. coli* viability was carried out by the resazurin assay, and the bacteria growth was monitored by reading the optical density at 550 nm in 96-well plates at 37 °C [19]. The resazurin assay (40 μg/mL) defined the viability of the bacteria after 2 h of incubation. *E.coli* (10^8 CFU/mL) was diluted to 10^3 CFU/in 10 μL plus 90 μL of different formulations of nanoparticles, or active Mygalin

Table 1 Mygalin and $AgNO_3$ initial concentrations

		Mygalin (μM)	$AgNO_3$ (mM)	Irradiation time (min)
	Mygalin	1000	-	-
	Myg + $AgNO_3$	10	3.5	-
$AgNO_3$ concentration	Myg[Ag]NP	10	[Ag1] 2.0	1
			[Ag2] 3.5	
			[Ag3] 5.2	
			[Ag4] 9.9	
			[Ag5]12.5	
Mygalin concentration	MygAgNP1	10	3.5	1
	MygAgNP2	20		
	MygAgNP3	30		
	MygAgNP5	50		
	MygAgNP6	100		
	PEGMygAgNP	10	3.5 (0.008 g PEG)	1
	MygAgNP4	10	3.5	7

(125–1000 μM) was added to the plate. Positive control of the reaction (100% death) was bacteria treated with H_2O_2 (125–1000 μM), and negative control (0% death) was untreated bacteria or treated with inactivated Mygalin (light exposure at 37 °C). The plates were incubated at 37 °C for 18 h, kept under stirring, and protected from light during the tests. The resazurin assay defined the viability of the bacteria, at the final concentration of 40 μg/mL after 2 h of incubation. The color change to blue meant cell death, as they incorporated the dye, while those that became purple were viable cells. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug that completely inhibited visible growth. In another assay, the effect of MygAgNP on *E. coli* growth was monitored by reading the optical density at 550 nm, after 24 h of treatment of the cells with MygAgNP (10, 50, and 100 μM) diluted 1/640, active Mygalin (125-1000 μM), or as a positive control, ciprofloxacin (0.19–0.78 μM).

2.4 Cell Culture

Two cell lines were used, one healthy, (NHI-3T3 murine fibroblast) and one tumoral (MCF-7 human breast tumor), to evaluate the cytotoxicity of MygAgNPs.

The cells were kept in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C with 5% CO_2. The cells were routinely subcultured every 3 days with 70–80% confluency and harvested using 0.25% trypsin.

2.5 Cell Viability

The cells (10^4 cells/well) were plated into flat-bottom 96-well plates and incubated in for 24 h for plate adhesion and growth. Then, the plates were divided into experimental groups, which received different concentrations of MygAgNP1 (80, 70, 60, 50, 40, 30, 20, 10, 5 e 2.5 μL), and the negative (0.9% NaCl) and positive (latex suspension, 0.5 g/L filter sterilized latex extract, 0.5 g/L in culture media, 24 h) controls. The plates were incubated for 24 h at 37 °C and 5% CO_2 atmosphere. The supernatant was removed; the cells were washed with PBS. Cell viability was assessed by MTS (CellTiter 96® AQueous MTS Reagent).

2.6 Statistical Analysis

Significances were calculated using the GraphPad Prism software. For antibacterial studies, Student's *t* test was used to determine statistical differences compared with those of untreated control. p values < 0.05 were considered significant.

For tumor cell death study, results were statistically compared (ANOVA and Dunnett's test). Cell survival percentage was calculated after background absorbance correction and blank absorbance subtraction as follows: % Cell viability = 100 × Experimental absorbance of the well/untreated absorbance control.

3 Results

3.1 MygAgNPs Synthesis and Characterizations

Figure 1 a shows the UV-vis spectra obtained for Mygalin solution and solutions containing a fixed concentration of Mygalin and variable concentrations of silver nitrate, irradiated with Xenon light for 1 min. As shown in Fig. 1a, Mygalin alone does not present absorption in UV-vis spectra. In the Mygalin silver nitrate solutions absorption band in the range, 200–300 nm appears due to spermidine and silver nitrate [20]. The presence of silver nanoparticles can be certified by the presence of the localized surface plasmon resonance (SPR), a specific wavelength of incidentlight that induces collective oscillation of the surface electrons of silver nanoparticles. This band depends on the silver nanoparticle size, shape, and agglomeration state. For larger particles, a secondary peak at lower wavelength appears a result of quadrupole resonance, in addition to the primary dipole resonance [21]. In Fig. 1a, the increase in the intensity of broadband with two shoulders at 390 nm and 550 nm in the function of the $AgNO_3$ concentration can be observed. In Fig. 1b, for the sample irradiated by 7 min, it is found at a peak around 425 nm due to surface plasmon resonance. In Fig. 1c, it is noted that SPR band was widened in the presence of PEG.

