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Bioeffects of silver nanoparticles (AgNPs) synthesized by producer of biosurfactant *Bacillus subtilis* strain: in vitro cytotoxicity, antioxidant properties and metabolic activities of mammalian cells

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Abstract: The present study is focused on the evaluation of bioeffects of silver nanoparticles (AgNPs) synthesized by *Bacillus subtilis* strain I'-1a, the producer of iturin A lipopeptide biosurfactant. The following properties of biologically synthesized silver nanoparticles (bio-AgNPs) were evaluated: in vitro cytotoxicity, antioxidant properties, and metabolic activities of mammalian cells. As a control, chemically synthesized silver nanoparticles (chem-AgNPs) were used. In vitro, antioxidant activity of bio-AgNPs showed a significant effect on the scavenging of free radicals. Bio-AgNPs can be potent natural antioxidants and can be essential for health preservation against oxidative stress-related degenerative diseases, such as cancer. The cell viability of human skin fibroblasts NHDF was remarkably inhibited in the presence of both AgNPs. However, bio-AgNPs were more active than chem-AgNPs. In our experiment, microarrays PM-M1–PM-M4 were used to evaluate the growth of NHDF fibroblast cells in the presence of bio-AgNPs and chem-AgNPs. The NHDF fibroblast cells were more active in the presence of bio-AgNPs than in chem-AgNPs. Probably, the presence of biosurfactant produced by *Bacillus subtilis* I'-1a significantly increased the stability of biogenic AgNPs and enhanced their biological activities and specific interaction with human DNA. Furthermore, the evaluated biological activities were enhanced for the biosurfactant-based AgNPs.

Introduction

Green nanotechnology (or green nanobiotechnology) uses bacteria, yeast, actinomycetes, fungi, plant extracts, biopolymers, vitamins, biosurfactants and enzymes for the synthesis of nanomaterials (or nanoparticles) (Pal et al. 2019, Zhang et al. 2020, Giri et al. 2022, Jadoun et al. 2022, Ying et al. 2022). Some of them, like biosurfactants, act as reducing and capping/stabilizing agents in the production of AgNPs. Various biotechnological processes are used in the synthesis of biogenic nanoparticles. The green synthesis technology is considered to be non-toxic, using non-toxic phytochemicals, avoiding dangerous ingredients that are used in chemical synthesis, ecofriendly – no wastes are produced, simple, cost-effective, therefore, it has been widely used in the recent research. A large number of nanoparticles generated by biological routes have been successfully used in various applications such as biomedical applications, electronics, mechanics, optics, in food, chemical and pharmaceutical industries, and environmental remediation (Selvakesavan and Franklin 2021, Shreyash et al. 2021, Santhosh et al. 2022). Unfortunately, nanoparticles have not only benefits but also bring risks. The small size allows for overcoming various structural barriers unnoticeably, providing easy penetration into the cells of living organisms, including humans. Exposure to nanoparticles has thus become a serious threat. Among all metal nanoparticles, silver nanoparticles (AgNPs) are the most studied ones because they have unique properties, such as catalytic properties and good conductivity, and a wide range of bioactivities, such as antifungal, anti-oxidant, antibacterial, anti-inflammatory and anticancer effects (Yugal et al. 2017, Chojniak et al. 2018, Ahn et al. 2019, Keshari et al. 2020, Shahzadi et al. 2022).

There are several hypotheses explaining the reaction of AgNPs with cells. AgNPs react with cell surface and inactivate processes by acting on the cell membrane or AgNPs penetrate the cell and release highly reactive forms which react with cell organelles. The nanoparticles get mainly into the mitochondria, which contributes to the increased production of reactive oxygen species (ROS), leading to the oxidative stress. This situation causes a lot of changes in the processes taking place in the cells, such as lipid peroxidation (changes in the properties

of biological membranes), lipoprotein modification or protein and nucleic acid damage (lack of enzymatic activity). Such dysfunctions of cells are considered to be the main causes of the cell death (apoptosis or necrosis) through the inactivation of DNA and ATP production. Due to these facts it has become necessary to verify the impact of biogenic silver nanoparticles (bio-AgNPs).

Hence, in this study, an attempt was made to evaluate the bioeffects of bio-AgNPs, *e.g.*, in vitro cytotoxicity, antioxidant properties and metabolic activities of mammalian cells of silver nanoparticles synthesized using biosurfactant produced by *Bacillus subtilis* strain I'-1a. In our previous study (Chojniak et al. 2018), it was observed that biogenic silver nanoparticles (bio-AgNPs) synthesized by biosurfactant produced by *Bacillus subtilis* strain I'-1a showed a broad spectrum of activity against environmental bacteria and fungi. Bio-AgNPs were most active against phytopathogenic fungi and Gram-positive bacteria, whereas less active against Gram-negative bacteria.

