

# *In Vitro* Effects of Silver Nanoparticles on Pathogenic Bacteria and on Metabolic Activity and Viability of Human Mesenchymal Stem Cells

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## Abstract

The rapid development of nanotechnology has led to the use of silver nanoparticles (Ag-NPs) in various biomedical fields. However, the effect of Ag-NPs on human mesenchymal stem cells (hMSCs) is not fully understood. Moreover, too frequent an exposure to products containing nanosilver in sublethal amounts raises widespread concerns that it will lead to the development of silver-resistant microorganisms. Therefore, this study aimed to evaluate the mechanism of action of Ag-NPs on hMSCs by analyzing the cellular uptake of Ag-NPs by the cells and its effect on their viability and to assess antimicrobial activity of Ag-NPs against emerging bacterial strains, including multidrug-resistant pathogens. For metabolic activity and viability evaluation, hMSCs were incubated with different concentrations of Ag-NPs (14 µg/mL, 7 µg/mL, and 3.5 µg/mL) for 10 min., 1 h and 24 h and subsequently analyzed for their viability by live-dead staining and metabolic activity by the MTS assay. The effect of Ag-NPs on bacterial pathogens was studied by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). In conclusion, it was observed that exposure of hMSCs to Ag-NPs of size <10 nm has no cytotoxic effect on the metabolic activity of the cells at the concentration of 3.5 µg/mL, with minimal cytotoxic effect being observed at the concentration of 14 µg/mL after 24 h of incubation. Our findings also confirmed that Ag-NPs at the concentration of 4 µg/mL are effective broad-spectrum bactericidal agents, regardless of the antibiotic-resistance mechanism present in bacteria.

## Keywords

Ag-NPs • hMSCs • Cytotoxicity • Nanoparticles • Multi drug-resistant bacteria

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## 1. Introduction

Nanomedicine is a fast-growing biomedical field that aims at enhancing the diagnosis and treatment of various diseases by applying nanotechnology. Nanoparticles, with their improved physical–chemical properties, such as nanoscale-small sizes, large surface area, and high surface reactivity, have opened up new horizons for diagnostic, therapeutic, and preventative applications (Hare et al. 2017). Studies report that silver nanoparticles (Ag-NPs) with their broad-spectrum antibacterial action and ability to reach complicated anatomies could be a promising alternative to antibiotics. The immunoregulatory role played by Ag nanoparticle-modified surfaces on immune cells has also been reported. In oncology, for instance, the concept of nanomedicine aims at modifying the pharmacokinetics and

tissue distribution of anti-cancer drugs, hence improving their therapeutic index (Weissleder et al. 2014). In inflammatory disorders, including cardiovascular diseases, rheumatoid arthritis, or inflammatory bowel disease, monocyte/macrophage-targeted nanomedicine can be implemented as a cell-specific drug-delivery system aiming to modulate the inflammation (Mulder et al. 2014). Ag nanoparticles have been frequently used in various antibacterial implants, such as wound dressings and catheters (Pauksch et al. 2014). In dentistry, Ag-NPs have been used in many forms, including irrigants (Kim et al. 2008; Tang and Zheng 2018), intracanal medicaments (Hsin et al. 2008), dental adhesives (Kim et al. 2008), and as an additive within sealers/restorative materials. Therefore, accumulation of nanoparticles in the targeted area, i.e., in organs or tissues is desired for an enhanced therapeutic effect (Kim et al. 2008).

The antibacterial properties of silver have been known for centuries; therefore, its use for its antimicrobial properties is not a latest development (Nowack et al. 2011). For ages, containers made of silver were used for storing water (Amato et al. 2011). The Macedonians used silver plates as a healing improving medium, and colloidal nanosilver has been used as a biocidal material in the United States since it was

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registered in 1954 (Nowack et al. 2011). Despite being widely used for so long, the exact mechanism of antimicrobial properties of Ag-NPs is not fully known (Du et al. 2018).

Silver ions exert antimicrobial effects on a wide spectrum of bacteria (*Staphylococcus aureus* or *Escherichia coli*) and fungi (Siddiqi et al. 2018; Yin et al. 2020; Rakowski et al. 2021). Also, the interaction with viruses has been reported (Samuel et al. 2020). Ag<sup>+</sup> has been reported to interact with a variety of microbial molecules such as DNA (Weissleder et al. 2014), cell wall components, or sulfhydryl groups of metabolic enzymes (Du et al. 2018; Siddiqi et al. 2018). Thereby, bacterial replication, membrane permeability, and different metabolic pathways are interrupted in this manner. However, silver-resistant bacterial strains, e.g. *Salmonella*, have also been found. The resistances result not from chemical detoxification but from an energy-dependent ion efflux from the cell by membrane proteins that function either as ATPase or as chemiosmotic cation/proton antiporters (Samuel et al. 2020). Nanoparticles are natural, incidental, or artificial materials made of particles, where at least half of the particles are in a size range 1–100 nm (Ema et al. 2017). Generally speaking, the mechanism of absorption of nanoparticles is more complex than one involving small molecules. Depending on the way the nanoparticles are administered, they can be absorbed by paracellular transport—when administered orally, or by macrophages and lymphatic uptake—when administered subcutaneously, intramuscularly, or inhaled (Lin et al. 2015; Li et al. 2017). The liver is the primary organ for silver-particle distribution in the organism, followed by the spleen and the kidneys (Lin et al. 2015). On the other hand, some medical devices release more Ag-NPs particles that can interact with human cells. This depends on the type of medical device used, the manufacturing technique, operator technique, or the lifespan of the medical device (Lin et al. 2015; Li et al. 2017).

Human mesenchymal stem cells (hMSCs) are naturally occurring adult multipotential cells that can be found in different tissues such as bone marrow, muscles, and fat. This cell type is intimately involved in tissue regeneration or repair. The mesenchymal stem cells are likely to come into close contact with any nanosilver-coated implants. Furthermore, due to their high differentiating capacity, these cells also represent an optimal cellular model to analyze a possible influence of nanosilver on cell differentiation (Liu et al. 2014). Some previous studies have shown that Ag-NPs exert cytotoxic effects on hMSCs at high concentrations, but also lead to cell activation at sublethal concentrations (Greulich et al. 2009).