Figure 2 a shows TEM of MygAgNP1, PEGMygAgNP, and MygAgNP4. MygAgNP1 exhibit nanosized, spherical particles ranging in size from 30 to 60 nm. With PEG, the particle size ranges from the 50–80 nm. Increasing irradiation time, nanoparticle sizes are ~ 7 nm. The polydispersity index of 0.848 was measured by dynamic light scattering (DLS) for MygAgNP1. This nanoparticle presented negative zeta potential (− 33.9 mV), as showed in Fig. 2b.

3.2 Antibacterial Activity

The microbicidal activity of MygAgNP was tested in *E. coli* DH5α, using the resazurin assay. Figure 3 a (plate 1) shows the viability of *E. coli* following treatment with MygAgNP preparations at different concentrations (10, 20, 30 μM) serially diluted in base 2 (1/4 to 1/4096). The microbicidal activity occurred until dilution 1/512. Subsequent dilutions showed no microbicidal activity, staining purple (rows A–H, columns 10–12). The PEGMygAgNP (rows G–H, columns 1–8) showed the same bactericidal activity as described for MygAgNP3. The minimum inhibitory concentration (MIC) of the

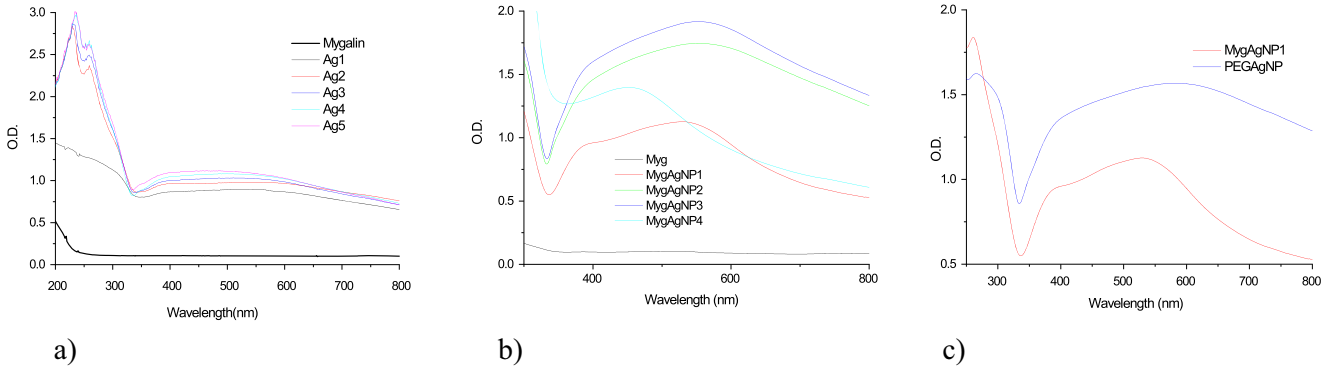


Fig. 1 UV-vis spectra. **a** $AgNO_3$ concentration study of Myg[Ag]NPs (Table 1) diluted 50%. **b** Mygalin concentration study of MygAgNP. **c** Presence of PEG in the composition

MygAgNPs ranged from 19 to 58 nM, depending on the concentration of Mygalin used. Results of plate 2 compare the microbicidal activity of MygAgNP4 and MygAgNP1 sample (rows A–D, columns 1–11). MygAgNP4 promoted bacterial death until 1/4096 dilution, which corresponds to MIC of 2.4 nM. All silver-decorated Mygalin (MygAgNPs) showed much higher antibacterial activity than the native (active) Mygalin, whose MIC was 1000 μM (plate 2, rows G–H, columns 5–8), although there are some viable bacteria at concentrations of 125–500 μM. The activity of 1000 μM of Mygalin was like treatment with H_2O_2 (1000 μM), positive reaction control. Treatment of *E. coli* with inactivated Mygalin, maintained at 37 °C and under light, (plate 2, rows E–F, columns 1–11), had no microbicidal effect at all dilutions tested (1/4 to 1/4096), staining purple as the untreated bacteria (rows G–H, columns 9–10).

The microbicidal activity of the nanoparticles was higher than the 6400-fold compared with that of the native (active) molecule, which had the highest effect in 1000 μM, proving the efficacy of these nano-formulations.

In another trial using a new MygAgNP (Myg-nano) sample (10, 50, and 100 μM, diluted 1/640) and active Mygalin (125, 250, 500, and 1000 μM), the effect of the formulations on inhibition of *E. coli* growth was tested after 24 h of treatment. Ciprofloxacin was used as bactericidal control to compare with the obtained data. The optical density reading was at 550 nm (Fig. 4). Data show that regardless of the final MygAgNP concentration (15, 78, and 156 nM), there was inhibition of bacterial growth in a similar pattern like 1000 μM of native (active) Mygalin and 0.78 μM ciprofloxacin. Although the maximum activity of Mygalin is 1000 μM, doses between 250 and 500 μM still inhibit bacterial growth. These results confirm that the construction of Mygalin nanoparticles increased the effectiveness of the native molecule, where 15 nM MygAgNPs was enough to inhibit *E. coli* growth by 100%, while with native Mygalin was 1000 μM.