Materials and methods

Growth of Bacillus subtilis strain I'-1a

Bacillus subtilis I'-1a was characterized and its biosurfactant production was described by Bernat et al. (2016) and Płaza et al. (2015). The bacterial culture was grown in liquid Luria-Bertani medium (Tryptone, 10 g/L; NaCl, 10 g/L; Yeast extract, 5 g/L) at 30°C, under aerobic conditions with constant shaking (100 rpm) (Innova 42 Incubator, New Brunswick Scientific, USA) for 96 h. After culturing, the bacterial culture was centrifuged at 10,000 g (Eppendorf) for 10 min. The supernatant was collected and filtered through sterile 0.22 µm filter (Whatman). Cell-free supernatant was used to synthesize bio-AgNPs.

Synthesis and characterization of bio-AgNPs

Biological synthesis of AgNPs was carried out as described by Płaza et al. (2016). The formation of silver nanoparticles was evaluated by UV-Vis absorption spectroscopy (Eppendorf). The absorption peak of AgNPs was monitored within the range of λ= 300–700 nm. Sizes of the silver nanoparticles were measured using the Dynamic light scattering (DLS) technique on a Brookhaven BI-200 goniometer. Zeta potential was measured with Zetasizer Nano ZS 90 (Malvern Instruments). Transmission electron microscopy (TEM) was used to evaluate the size and shape of nanoparticles. The concentration of AgNPs was evaluated by atomic absorption spectroscopy (AAS). The chemical silver nanoparticles (chem-AgNPs) were used as a control. The synthesis of chem-AgNPs was performed as described by Mendrek et al. (2016).

Antioxidant assay

Antioxidant properties of AgNPs were evaluated as the scavenging activity of *1,1-diphenyl-2-picrylhydrazyl* (*DPPH*) radical, as described by Mensor et al. (2001). 1 mL of 0.1 mM DPPH solution of a purple color was added to 1 mL of AgNPs solution (1:1). The mixture was incubated for 30 minutes in the dark. Afterwards, the absorbance at a wavelength of 517 nm was measured. As a control, 2 ml of methanol was added instead of silver nanoparticles.

Antioxidant activity was calculated according to the following formula:

$$DPPH\ activity\ (\%) = \frac{[(Abs\ control - Abs\ sample)]}{\text{Abs control}}\ x\ 100$$

Abs control is DPPH solution + methanol (1:1 ratio)
Abs sample is the absorbance of DPPH radical + nanoparticles

Cell culture

The normal human skin fibroblasts NHDF (CC-2511 Lonza, Basel, Switzerland) obtained from the Department of Biochemistry at the Medical University of Silesia were used. The cells were incubated in the atmosphere of 5% CO_2, at 37°C in Direct Heat CO_2 Incubator (Thermo Scientific, Waltham, MA, USA), in the FBM (Fibroblast Basal Medium; Lonza, Basel, Switzerland), enriched with hFGF-B (Human Fibroblast Growth Factor-basic), insulin and gentamicin (FGMTM SingleQuotsTM, Lonza, Basel, Switzerland). The cells were seeded onto the plates at the density of around 1×10^6 cells per well and incubated for 24 h prior to the experiment.

MTT cell viability assay

To determine the cytotoxic effects of AgNPs, cell viability study was conducted using the conventional MTT reduction assay. This is a colorimetric assay based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) (Sigma-Aldrich) to purple formazan crystals by metabolically active cells.

The 24 hour culture of NHDF fibroblast cells was incubated as described in "Cell culture" section. After incubation, the attached cells were trypsinizated for 3–5 min in 37°C, 5% CO_2 to get the individual cells (0.05% solution of EDPS trypsin, Gibco, USA). Then, they were centrifuged (800 rpm, 10 min). The number of cells in 1 ml of culture medium was determined using a light microscope (Zeiss). The inoculum size was optimized before the start of the experiment. Different volumes of AgNPs solutions were used to check the toxic dose for the cells tested. 1.2, 5, 10, 15, 20, 30, 50, 75, 100, 120 µL of each solution were tested in the study. Three independent replicates were used for each dose. 1000 µl of medium with 17,000 cells was applied to the titration plates. Then, the plates were incubated for 24 hours. Cell viability in the presence of AgNPs was evaluated with the absorbance value obtained by measuring the color formed during the cell growth. The optical density of the formazan product was taken at 595 nm in the microplate reader (Biolog, Hayward, CA, USA). The cell growth was also checked by a light microscope (Zeiss).