Ag-NPs are produced and used in a wide range of commercial products, mainly due to their well-known antiseptic effects, but their effect on hMSCs is not fully understood. Therefore, the aim of this study is to investigate the mechanism of action of Ag-NPs on hMSCs by analyzing the cellular uptake of Ag-NPs by hMSCs and its impact on the viability of

these cells depending on different exposure times and Ag-NP concentrations. Considering the broad antimicrobial potential of Ag-NPs, this study also evaluated their bacteriostatic and bactericidal effects on pathogens that are frequently found in healthcare settings.

## 2. Materials and Methods

### 2.1. Characterization of Ag-NPs

The colloidal solution of Ag-NPs (20 µg/mL, cat. no. 730785) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The colloidal solution of Ag-NPs was tested for particle size and shape. All data have been obtained by computer-aided processing of the live images. The first step was binarization, followed by operations of mathematical morphology (Kurzydłowski and Ralph 1995).

### 2.2. Cell culture

Poietics™ normal human bone marrow derived mesenchymal stem cells (hMSC, cat. no.: PT-2501) were purchased from Lonza (Basel, Switzerland). The cells were cultured in expansion medium, i.e. minimum essential medium alpha (a-MEM, Gibco, Thermo Fisher Scientific, Paisley, UK) supplemented with 10% of fetal bovine serum (FBS; EuroClone, Milan, Italy), antibiotics (5 µg/mL penicillin, 5 µg/mL streptomycin, and 10 µg/mL neomycin, Gibco), and 1 ng/mL human growth factor (FGF2; Sigma-Aldrich) at 37°C and 5% CO<sub>2</sub>.

### 2.3. MTS assay and live-dead staining

hMSCs were seeded at a density of  $1.5 \times 10^4$  per well of a 24-well plate (MTS assay) or  $7.5 \times 10^4$  per Petri dish ( $\varnothing = 60$  mm, live/dead staining). Cells were allowed to attach for one day before treatment. Subsequently, they were exposed to different concentrations of Ag-NPs, i.e. 14 µg/mL, 7 µg/mL, and 3.5 µg/mL for 10 min (group A), for 1 h (group B) and 24 h (group C). Subsequently, the cells were washed and cultured in expansion medium for up to 24 h (live/dead staining and MTS assay) since the addition of the Ag-NPs.

For the live/dead staining, the cells were washed with phosphate buffered saline (PBS) and stained with 2.5 mL buffer solution of acridine orange (green fluorescence, all cells) and propidium iodine (red fluorescence, dead cells) for 2.5 min. Subsequently, the dye solution was removed, and the cells were rinsed with PBS again. The cells were examined using a Leica TCS SP8 fluorescence microscope at 100× magnification and excitation of 450–490 nm (all cells) and 515–560 nm (dead cells).

For the MTS assay, hMSCs were rinsed with a-MEM and incubated with 0.5 mL of a-MEM w/o FBS, with addition of 100 µL of MTS (CellTiter 96 AQueous One Solution Cell

Proliferation Assay, Promega, Madison, WI, USA) for 1 h and the absorbance value was read at 490 nm. Cells cultured without the addition of Ag-NPs served as controls.

#### 2.4. Bacterial strains and culture conditions

For antibacterial activity of Ag-NPs, four drug-sensitive bacterial strains, consisting of methicillin-susceptible *Staphylococcus aureus* (MSSA, ATCC 29213), *E. coli* (ATCC 25922), *Lactobacillus acidophilus* (ATCC 314), and *Streptococcus mutans* (ATCC 25175) and two multidrug-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA, NTC 12493) and clinical isolate of multidrug-resistant, inducible macrolide-lincosamide-streptogramin B-resistant *Staphylococcus haemolyticus*, were used in this study. All bacterial strains, except *L. acidophilus*, were grown on Mueller Hinton Agar (MHA) at 37°C. *L. acidophilus* was cultured on deMan–Rogosa–Sharpe (MRS) agar at 37°C.

#### 2.5. Well-diffusion method

Agar well-diffusion method is widely used to evaluate the antimicrobial activity of antimicrobial products and is a modification of the reference disc-diffusion method (European Committee on Antimicrobial Susceptibility Testing [EUCAST]). For the well-diffusion method, bacterial suspensions (MacFarland = 0.5) were inoculated on MHA or MRS. After 15 min of being allowed to dry, 6-mm diameter wells were punched out of the agar using a cork border. Each well was filled with 50 µL of Ag-NPs dilutions. A gentamycin disc (30 µg/mL) was used as a reference antibiotic against bacterial species (positive control). The plates were incubated for 24 h at 35°C.

#### 2.6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The standard microdilution method (according to EUCAST) was used for evaluating the MIC of Ag-NPs on tested bacterial strains. Serial two-fold dilutions of Ag-NPs in concentrations ranging from 20 µg/mL to 0.019 µg/mL were prepared in a 96-well micro titer plate. Bacterial suspensions were prepared in Mueller-Hinton Broth (MHB, BioMérieux, Lyon, France) and adjusted to a density of 0.5 on the McFarland scale ( $1.10^8$  colony forming units [CFUs]/mL). This suspension was then diluted  $20 \times (1/20)$  to a density of  $5 \times 10^6$  CFU/mL. About 180 µL of the tested substance in MHB and 20 µL of the bacterial suspension were added to the wells of the microplate, obtaining a final concentration in the well of  $5 \times 10^5$  CFU/mL (dilution 1/10). The last three wells were positive and negative controls, respectively. The positive control was inoculated with bacterial suspension only, while the

negative well was left blank without inoculum. The plate was incubated for 18–24 h at 35°C. The MIC was recorded as the lowest concentration of Ag-NPs where no visible growth is seen in the wells of the 96-well micro titer plate.

After the MIC determination of the Ag-NPs, aliquots of 10 mL out of each well were inoculated onto the MHA or MRS agar plates and incubated for 24 h. The lowest concentration that showed no growth after 24 h gave the MBC value.

#### 2.7. Statistical analysis

All numerical data are presented as the mean  $\pm$  SD and were subjected to post hoc statistical analysis of variance (ANOVA with a Tukey–Kramer pair-wise comparison test; (KyensLab Inc.) and significance was set at  $P < 0.05$ . All experiments were performed in three independent replicates.

### 3. Results

#### 3.1. Nanoparticles size and shape check

The nanoparticle size plays a crucial role for their effective biological applications. Therefore, to analyze the size of the Ag-NPs, the TEM image of the nanoparticles provided by the manufacturer (Sigma-Aldrich; Figure 1a and 1b) was digitized, filtered to binary forms, and analyzed using a computer image analyzer. The obtained experimental size distribution of the particles is shown in Figure 1c. By measuring the size of the particles, it was determined that the average size of >50% of the particles was between 7 nm and 8 nm, ranging 4–12 nm.