3.3 Effects of the Silver Nanoparticles on NHI-3T3 and MCF-7 Cells

The cytotoxicity effects of Silver Nanoparticles in cells can be observed in Fig. 5, where the percentage of cell viability is plotted in function of the volume of solution added in each well. The concentrations used (10 to 80 μL) led to the death of almost 95% of the cells (healthy Fig. 5a and tumor Fig. 5b).

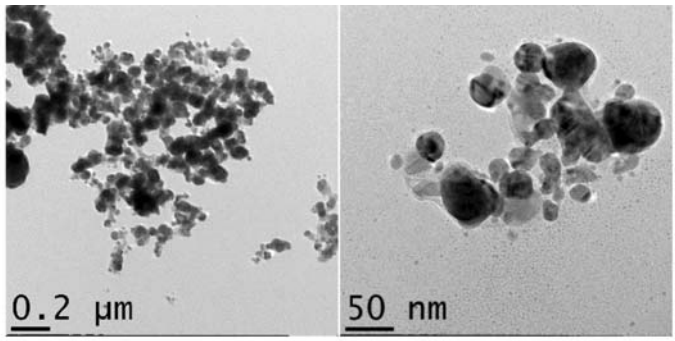
By analyzing the two cytotoxicity tests with the MygAgNP1, it was possible to reach a concentration that was not toxic to healthy cells (NHI-3T3) and, at the same time, toxic to the tumor line (MCF-7). Figure 5 c presents the results with 5 and 2.5 μL of the nanoparticle solution. The volume of 5 μL led to the death of ~ 13.0% of healthy cells, and in contrast, with the tumor lineage, the death rate was ~ 52.0%.

4 Discussion

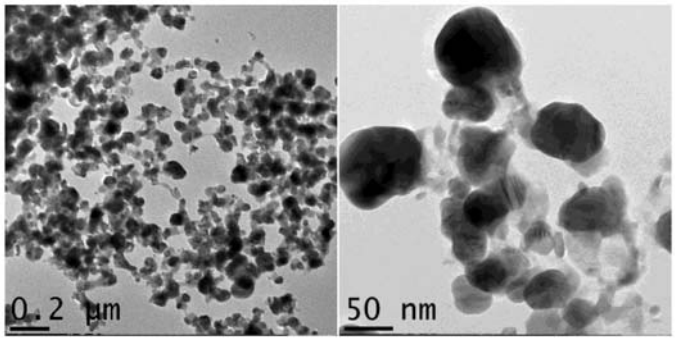
In recent years, the assembly of antibacterial drugs in nanoparticle delivery systems has been shown as a promising alternative to increase therapeutic efficacy and to minimize the undesirable effects of drugs. Among the advantages attributed to the use of this system, we can mention the improvement of the solubility of the drug, increase in the time of permanence in the organism, and use of suitable concentrations to find the desired target, besides the delivery of multiple drugs simultaneously. Several formulations are being tested for disease control, including bacterial infections [22].

Mygalin (acylpolyamine) is a synthetic analog of polyamine spermidine that has been described with bactericidal properties for Gram-negative bacteria [23]. In this work, this strategy was used to improve the efficiency of Mygalin. The data obtained showed that it was possible to get nanoparticles of Mygalin in silver nanoparticle system (Figs. 1 and 2).

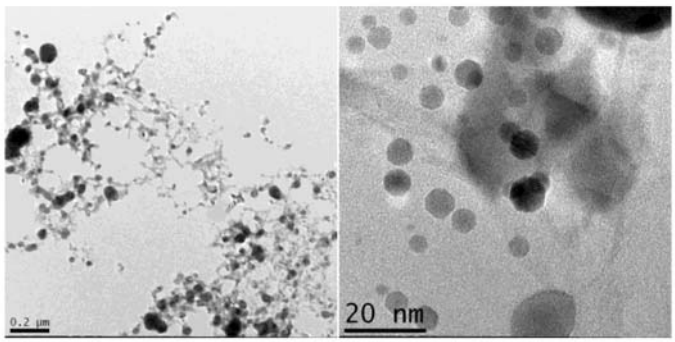
The solution containing $AgNO_3$ and Mygalin is colorless. Mygalin (10 μM) and silver nitrate (0.35 μM) water solution do not show SPR absorption bands, characteristic of silver