Phenotype microarrays PMM

The Phenotype Mammalian Microarrays (PM-M) developed by Biolog company (Hayward, CA, USA) were used in the study. All reagents for the experiment were purchased from Biolog company (Hayward, CA, USA). The procedure was made according to a protocol developed by Bochner et al. (2011) and Biolog company (Hayward, CA, USA).

NHDF fibroblast cell suspension was inoculated in IF-M1 or IF-M2 fluids without carbon and energy sources. Then, 50 mL suspension (20,000 cells per well) was dispensed

into the four 96-well microplates from PM-M1 to PM-M4 (Biolog, Hayward, CA, USA) providing 367 chemicals. PM-M1 contains a range of diverse carbon sources including simple sugars, polysaccharides, and carboxylic acids. PM-M2 toPM-M4 contain lipids and protein-derived nutrients, primarily amino acids and dipeptides.

In the experiment, the concentration of bio-AgNPs and chem-AgNPs was determined on the basis of MTT assay. 30 µL of NPs solution was added to each well.

Finally, Biolog Redox Dye was added to the used PM-M plates. The plates were incubated in the Omnilog automated incubator-reader (Biolog, Hayward, CA, USA) for 24 hours at 37°C. During the incubation, tetrazolium dye was reduced to purple formazan. The color change was measured at 590 nm absorbance in 15-minute intervals using Biolog's Omnilog® instrument and software. The formazan production was also measured by an endpoint absorbance at 590 nm with a microplate reader (Biolog, Hayward, CA, USA). The results are presented as "Omnilog units" (OLU) and are generated by the Biolog Omnilog PM software from readings of the color intensity.

Interaction of AgNPs with human DNA

The commercially purchased human DNA of 10 ng/µL concentration (ThermoFisher, Applied Biosystems™ TaqMan™, catalog no. 4312660) was used. The reaction mixture contained 10 µl of AgNPs and 5 µl of human DNA and was incubated at 37°C for 30 min.

Cryogenic Transmission Electron Microscopy images were obtained using a Tecnai F20 X TWIN microscope (FEI Company, Hillsboro, Oregon, USA). Images were recorded on the Rio 16 CMOS camera (Gatan Inc., Pleasanton, USA) and processed with DigitalMicrograph software (Gatan Inc., Pleasanton, USA). Specimen preparation was done by vitrification of the aqueous solutions on grids with holey carbon film (Quantifoil R 2/2; Quantifoil Micro Tools GmbH, Großlöbichau, Germany). Prior to use, the grids were activated for 15 seconds in oxygen plasma using a Femto plasma cleaner (Diener Electronic, Ebhausen, Germany). Cryo-samples were prepared by applying 3 µL of the solution to the grid, which were then blotted with filter paper and immediately frozen in liquid ethane using a fully automated blotting device Vitrobot Mark IV (FEI Company, Hillsboro, Oregon, USA). After preparation, the vitrified specimens were kept under liquid nitrogen until they were inserted into a cryo-TEM holder Gatan 626 (Gatan Inc., Pleasanton, USA) and analyzed in the TEM at -178°C.

Statistical analysis

Statistical analyzes were performed using the TIBCO Statistica 13.3 software. Basic descriptive statistics was analyzed together with Shapiro-Wilk tests as well as multivariate analysis of variance in a mixed pattern. The classic threshold $\alpha = 0.05$ was assumed as the level of significance.

Results and discussion

Characterization of the AgNPs

The cell-free supernatant of *Bacillus subtilis* I'-1a was used for synthesis of biogenic AgNPs. The lipopeptide biosurfactant was produced by *Bacillus subtilis* I'-1a as it was previously described (Bernat et al. 2016). The UV-Vis spectra of bio-AgNPs and chem-AgNPs are shown in Figure 1. The change of color of reaction mixture to yellowish-brown confirmed the production of AgNP. Bio-AgNPs had the maximum absorbance at 425 nm. Similar results were obtained by Giri et al. (2022). The spectral analysis showed that the highest peak of AgNPs was obtained at 417 nm. Chem-AgNPs had the maximum absorbance at 400 nm. DLS study showed small size of bio-AgNPs ranging from 5 to 52 nm. Size of chem-AgNPs was greater – from 20 to 100 nm. The zeta potential of both bio-AgNPs and chem-AgNPs ranged from -23 to -26 mV. The synthesized AgNPs with the zeta potential of around -30 mV can be considered to be an adequately stable colloidal system and this proves the good stability of silver nanoparticles (Keat et al. 2015). The role of biosurfactants in NPs production has already been described in the literature (Reddy at al. 2009, Jimoh & Lin 2019, Durval et al. 2021). Biosurfactants are involved in the reduction of metal precursors and are used as stabilizing agents in the synthesis of metal NPs. Reddy et al. (2009) used surfactin, as a template and stabilizing agent in the green synthesis of metal NPs.