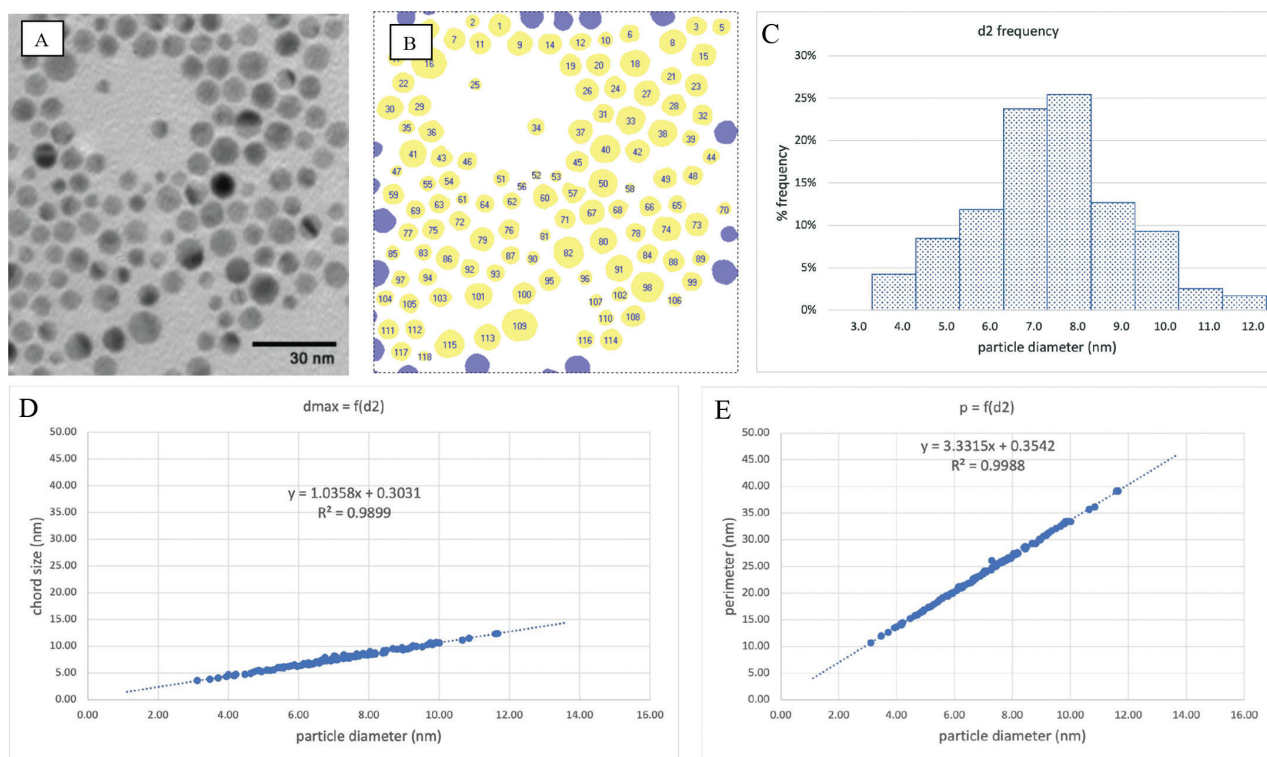
The shape of the particles has been quantified using two shape factors defined as:

- A) Maximum chord length to equivalent diameter
- B) Perimeter of the projected image to equivalent diameter

These two parameters were equal to 1 and Pi, respectively, for spheres, and achieve higher values with elongation of the particles. The measured values of the maximum chord and the perimeter are plotted against the equivalent diameter for individual particles in Figure 1d and 1e. The results presented in Figure 1d and 1e clearly prove the near-spherical shape of the tested particles.

#### 3.2. MTS assay

The test involves the reduction of the MTS compound to a water-soluble formazan by metabolically active cells. Thus, the concentration of the reaction product depends on both the number of viable cells and their metabolic activity. As presented in Figure 2a–2c, the dose-dependent effect of Ag-NPs on the metabolic activity of hMSCs was visible only



**Fig 1.** Nanoparticles size and shape analysis (A) Representative micro-graphs (TEM) of Ag-NPs provided by the manufacturer (<https://www.sigmaaldrich.com/PL/pl/product/aldrich/730785>) and (B) computer-processed image for size measurements. (C) Histogram of size distribution function with size defined as an equivalent diameter. (D) Values of a maximum chord plotted against the equivalent diameter for individual particles. (E) Values of a perimeter plotted against the equivalent diameter for individual particles. Ag-NPs, silver nanoparticles.

after 24 h of incubation with the nanoparticles. At shorter exposure times, i.e. for 10 min and 1 h, the metabolic activity of hMSCs was similar for the concentrations of 7 µg/mL and 14 µg/mL, and in both cases, it was significantly lower than for the concentration of 3.5 µg/mL. Of note, the concentration of Ag-NPs equal to 3.5 µg/mL seemed to have a stimulatory effect on hMSCs' activity (higher at each incubation interval than in the control); moreover, the metabolic activity increased with the time of incubation from  $106 \pm 4\%$  after 10 min to  $122 \pm 9\%$  after 24 h).

To sum up, the results obtained in the MTS assay show that Ag-NPs do not have a negative impact on the metabolic activity of the hMSCs at any of the tested concentrations. Minimal cytotoxic effect was observed at a concentration of 14 µg/mL after 10 min and 24 h of incubation.

### 3.3. Staining with acridine orange and propidium iodide (live/dead staining)

Acridine orange is a dye that can penetrate the cell membrane of both living and dead cells. Propidium iodide, however, can only enter cells through the disintegrated cell membrane and stains the nucleus of a dead cell. Figure 3a–3c show