MygAgNP1

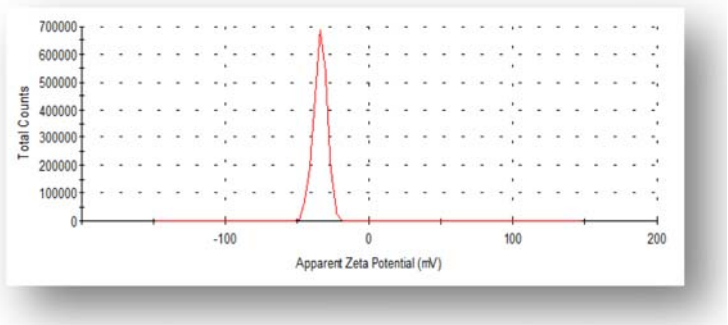


PEGMygAgNP



MygAgNP4

a)



b)

Fig. 2 **a** Transmission electron and scanning electron microscopies of MygAgNP1, PEGMygAgNP, and MygAgNP4. **b** Zeta potential distribution of MygAgNP1

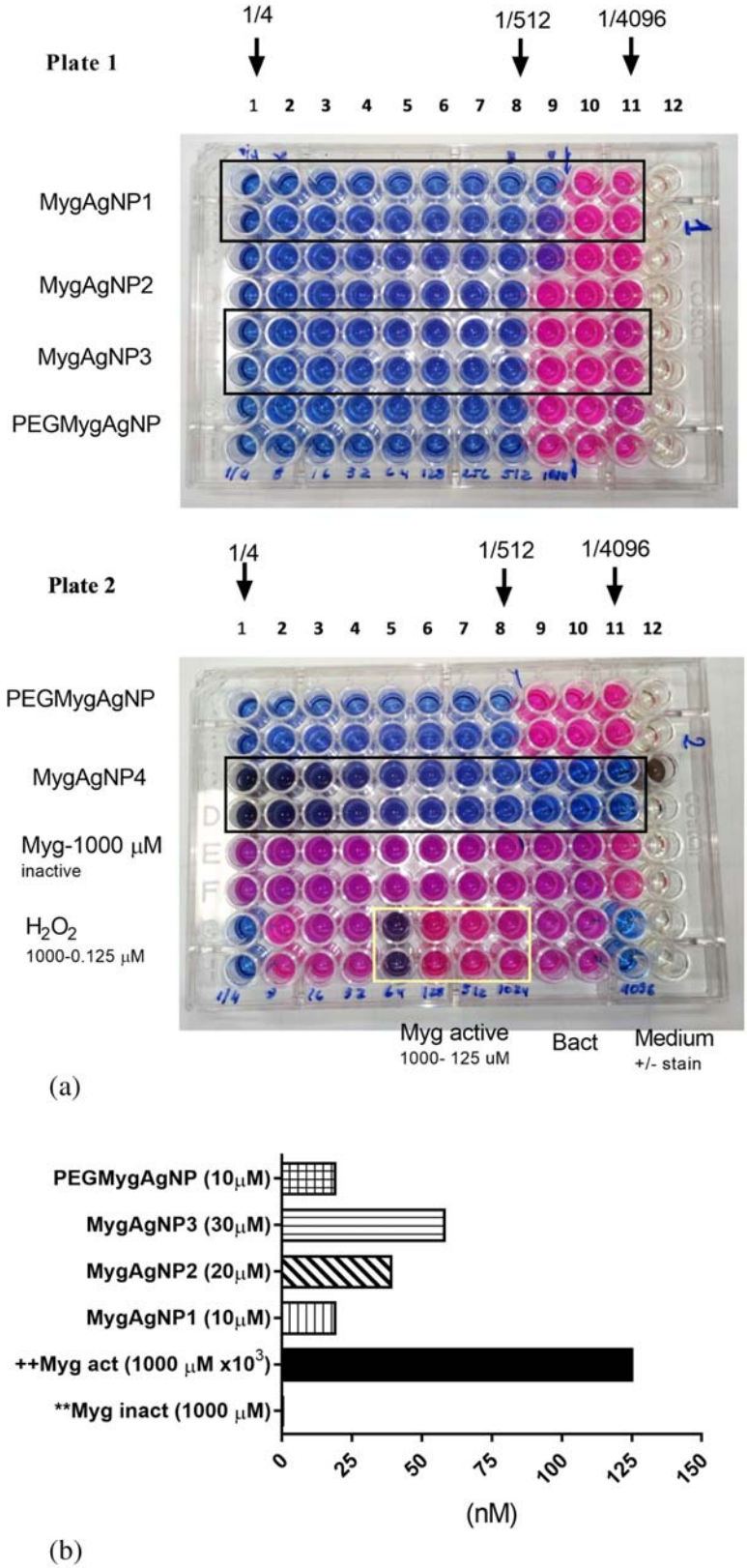


Fig. 3 **a**) Effect of different formulations of MygAgNPs on *E. coli* viability by the resazurin assay. *E. coli* (10^3 CFU/well) cultured for 24 h in the presence of serially diluted (1/4 to 1/4096) MygAgNP1 (10 μM), MygAgNP2 (20 μM), MygAgNP3 (30 μM), PEGMygAgNPs, MygAgNP4, and active Mygalin (125–1000 μM). Positive controls, H_2O_2 (125–1000 μM). Negative control: bacteria without treatment, inactive Mygalin (1000 μM), and medium ± dye without bacteria. **b**) The minimum inhibitory concentration (MIC) of MygAgNPs and the native (active) molecule