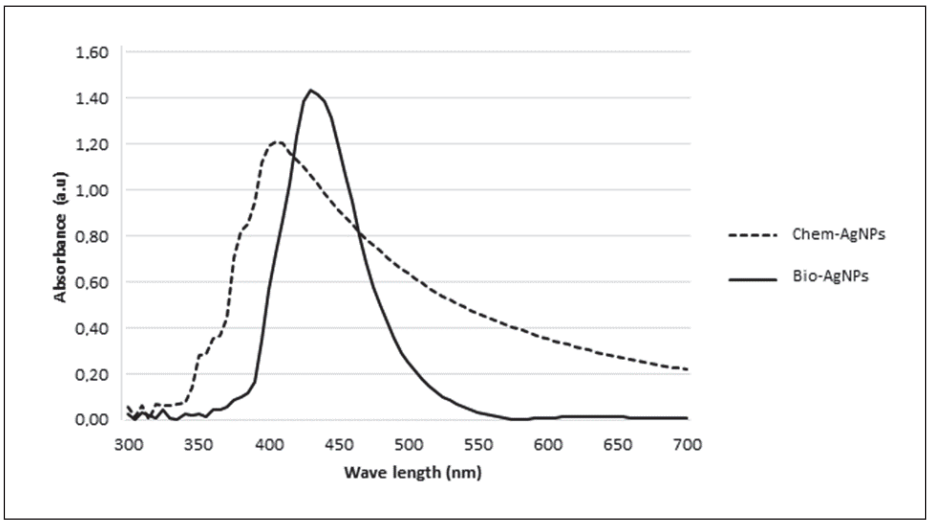


Fig. 1. UV-Vis spectra for bio-AgNPs and chem-AgNPs

Analysis using the transmission electron microscope (TEM) showed different shapes of AgNPs. Bio-AgNPs were oval and triangular, and these shapes are typical for biologically synthesized nanoparticles. The green-synthesized AgNPs are different in shape and size, but the most common forms are spherical, triangular, and hexagonal (Ying et al. 2022). However, chem-AgNPs had rectangular, triangular, and hexagonal shape. In Table 1, the properties of bio-AgNPs and chem-AgNPs are presented.

Evaluation of antioxidant properties of bio-AgNPs

The antioxidant potential was evaluated by DPPH radical scavenging assay. The change of color of the solution containing DPPH indicated the scavenging of free radicals and antioxidant activity. The results are compiled in Table 2 and they confirm that the bio-AgNPs had greater antioxidant activity in comparison to chem-AgNPs. Silver nanoparticles are known to exhibit high catalytic activity in certain oxidation reactions, such as CO oxidation, while silver as a compact metal is chemically inactive due to the size and nature of the interaction. Due to this information, biologically synthesized silver nanoparticles can lead to the increased production of reactive oxygen species, which in turn brings about the oxidative stress. The prolonged oxidative stress changes the properties of biological membranes, damages proteins and nucleic acids. These changes often lead to apoptosis or necrosis of the cell via the microchondrial cell death pathway. Our results were found to be similar to the results described in the literature for antioxidant activity of biologically synthesized AgNPs (Priya et al. 2015, Yugal et al. 2017, Keshari et al. 2020, Shahzadi et al. 2022). Bio-AgNPs, which are formed in the presence of biosurfactants as capping and stabilizing agents, showed higher antioxidant activity. Similar results were obtained by Priya et al. (2015) where the higher inhibitory action of biosynthesized AgNPs depended not only on the size of the nanoparticles, but also on capping agent of the nanoparticles. According to the literature, surface and chemical properties of NPs depend on capping and stabilizing agents which play essential role in free radical scavenging.

The cytotoxicity of bio-AgNPs

The results of cytotoxicity of bio-AgNPs on NHDF fibroblast cells are presented in Figure 2.