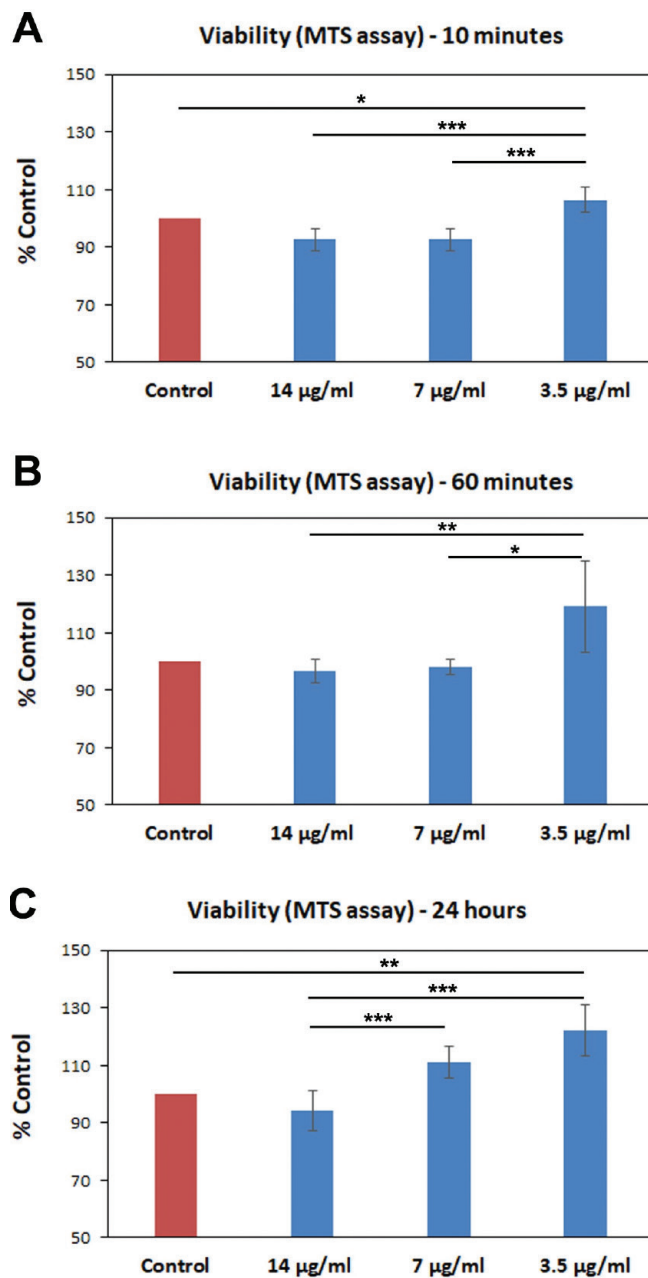
examples of control cells stained with acridine orange and propidium iodide.

Comparing the images of the cells incubated with Ag-NPs and controls, it can be seen that 1 day after adding Ag-NPs to the cell cultures, no dead cells were observed (no red-stained cell nuclei in Figures 4–6b,d, and f). Viable cells had normal morphology. Agglomerates of Ag-NPs absorbed by the cells are visible as brighter points surrounding the cell nucleus (Figures 4–6).

### 3.4. Antimicrobial activity of Ag-NPs

The antimicrobial potential of Ag-NPs against the tested bacterial pathogens was first evaluated by the well-diffusion method. Initial screening revealed that Ag-NPs have good antibacterial activity against drug-sensitive and multidrug-resistant pathogens (Figure 7). The compound was most effective against *S. aureus*, both drug-sensitive (MSSA) and drug-resistant (MRSA) strains and multidrug-resistant *S. haemolyticus*, as the lowest concentration of 5 µg/mL successfully inhibited the growth of the bacteria. The highest concentration of Ag-NPs was needed to inhibit the growth of the remaining tested bacteria (*E. coli*, *S. mutans*, and *L.*