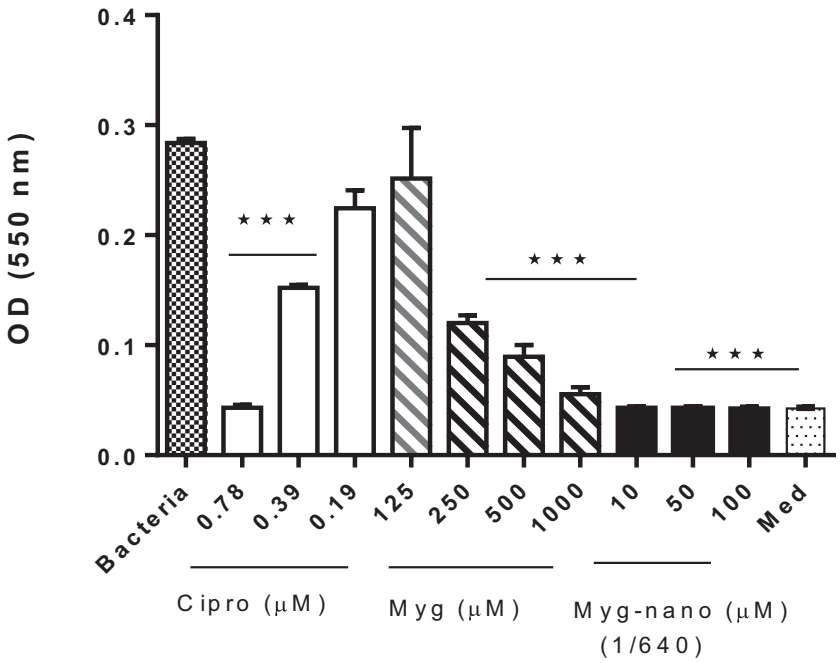


Fig. 4 Increased microbicidal activity of Mygalin by silver nanoparticles. *E. coli* was treated with Mygalin or MygAgNPs (Myg-nano 1/640) for 24 h. Absorbance readings at 550 nm monitored growth. Ciprofloxacin (Cipro) was used as a positive control. Data represents ± SEM three independent experiments compared with non-treatment ***$p < 0.001$ (Student's t test). Med = culture medium

nanoparticles immediately after the preparation, but after approximately 7 days, the SPR band appears indicating that Mygalin by itself is capable of reducing silver. After light irradiation, the solutions change to brownish/yellow hues indicating changes in the sample transmission due to the presence of silver nanoparticles in the suspensions. The absorbances of the silver colloidal Mygalin solutions with varying silver nitrate concentrations irradiated for 1 min have shown an increase in the number of AgNPs in the solution. In other words, Mygalin releases H^+ and allows the reduction of silver ions (Ag^+) to metallic silver (Ag^0). The combination of the photons and temperature supplied by the xenon lamp facilitates the oxidation and the reduction of the silver ions forming nanoparticles. Increase in the illumination time impige in a decrase in the particles size. After the reduction, silver clusters are formed with the subsequent nucleation and growth of the nanoparticles. Thus, light is a catalyst for the formation of the AgNPs. In this process, spermidine is exposed to the surface of the nanoparticle, as observed in Fig. 1a. The average size of particles increases in the presence of PEG.

MygAgNPs were tested for their microbicidal activity against *E. coli*, DH5α. Data presented in Figs. 3 and 4 suggested that the MygAgNPs enhanced the microbicidal activity of Mygalin by approximately 6400-fold. Thus, we verified the feasibility of this nanostructure as a promising alternative to be explored in infections against multidrug-resistant bacteria. It was demonstrated that the microbicidal mechanism of Mygalin against *E. coli* involves the generation of oxidative stress, DNA damage, and changes in the cell wall [18]. Finally, an important point to be mentioned is that the active form of this molecule did not present a cytotoxic effect to murine splenocytes and induced an innate immune response. In the future, these studies will be expanded by evaluating other parameters such as action on different species of bacteria, a possible mechanism of action, and their ability to interact with eukaryotic cells.