The cell viability of the tested cell line was remarkably inhibited in the presence of both AgNPs at a concentration of 30 µL/mL or higher. The decreased trend of cell viability on increasing volume of both AgNPs was observed, but bio--AgNPs were more active than chem-AgNPs. Bio-AgNPs significantly inhibited the cell viability. The changes in cell morphology were observed from a minimal dose of 5 µL/mL under a phase-contrast inverted microscope (20 × magnification). The findings are comparable to the previous reports stating that biosynthesized silver nanoparticles work effectively (Tariq et al. 2022). This activity might be due to the synergetic impact of AgNPs and bioactive compounds like biosurfactants which adhere to the nanoparticles' surfaces. The review of Liao et al. (2019) provides a state-of-the-art review on the synthesis of AgNPs and their bactericidal activity, and

Table 1. The properties of bio-AgNPs and chem-AgNPs

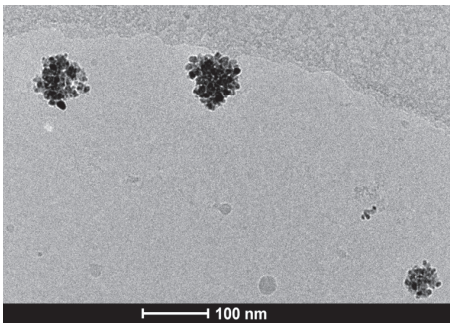
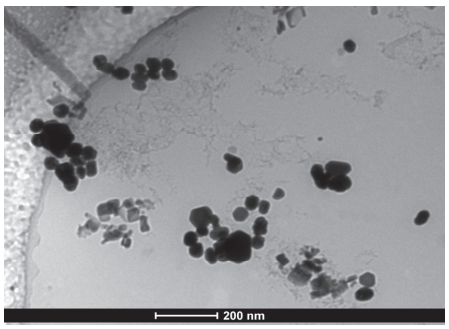
Properties	Bio-AgNPs	Chem-AgNPs
DLS (nm)	5–52	20–100
Zeta potential (mV)	-23	-26
TEM image		
AAS (mg/L)	165.5	90.81

Table 2. Antioxidant activity of biol-AgNPs and chem-AgNPs

AgNPs	pH solution	DPPH Scavenging activity (%)
Biologically synthesized nanoparticles (bio-AgNPs)	8.34	67.4 ± 0.28
Chemically synthesized nanoparticles (chem-AgNPs)	7.67	51.02 ± 0.37

cytotoxic effect in mammalian cells. The obtained results show that the biologically synthesized silver nanoparticles have an impact on NHDF fibroblast cells, however, additional studies are required to validate these results in vivo models.

Evaluation of metabolic ability of mammalian cells using Phenotype microarrays PMM

There are only a few papers describing the application of PMM to evaluate metabolic activities of different mammalian cells in the presence of various chemicals. PM-Ms were used to provide cellular fingerprint that was used to identify, for example, biomarkers, investigate gene function, validate drug targets, and streamline lead validation, optimization, and toxicology studies (Bochner et al. 2011, Pauly et al. 2021).

In our experiment, microarrays PM-M1–PM-M4 were used to evaluate the growth of NHDF fibroblast cells in the presence of bio-AgNPs and chem-AgNPs. PM-M1 contains primarily carbohydrate and carboxylate substrates, whereas PM-M2, -M3, and -M4 contain individual L-amino acids and most dipeptide combinations. The concentrations of substrate nutrients in PM-M1 to M4 are low, about 1 millimolar.

To evaluate the metabolic ability of the cell line, the statistical analyzes were performed using the TIBCO Statistica 13.3 software. It was used to analyze basic descriptive statistics together with Shapiro-Wilk tests as well as multivariate analysis of variance in a mixed scheme. The classical threshold of $\alpha = 0.05$ was assumed as the level of significance. The results of the carried out work are presented in Tables 3 and 4, and Figure 4.

In the first step, the basic descriptive statistics of the examined quantitative variables were calculated along with the Shapiro-Wilk test checking the normality of the distribution of these variables. This test showed that the distribution of measurement results at all 3 time points differs significantly from the normal distribution. In this case, however, the value of the skewness should be analyzed. If its absolute value does not exceed 2, it can be assumed that the distribution is close to normal distribution (George and Mallery 2010). Such a situation was noted in the case of measurements at each of the 3 time points. Therefore, it can be concluded that the studied distributions are not significantly asymmetric in relation to the mean. Therefore, in this paper, parametric tests