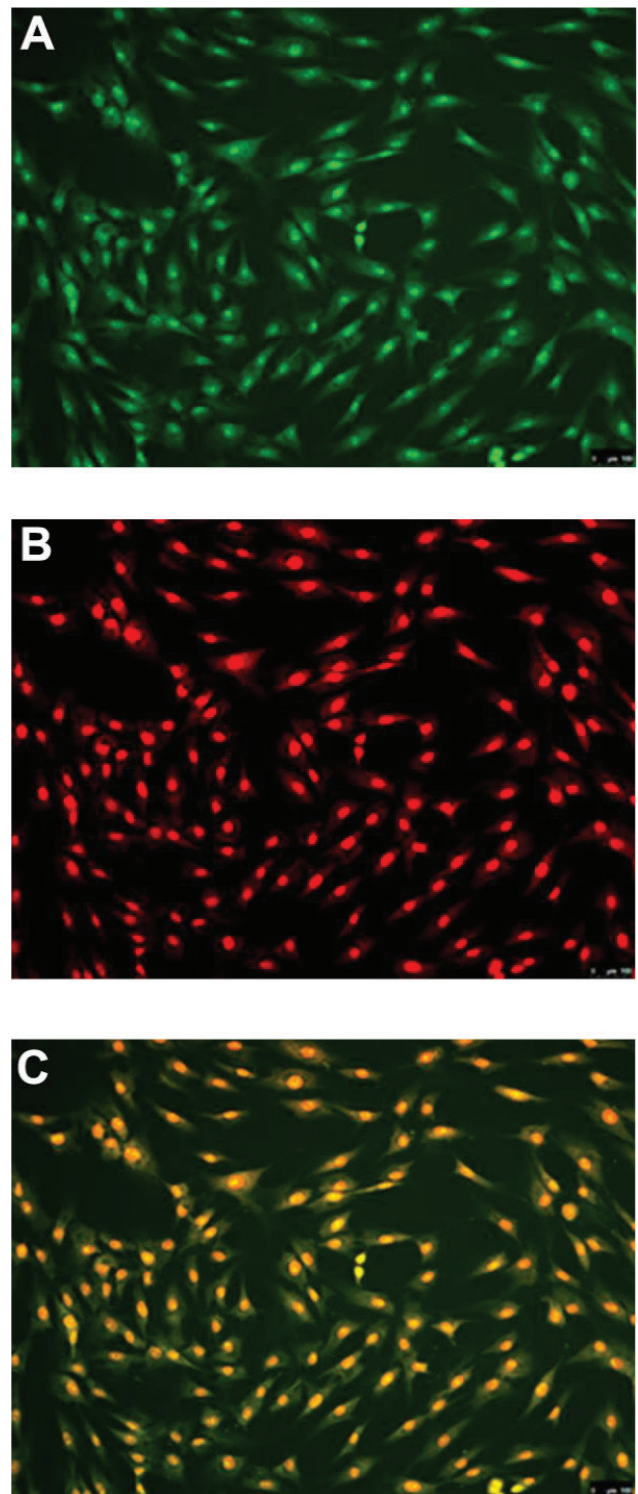




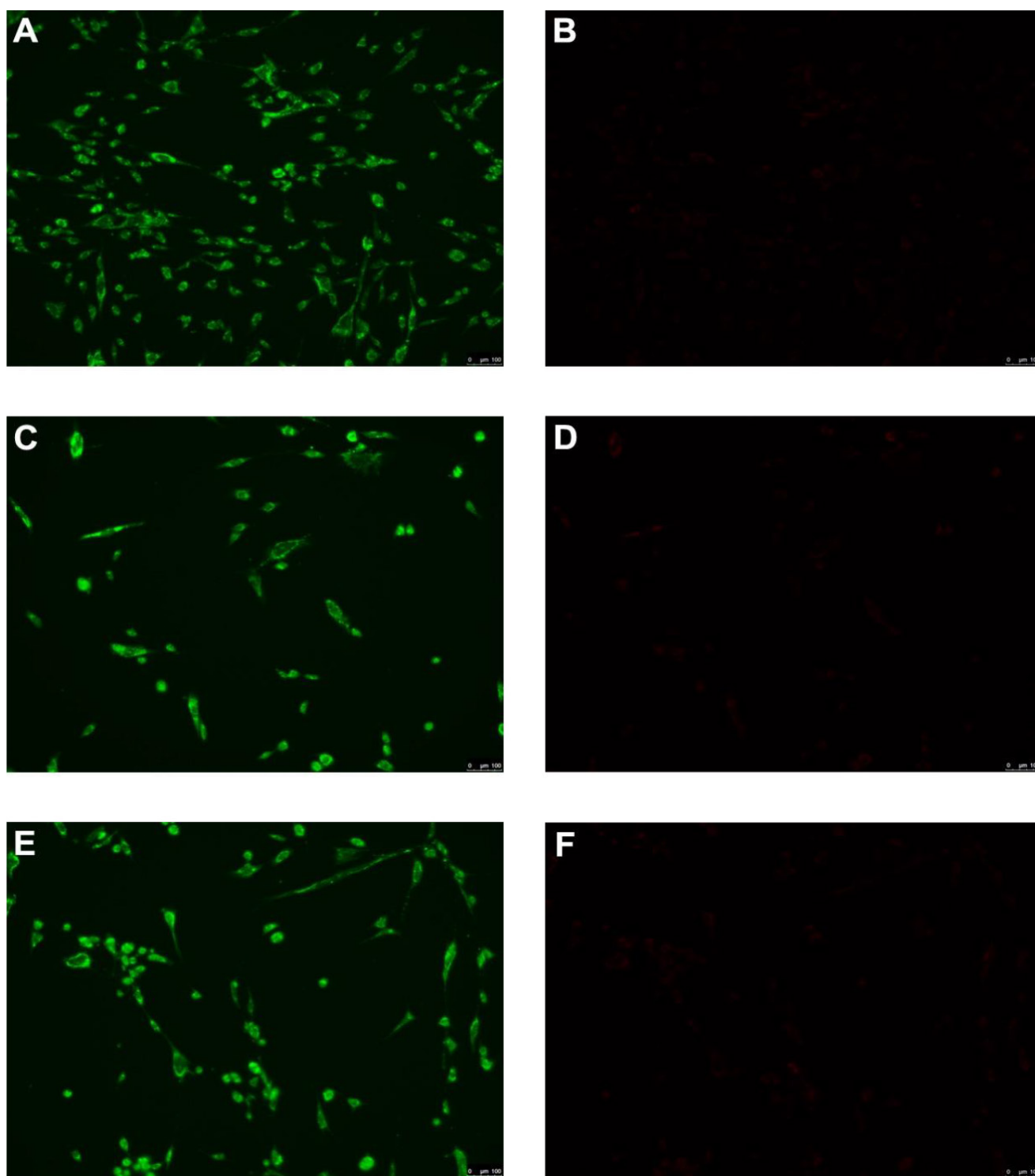
**Fig 2.** Metabolic activity of hMSCs incubated with Ag-NPs at a concentration of 0 µg/mL, 3.5 µg/mL, 7 µg/mL, and 14 µg/mL for (A) 10 min, (B) 60 min, and (C) 24 h. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Ag-NPs, silver nanoparticles.

*acidophilus*). Growth inhibition here was observed at the 10 µg/mL concentration well.

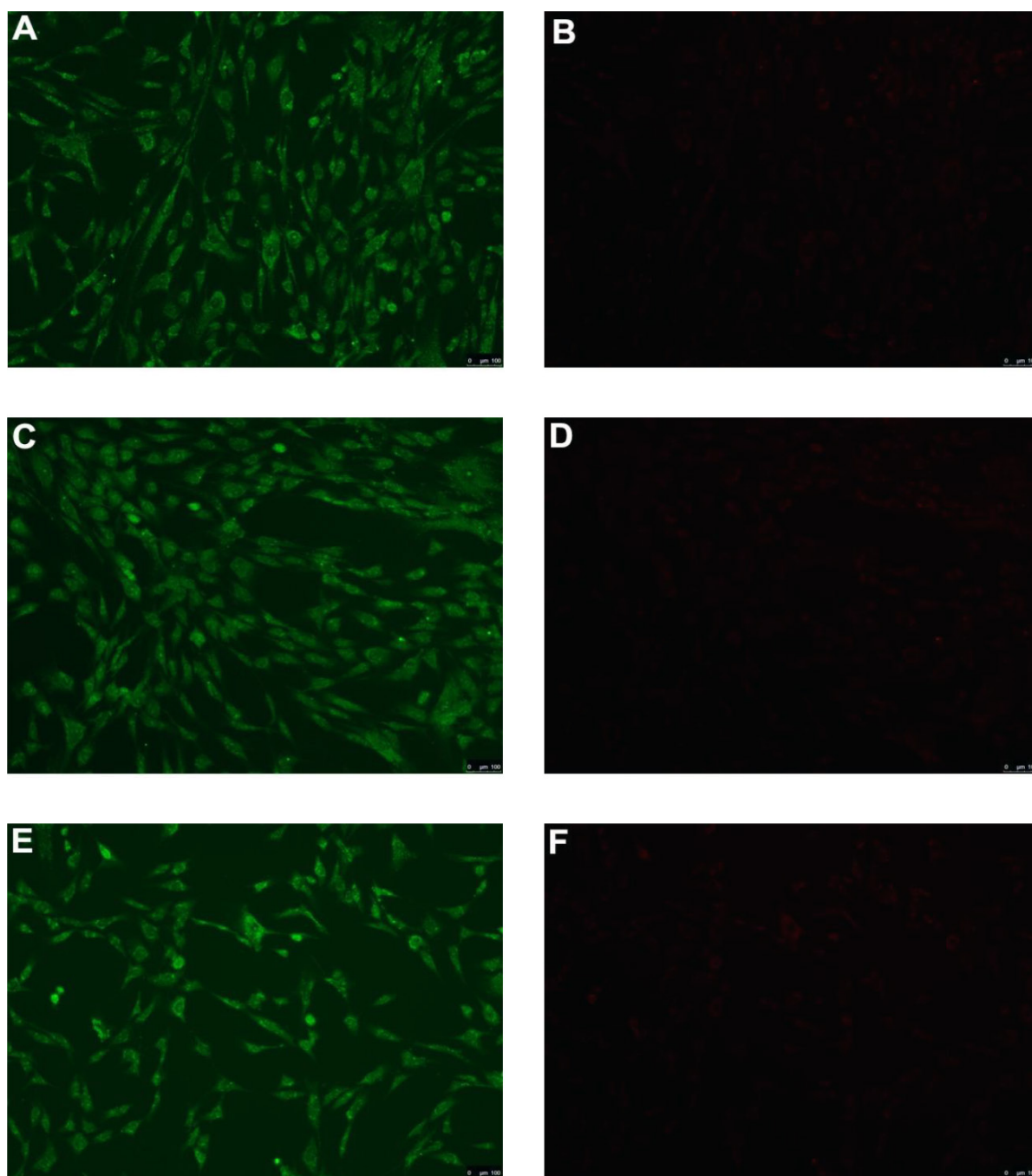
To investigate the potential bacteriostatic/bactericidal effect of the tested agent on bacterial pathogens *in vitro*, in the next stage of the study, its MIC and MBC values were determined in relation to the tested bacterial strains.