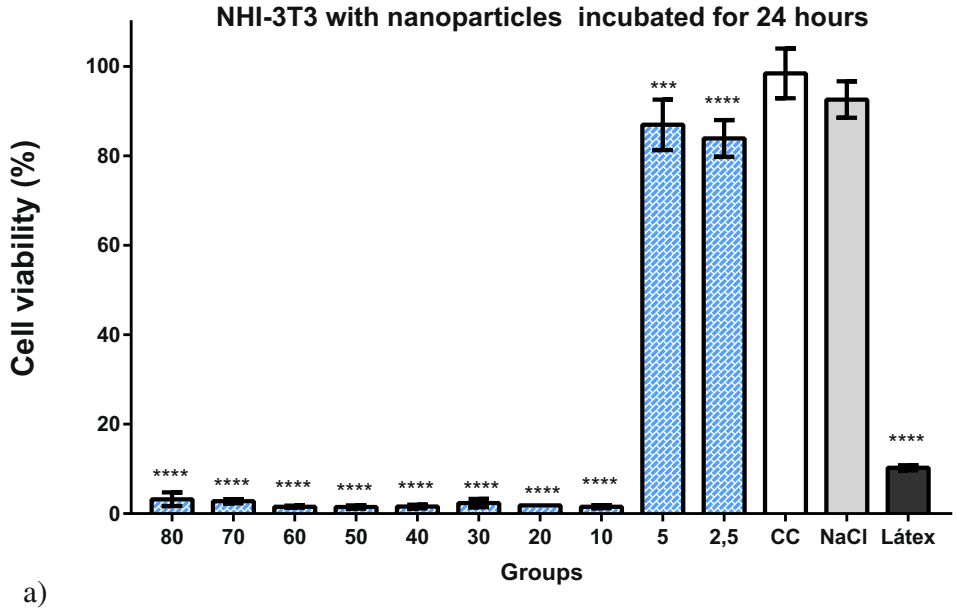
There are several approaches regarding the mechanism of action of AgNPs with bacteria [24]. The antibacterial potential of NP compounds may depend on their sizes, charges, and stability [7, 25]. Silver nanoparticles can bind cell membrane structures, destabilizing the membrane potential and causing proton leakage [26, 27].

Many studies have shown that silver nanoparticles present therapeutic applications as antitumor agents [25, 28]. Several in vitro studies have indicated that silver nanoparticles can enter in cells by endocytosis and promote oxidative stress, induction of apoptosis, and mitochondrial damage in cancer cells [25, 29–32].

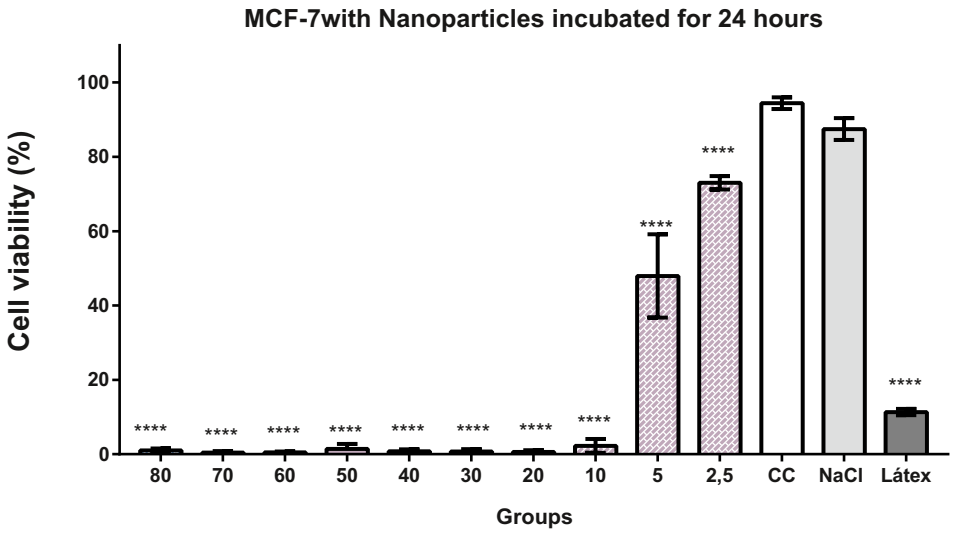
MyAgNPs presented an anticancer effect against the MCF-7 breast cancer cell line, and a low concentration (< 5 μL) induced 52% of cancer cell death (Fig. 5). Interestingly, for this concentration, more than 90% of healthy cells survived.