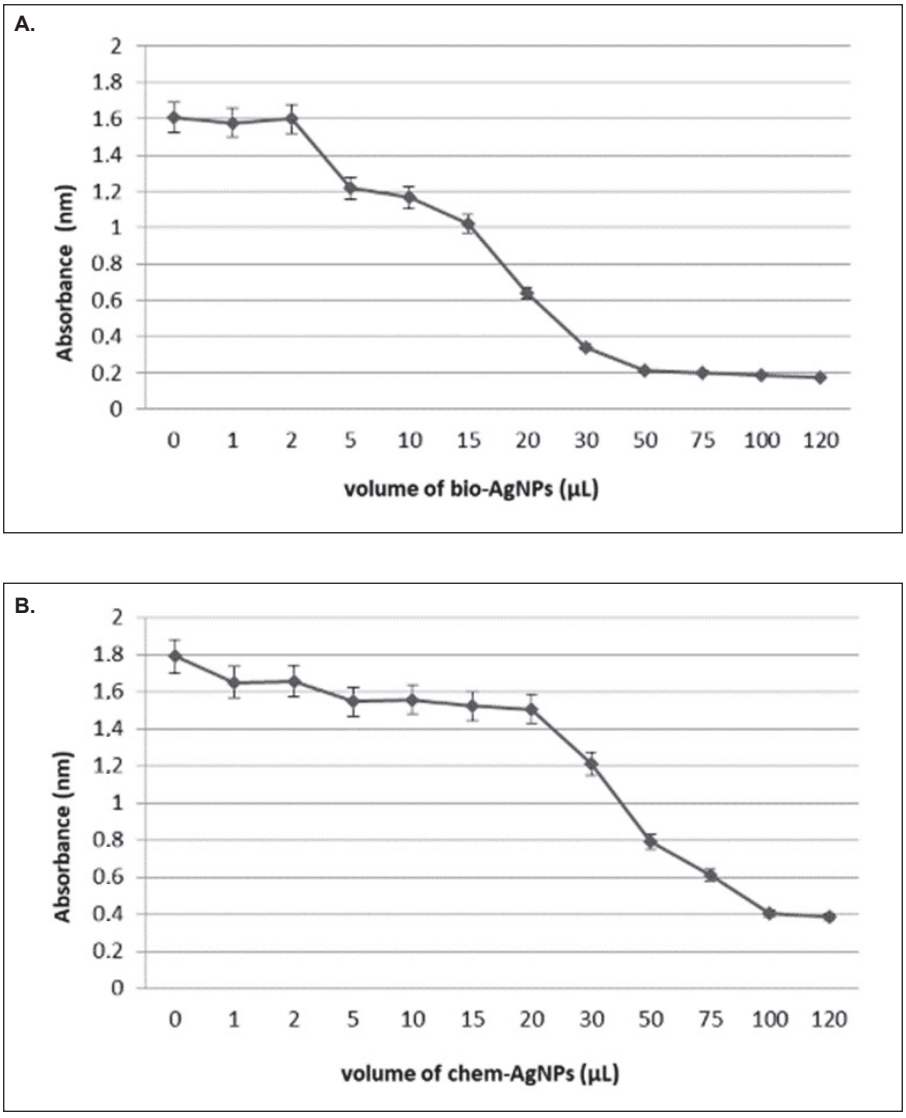


Fig. 2. Effect of bio-AgNPs and chem-AgNPs on NHDF fibroblast cells (MTT assay).
A. biologically synthesized nanoparticles (bio-AgNPs); B. chemically synthesized nanoparticles (chem-AgNPs)

were performed, as long as their other assumptions were met. The results of the analysis are summarized in Table 3.

In order to evaluate the effect of AgNPs on human cell growth, the cell growth measurement results obtained after 1, 12 and 24 hours of the experiment (expressed in Omnilog units) were compared. The results are presented in Table 4 and Figure 3. An analysis of variance was performed in a mixed scheme 3 × 3: three time measurement points and 3 groups depending on the presence of nanoparticles. The main effect of the within-object variable turned out to be statistically significant: $F(2; 1436) = 6167.01$; $p < 0.001$; $\omega^2 = 0.90$. The strength of the observed effect was very high. The main effect of the inter-object variable also turned out to be statistically significant: $F(2; 1437) = 74.74$; $p < 0.001$; $\omega^2 = 0.09$. The strength of the observed effect was moderate. Also, the interactive effect of both variables turned out to be statistically significant: $F(4; 2{,}872) = 44.24$; $p < 0.001$; $\omega^2 = 0.06$. The strength of the observed effect was low.