**Fig 3.** Representative images of hMSC cells stained with acridine orange and propidium iodide: all cells (A) (green); dead cells (red) (B) and overlap of both (C). hMSCs were fixed with MeOH, which caused cell death and membrane permeabilization.

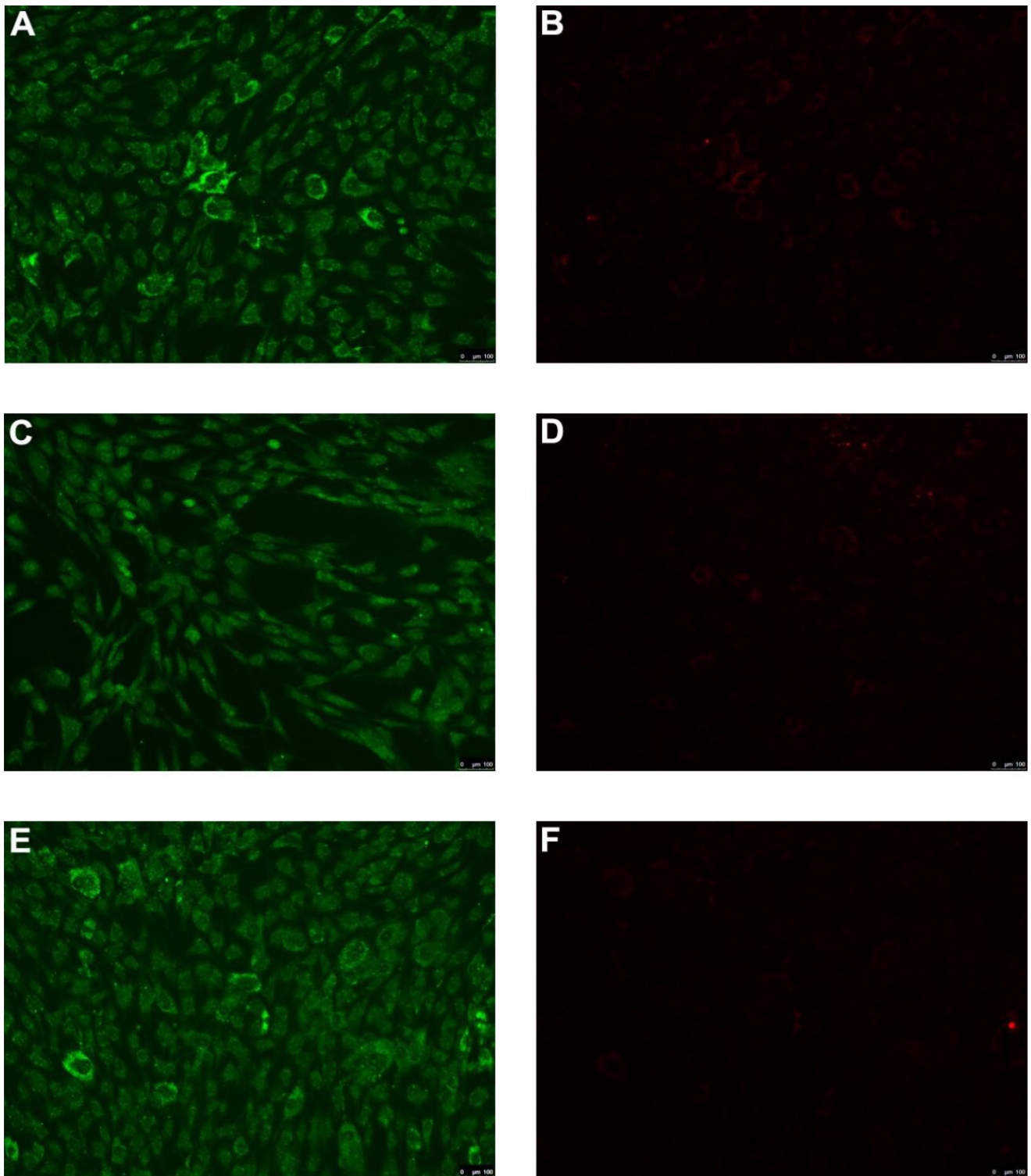


**Fig 4.** hMSCs incubated with Ag-NPs at a concentration of 14 μg/mL (A,B); 7 μg/mL (C,D); and 3.5 μg/mL (E,F) for the duration of 10 min. Green color—all cells (a,c,e); Red color—dead cells (b,d,f). Ag-NPs, silver nanoparticles.