5 Conclusions

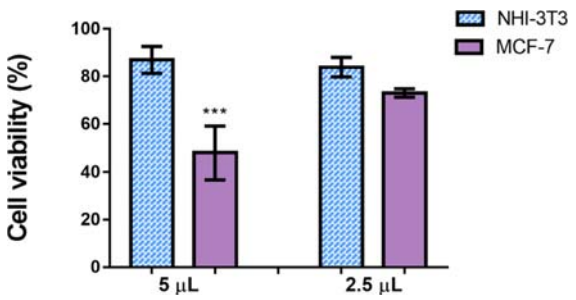
Mygalin silver nanoparticles were successfully synthesized by the photoreduction method, generating spherical particles smaller than 60 nm. The synthesized silver nanoparticles have microbicidal activity much higher than the molecule in its active form, suggesting that this system can be exploited for the control of bacterial infections and other aspects related to



a)



b)



c)

Fig. 5 **a** Cell viability test in healthy murine fibroblast cells (NHI-3T3), incubated for 24 h with MygAgNPs. Data were compared using the ANOVA test followed by the Dunnett test, with $p < 0.0001$. **b** Cell viability test in breast tumor cells (MCF-7), incubated for 24 h with MygAgNPs. Data were compared using the ANOVA test followed by the Dunnett test, with $p < 0.0001$. **c** Viability test comparing the two cell lines. The concentration of 5 µL presents toxicity to the breast tumor cells, showing a significant result when compared with that of the other groups. Data were analyzed using the ANOVA test followed by the Dunnett test, with $p < 0.0001$

its interaction with eukaryotic cells. Further, the present work reveals the potential application of MygAgNPs as an anticancer agent against human breast cancer.

Acknowledgments The authors thank the Center for Research on Toxins, Immune-Response and Cell Signaling (CeTICS –CEPID) and the Special Laboratory for Applied Toxicology (LETA).

Funding Information São Paulo Research Foundation FAPESP funded this research, grant number 2017/23686-6, and 2014/04307-6, PhD. Scholarship was provided by CONCYTEC-FONDECYT Institution of Peruvian State (N092-2016) and the Butantan Foundation.