The results showed that Omnilog values changed over the time which is presented in Figure 4. Therefore, post-hoc pairwise comparisons were also made using the LSD-Fisher test. This test showed that significantly higher Omnilog values were observed after 12 hours of the experiment than after 1 hour ($p < 0.001$). In the case of biologically synthesized

Table 3. Basic descriptive statistics of growth measurement in PM-M1–PM-M4 microplates

Time (hours)	*M*	*Me*	*Min.*	*Max.*	*SD*	*Sk.*	*Kurt.*	*W*	*p*
1	173.17	171.00	69.00	340.00	39.57	0.48	1.68	0.97	<0.001
12	260.22	264.00	86.00	363.00	51.33	-0.55	0.20	0.98	<0.001
24	264.69	265.00	91.00	365.00	48.70	-0.44	0.20	0.98	<0.001

M – average; Me – median; Min and Max – the lowest and highest value of the distribution; SD – standard deviation; Sk. – skewness; Kurt. – kurtosis; W – Shapiro-Wilk test result; p – significance level

Table 4. The effect of bio-AgNPs and chem-AgNPs on the growth of NHDF fibroblast cells

				95% CI	
	Time (hours)	*M*	*SE*	*LL*	*UL*
Bio-AgNPs	1	184.5	2.55	179.44	189.46
	12	286.29	2.92	280.54	292.04
	24	293.5	2.53	288.17	298.12
Chem-AgNPs	1	172.2	1.76	168.86	175.78
	12	256.4	2.32	252.28	261.39
	24	258.44	2.22	254.08	262.80

M – average; SE – standard error; 95% CI – confidence interval for the difference between means; LL and UL – lower and upper limits of the confidence interval

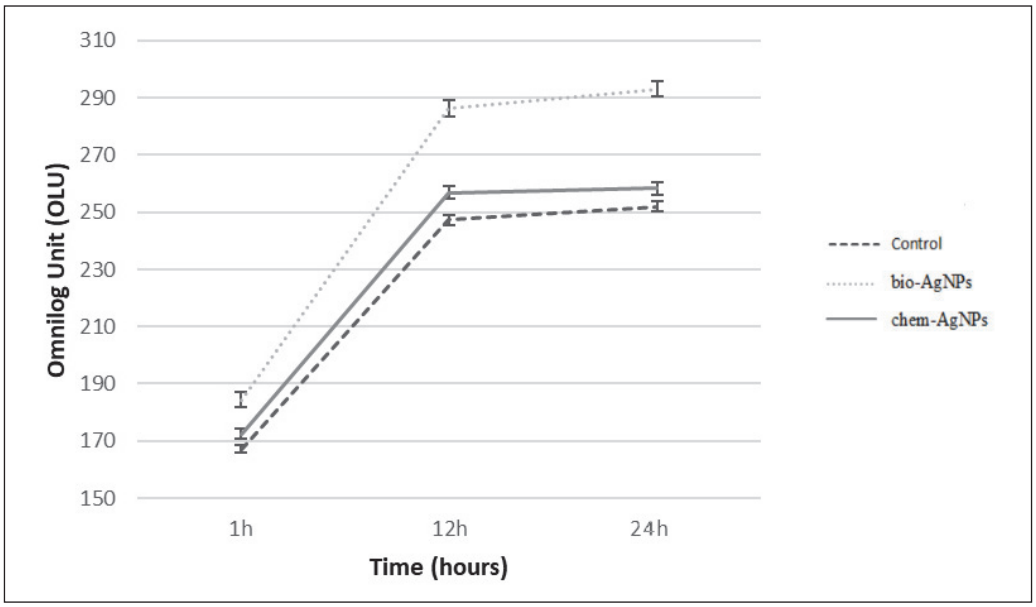


Fig. 3. Effect of bio-AgNPs and chem-AgNPs on NHDF fibroblast cells by PMMs (Biolog, Hayward, CA, USA)