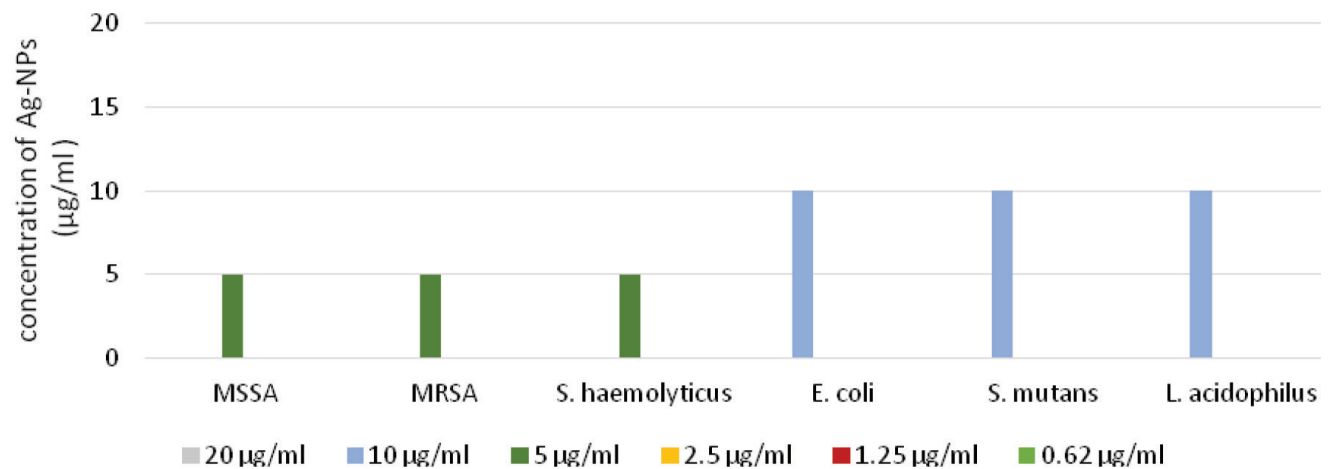
**Fig 5.** hMSCs incubated with Ag-NPs at a concentration of 14 µg/mL (A,B); 7 µg/mL (C,D); and 3.5 µg/mL (E,F) for the duration of 1 h. Green color—all cells (a,c,e); Red color—dead cells (b,d,f). Ag-NPs, silver nanoparticles.





**Fig 6.** hMSCs incubated with Ag-NPs at a concentration of 14 µg/mL (A,B); 7 µg/mL (C,D); and 3.5 µg/mL (E,F) for the duration of 24 h. Green color—all cells (a,c,e); Red color—dead cells (b,d,f). Ag-NPs, silver nanoparticles.





**Fig 7.** Antimicrobial activity of Ag-NPs against bacterial strains determined with the well-diffusion method. Bars represent the zones of inhibition of different concentrations of Ag-NPs for the tested bacterial strains. The data represent mean  $\pm$  SD ( $n = 3$ ). Ag-NPs, silver nanoparticles; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

**Table 1.** MIC and MBC values ( $\mu\text{g/mL}$ ) of Ag-NPs for different bacterial strains

	Drug-sensitive strains			Multidrug-resistant strains		
	<i>S. aureus</i> MSSA	<i>S. mutans</i>	<i>E. coli</i>	<i>L. acidophilus</i>	<i>S. aureus</i> MRSA	<i>S. haemolyticus</i>
MIC $\mu\text{g/mL}$	5	10	5	5	5	5
MBC $\mu\text{g/mL}$	20	10	20	5	20	5
MBC/MIC ratio	4	1	4	1	4	1

Ag-NPs, silver nanoparticles; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*.

As indicated in Table 1, the MICs for the bacteria tested ranged from 5  $\mu\text{g/mL}$  to 10  $\mu\text{g/mL}$ . It was also found that the MBC/MIC ratio of Ag-NPs against drug-sensitive and against multidrug-resistant bacteria tested was between 1 and 4. This indicates a good bactericidal effect of the Ag-NPs against these strains.

MIC is the lowest concentration of Ag-NPs that inhibits bacterial growth and MBC is the lowest concentration of Ag-NPs that kills bacteria. The table shows the concentration ( $\mu\text{g/mL}$ ) of Ag-NPs that inhibited (MIC) or killed (MBC) the tested organisms. The values are given as the highest value out of three replicates.

## 4. Discussion

Due to their unique properties, Ag-NPs are widely used in various fields, including food storage, medical, and the health care industry. According to Liu et al. (2015), 383 consumer products that are currently on the market contain Ag-NPs, inclusive of furniture, textiles, kitchen clothes, refrigerators, cosmetic products, towels, and medical devices such as wound dressings and implant surfaces (Sharma and Sharma 2007). Thus, prolonged and frequent exposure to the Ag-NPs

in daily life is common. However, the widespread use of Ag nanoparticles in commercial products raises concerns about their potential risks to human health and the environment. Indeed, most Ag-NPs have a toxic effect on different cell types *in vitro*, including macrophages, bronchial epithelial cells, hepatocytes, and skin keratinocytes where, by affecting the cellular DNA, they lead to its damage and, consequently, to cell death by apoptosis (Ferdous and Nemmar 2020). hMSCs may also come into close contact with Ag-NPs during various medical procedures, including the implantation of Ag-NP-coated devices (Greulich et al. 2012). Despite the high degree of commercialization of Ag-NPs, the effect of Ag-NPs on human mesenchymal stem cells is not fully understood; therefore, the aim of this study was to investigate the mechanism of action of Ag-NPs on hMSCs by analyzing the cellular uptake of Ag-NPs by the cells and its effect on the viability of these cells in relation to different exposure times and concentrations of Ag-NPs.

Ag-NPs are widely known for their strong, broad-spectrum antibacterial activity. Their antibacterial properties are used to control the presence of bacteria in a variety of applications, including dentistry, surgery, and in the treatment of wounds and burns (Chalas et al. 2015; Stachurski et al. 2023).

However, too frequent exposure to products containing nanosilver in sublethal amounts raises widespread concerns that it will lead to the development of silver-resistant microorganisms (Durán et al. 2010; Shih et al. 2023). Therefore, our next goal was to investigate the antibacterial properties of Ag-NPs against emerging bacterial strains, including multidrug-resistant pathogens, by determining the minimum bacteriostatic and bactericidal concentrations of the compound. When considering these issues, it is then crucial to determine the appropriate concentration of Ag-NP that will have antibacterial properties and at the same time will not be toxic to the surrounding stem cells.