References

1. Mahlapuu, M., Hakansson, J., Ringstad, L., et al. (2016). Antimicrobial peptides: An emerging category of therapeutic agents. *Frontiers in Cellular and Infection Microbiology, 6*, 194. https://doi.org/10.3389/fcimb.2016.00194.
2. Lei, J., Sun, L. C., Huang, S. Y., et al. (2019). The antimicrobial peptides and their potential clinical applications. *American Journal of Translational Research, 11*, 3919–3931.
3. Mafra, D. G., da Silva, P. I., Galhardo, C. S., et al. (2012). The spider acylpolyamine Mygalin is a potent modulator of innate immune responses. *Cellular Immunology, 275*, 5–11. https://doi.org/10.1016/j.cellimm.2012.04.003.
4. Rafique, M., Sadaf, I., Rafique, M. S., et al. (2017). A review on green synthesis of silver nanoparticles and their applications. *Artificial Cells Nanomedicine and Biotechnology, 45*, 1272–1291. https://doi.org/10.1080/21691401.2016.1241792.
5. Velusamy, P., Kumar, G. V., Jeyanthi, V., et al. (2016). Bio-inspired green nanoparticles: Synthesis, mechanism, and antibacterial application. *Toxicological Research, 32*, 95–102. https://doi.org/10.5487/tr.2016.32.2.095.
6. Mandava, K. (2017). Biological and non-biological synthesis of metallic nanoparticles: Scope for current pharmaceutical research. *Indian Journal of Pharmaceutical Sciences, 79*, 501–512. https://doi.org/10.4172/pharmaceutical-sciences.1000256.
7. Franci, G., Falanga, A., Galdiero, S., et al. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules, 20*, 8856–8874. https://doi.org/10.3390/molecules20058856.
8. Panacek, A., Smekalova, M., Vecerova, R., et al. (2016). Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. *Colloids and Surfaces. B, Biointerfaces, 142*, 392–399. https://doi.org/10.1016/j.colsurfb.2016.03.007.
9. Maillard, J.-Y., & Hartemann, P. (2013). Silver as an antimicrobial: Facts and gaps in knowledge. *Critical Reviews in Microbiology, 39*, 373–383. https://doi.org/10.3109/1040841x.2012.713323.
10. Baptista, P. V., McCusker, M. P., Carvalho, A., et al. (2018). Nano-strategies to fight multidrug resistant bacteria-"a battle of the titans". *Frontiers in Microbiology, 9*, 1441. https://doi.org/10.3389/fmicb.2018.01441.
11. Salas-Orozco, M., Nino-Martinez, N., Martinez-Castanon, G. A., et al. (2019). Mechanisms of resistance to silver nanoparticles in endodontic bacteria: A literature review. *Journal of Nanomaterials, 2019*, 11. https://doi.org/10.1155/2019/7630316.
12. Kaur, A., & Kumar, R. (2019). Enhanced bactericidal efficacy of polymer stabilized silver nanoparticles in conjugation with different classes of antibiotics. *RSC Advances, 9*, 1095–1105. https://doi.org/10.1039/c8ra07980c.
13. Akita, H., Fujiwara, T., Santiwarangkool, S., et al. (2015). Transcytosis-targeting peptide: A conductor of liposomal nanoparticles through the endothelial cell barrier. *Small, 2*, 1212–1221. https://doi.org/10.1002/smll.201500909.
14. Ban, R., Abdel-Halim, E. S., Zhang, J., et al. (2015). β-Cyclodextrin functionalised gold nanoclusters as luminescence probes for the ultrasensitive detection of dopamine. *Analyst, 140*, 1046–1053. https://doi.org/10.1039/c4an02161d.
15. Xie, J., Lee, S., & Chen, X. (2010). Nanoparticle-based theranostic agents. *Advanced Drug Delivery Reviews, 62*, 1064–1079. https://doi.org/10.1016/j.addr.2010.07.009.
16. Ranghar, S., Sirohi, P., Verma, P., et al. (2014). Nanoparticle-based drug delivery systems: Promising approaches against infections. *Brazilian Archives of Biology and Technology, 57*, 209–222.
17. E. Atherton RCS. Solid phase peptide synthesis : A practical approach 1989.
18. Espinoza-Culupu, A., Mendes, E., Vitorino, H. A., et al. (2020). Mygalin: An acylpolyamine with bactericidal activity. *Frontiers in Microbiology, 10*, 2928. https://doi.org/10.3389/fmicb.2019.02928.
19. Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods, 42*, 321–324. https://doi.org/10.1016/j.ymeth.2007.01.006.
20. Khomutov, A. R., Grigorenko, N. A., & Skuridin, S. G. (2007). Novel approach to design an isosteric charge-deficient analogue of spermine and its biochemically important derivatives. *Biochemical Society Transactions, 35*, 369–373. https://doi.org/10.1042/BST0350369.
21. Gomez, F. R., Rubira, R. J. G., Camacho, S. A., et al. (2018). Surface plasmon resonances in silver nanostars. *Sensors, 18*, 9. Article. https://doi.org/10.3390/s18113821.
22. Pelgrift, R. Y., & Friedman, A. J. (2013). Nanotechnology as a therapeutic tool to combat microbial resistance. *Advanced Drug Delivery Reviews, 65*, 1803–1815. https://doi.org/10.1016/j.addr.2013.07.011.
23. Pereira, L. S., Silva, P. I., Miranda, M. T. M., et al. (2007). Structural and biological characterization of one antibacterial acylpolyamine isolated from the hemocytes of the spider Acanthocurria gomesiana. *Biochemical and Biophysical Research Communications, 352*, 953–959. https://doi.org/10.1016/j.bbrc.2006.11.128.
24. Ivask, A., ElBadawy, A., Kaweeteerawat, C., et al. (2014). Toxicity mechanisms in Escherichia coli vary for silver nanoparticles and differ from ionic silver. *ACS Nano, 8*, 374–386. https://doi.org/10.1021/nn4044047.
25. Courrol, D. D., Lopes, C. R. B., Pereira, C. B. P., et al. (2019). Tryptophan silver nanoparticles synthesized by photoreduction method: Characterization and determination of bactericidal and anti-biofilm activities on resistant and susceptible bacteria. *International Journal of Tryptophan Research, 12*, 1178646919831677. https://doi.org/10.1177/1178646919831677.
26. Morones, J. R., Elechiguerra, J. L., Camacho, A., et al. (2005). The bactericidal effect of silver nanoparticles. *Nanotechnology, 16*, 2346–2353. https://doi.org/10.1088/0957-4484/16/10/059.
27. Kim, J. S., Kuk, E., Yu, K. N., et al. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine, 3*, 95–101. https://doi.org/10.1016/j.nano.2006.12.001.
28. Zhang, X. F., Liu, Z. G., Shen, W., et al. (2016). Silver nanoparticles: Synthesis, characterization, properties, applications, and therapeutic approaches. *International Journal of Molecular Sciences, 17*, 34. Review. https://doi.org/10.3390/ijms17091534.
29. Ortega, F. G., Fernandez-Baldo, M. A., Fernandez, J. G., et al. (2015). Study of antitumor activity in breast cell lines using silver nanoparticles produced by yeast. *International Journal of Nanomedicine, 10*, 2021–2031. https://doi.org/10.2147/ijn.s75835.

30. Satapathy, S. R., Mohapatra, P., Preet, R., et al. (2014). Silver-based nanoparticles induce apoptosis in human colon cancer cells mediated through p53. *Anticancer Research, 34*, 6155–6156.
31. Zhang, X. F., Shen, W., & Gurunathan, S. (2016). Silver nanoparticle-mediated cellular responses in various cell lines: An in vitro model. *International Journal of Molecular Sciences, 17*, E1603. https://doi.org/10.3390/ijms17101603.
32. Sre, P. R. R., Reka, M., Poovazhagi, R., et al. (2015). Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam. *Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy, 135*, 1137–1144. https://doi.org/10.1016/j.saa.2014.08.019.