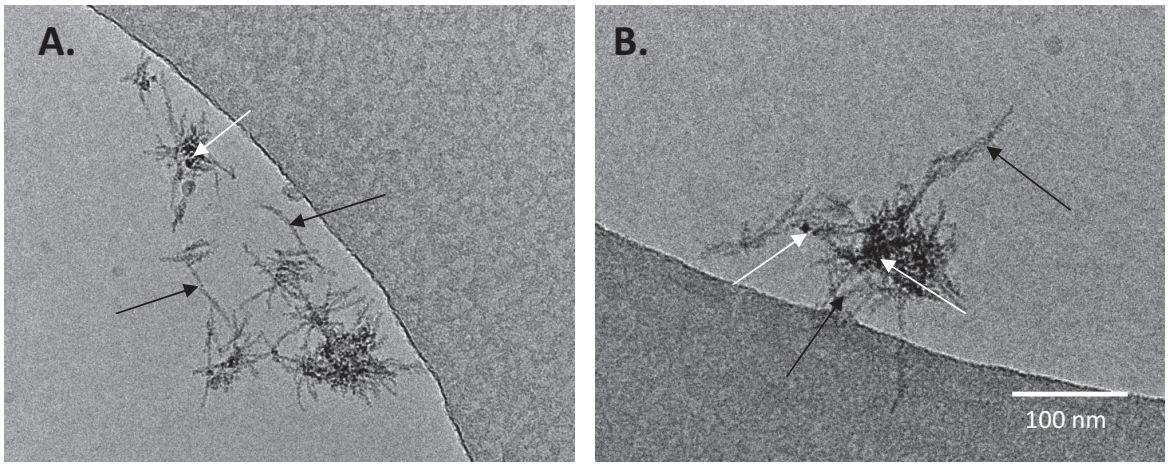


Fig. 4. Cryogenic transmission electron micrographs present the link between DNA and AgNPs. A. biological AgNPs; B. chemical AgNPs; The white arrow indicates AgNPs; the black arrow indicates fragments of human DNA

nanoparticles, the same pattern was observed, *e.g.*: the Omnilog values were significantly higher after 12 hours of the experiment than after 1 hour ($p < 0.001$).

After adding chemically synthesized nanoparticles, the Omnilog values increased significantly between the 1st and 12th hour of the experiment ($p < 0.001$). There was no statistically significant difference between the measurement performed at 12th and 24th hours ($p = 0.224$).

In the first analyzed measurement (after 1 hour), the highest Omnilog values were observed for biologically synthesized nanoparticles – they were significantly higher than those for chemically synthesized nanoparticles ($p < 0.001$). However, when comparing the latter two groups, the difference did not turn out to be statistically significant ($p = 0.072$). On the other hand, the Omnilog values for chemically synthesized nanoparticles were significantly lower than those for biologically synthesized nanoparticles ($p < 0.001$). A similar situation as above was observed in the measurement performed after 24 hours.

Interaction of bio-AgNPs and human DNA

The interactions of DNA and its components (bases and nucleosides) with metallic nanoparticles are the subject of great interest to researchers in nanobiotechnology (Li et al. 2014, Rai et al. 2021). The interaction between nanoparticles (NPs) and DNA plays an important role in the evaluation of genotoxicity of NPs. Li et al. (2014) investigated the relationship between the binding affinity of silver NPs, hematite NPs, gold NPs and DNA. The inhibition effects of NPs on DNA replication were observed. NPs with a high binding affinity for DNA molecules exhibited higher inhibition on DNA replication. In our experiment the link between bio-AgNPs and DNA strands was evaluated and observed. The morphologies of structures formed by DNA and silver nanoparticles are provided in Figure 4. DNA particles were observed to be densely accumulated around the biogenic AgNPs. The presence of biosurfactant produced by *Bacillus subtilis* I'-1a significantly increased the stability of biogenic AgNPs, however biosurfactant-based AgNPs has low aggregation capacity. They may cause some damage to the DNA strand, but not related to its folding.

Conclusions

Recently, the green synthesis of nanomaterials has attracted the attention of nanotechnologists. The new green routes of synthesis of biogenic NPs are developed. Biogenic AgNPs are reported to have some good properties such as biocompatibility and enhanced biological properties so in the future they may take the place of chemically synthesized NPs.

Based on the results reported in the present study, the cell-free supernatant from *Bacillus subtilis* strain I'-1a producer of iturin A lipopeptide biosurfactant is a good source for synthesizing stable AgNPs in short time. As it has already been presented by Chojniak et al. (2018), the synergistic effect of AgNPs and biosurfactants has already been observed earlier. The combination of biogenic AgNPs with the biosurfactant increased antibacterial and antifungal activities. The new roles of biosurfactants as stabilizers of biogenic AgNPs and enhancers of their antimicrobial properties were evaluated. The bio-AgNPs can be potent natural antioxidants and can be essential for health preservation against oxidative stress-related degenerative diseases, such as cancer. Bio-AgNPs showed cytotoxicity effect on NHDF fibroblast cells, and affected mammalian cell in the microplates PMM assay. Moreover, the specific interaction of bio-AgNPs with human DNA was also observed. The interaction between AgNPs and cell cultures depend on physical and chemical nature of the AgNPs and capping agents like biosurfactant. Thus, there is a pressing need to predict toxicological impacts of biogenic nanomaterials.

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