With an increase in antibiotic resistance, the development of new antimicrobial therapeutic agents, especially against multidrug-resistant pathogens, is of great concern (Durán et al. 2010). The ability of Ag-NPs to inhibit the growth of drug-sensitive and multidrug-resistant bacteria confirmed that the nanoparticles had good potential to be used as antimicrobial agents against pathogenic microorganisms. Although the exact antibacterial mechanism of Ag-NPs has not been elucidated yet, the most popular theory is based on the assumption that Ag-NPs interact with sulfur-containing groups in bacterial cell wall proteins thus blocking respiratory enzymes, inhibiting DNA replication, and eventually leading to cell death (Parvekar et al. 2020; Shih et al. 2023). An important advantage of Ag-NPs over most antibiotics is that it has a multitargeted antibacterial mechanism and is therefore effective against a broad spectrum of bacteria (McNeilly et al. 2021).

The antimicrobial activity of Ag-NPs is highly dependent on the particle size, shape, stability, and concentration. For spherical particles of smaller size, ranging 1–10 nm, the greatest antibacterial effect is gained. This is associated with better physical nanoparticle-to-cell contact and, consequently, better penetration of silver ions into the bacterial cell leading to structural and morphological changes, which can lead to cell death (Salomoni et al. 2017; McNeilly et al. 2021). Based on these facts, it can be concluded that with the decreasing nano particle size, better antibacterial effect can occur at a lower concentration (Parvekar et al. 2020; McNeilly et al. 2021).

In the present study, commercially available Ag-NPs of nearly spherical shape and the size of <10 nm were used. Consistent with previous reports, they had dose-dependent inhibitory effects on the bacterial species tested, demonstrating the maximum activity against *S. aureus* MSSA, *L. acidophilus*, *E. coli*, and drug-resistant MRSA and *S. haemolyticus* at 5 µg/mL. This result is in line with the studies of Salomoni et al. (2017), who found that the antibacterial effectiveness of Ag-NPs against clinical strains of *Pseudomonas aeruginosa* with a high degree of antibiotic resistance was excellent at 5 µg/mL. Similarly, Kvitek et al. (2011) demonstrated that silver NPs with 25 nm diameter were most effective against important drug-sensitive and multidrug-resistant bacteria at the MIC around 4 µg/mL when compared to particles measuring 35–50 nm. Therefore, by

controlling the physicochemical characteristics and the concentration of the Ag-NPs used, the antibacterial activity of nanosilver can be controlled.

The MBC/MIC ratio defines the bacteriostatic or bactericidal character of the tested compound. If the ratio is <4, then the antibacterial agent is considered bactericidal. The MBC/MIC ratio of the Ag-NP in our setting had a tolerance level  $\leq 4$ , which confirms its excellent bactericidal effect against all tested bacterial strains. Of note, there was no significant difference in the antimicrobial activity of Ag-NPs against drug-sensitive strains when compared to drug-resistant pathogens, suggesting that the antibiotic-resistance mechanisms have no impact on the susceptibility to Ag-NPs. This is in agreement with other studies showing the bactericidal nature of the nanoparticles against different groups of bacterial pathogens (Salomoni et al. 2017; Mondal et al. 2020; McNeilly et al. 2021). As confirmed by Morones et al. (2005), the bactericidal properties of nanoparticles depend largely on their size, since the only nanoparticles that show direct interaction with bacteria preferentially have a diameter of ~1–10 nm.

Due to their good antiseptic properties, silver nanoparticle-based biomaterials, such as implant scaffolds, are commonly used in tissue engineering or regenerative medicine, so that they may come into close contact with mesenchymal stem cells. Thus, their potential negative impact on their viability and metabolism should be minimal. The toxicity of Ag-NPs to eukaryotic cells is determined by the same factors as in the case of bacterial cells and depends, among others, on the size of the particles, their concentration, and the time of incubation (Sengstock et al. 2014; Salomoni et al. 2017; Yang et al. 2020; Algazlan et al. 2022). It has been proven that silver particles can easily penetrate biological membranes, accumulating in the cell cytoplasm. In response, the cell generates reactive oxygen species, mainly responsible for the cytotoxic effect of Ag-NPs (Sengstock et al. 2014; Yang et al. 2020). In this study, we have shown that the Ag-NPs of size <10 nm do not have a negative effect on the metabolic activity of hMSCs only at a concentration of 3.5 µg/mL, with the minimal cytotoxic effect being observed at a concentration of 14 µg/mL after 24 h of incubation. Several previous reports have investigated the cytotoxicity of Ag-NPs toward various cell types *in vitro* and *in vivo*. The common conclusion is that the toxicity of Ag-NPs increases with decreasing particle size, but their toxicity is also dose-dependent. Park et al. (2011) demonstrated that exposure of the murine fibroblasts L929 cell line to Ag-NPs of 20 nm resulted in cell-membrane damage and a decrease in metabolic activity depending on the concentration (Park et al. 2011). Greulich et al. (2012) reported that Ag-NPs (70 nm) at concentrations of 12.5 µg/mL to 50 µg/mL showed toxic effects toward human mesenchymal stem cells and peripheral blood mononuclear cells. In another study, 30 nm Ag-NPs were reported to

cause a reduction in the viability and mitochondrial membrane potential of hMSC in a dose-dependent manner (He et al. 2016).

## 5. Conclusions

In conclusion, our results indicate that the exposure of hMSCs to Ag-NPs of size <10 nm has no cytotoxic effect on the metabolic activity of the cells at the concentration of 3.5 µg/mL, with the minimal cytotoxic effect being observed at the concentration of 14 µg/mL after 24 h of incubation. Our findings also confirmed that Ag-NPs measuring <10 nm and at the concentration of 4 µg/mL are effective broad-spectrum bactericidal agents, regardless of the antibiotic-resistance mechanism present in the bacteria. Our results therefore prove that at suitable concentrations, Ag-NPs do not cause cytotoxicity to hMSCs, but eradicate microorganisms. The next step will be to study the effect of Ag-NPs on the proliferation and differentiation of hMSCs.

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## Author Contributions

Conceptualization, R.C.; methodology, R.C., A.M. and J.I.; software, K.J.K.; validation, R.C., M.K. and K.J.K.; formal analysis, R.C., J.I., P.M., M.P., A.M. and K.J.K.; investigation, J.I., K.J.K., A.M. and M.K.; resources, R.C.; data curation, J.I. and K.J.K.; writing—original draft preparation, J.I., M.K., A.M. and K.K.; writing—review and editing, R.C., P.M., M.P.; visualization, R.C., J.I. and K.J.K.; supervision, R.C.; project administration, R.C.; funding acquisition, R.C.

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## Conflict of Interest

The authors declare no conflict of interest.

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