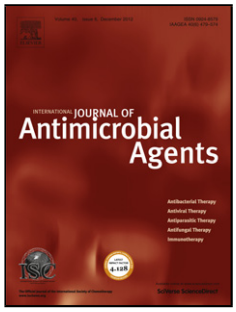

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Chemical and biological metal nanoparticles as antimycobacterial agents: a comparative study

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Highlights

- Metals nanoparticles as antitubercular agents were examined using in vitro and ex vivo assays.
- Activity was compared for chemical and biological gold and silver nanoparticles.
- Mycobacterial inhibition was achieved without cytotoxicity with silver nanoparticles.
- Specificity and selectivity of nanoparticles towards mycobacteria was established.
- Great potential of these nanoparticles to combat TB drug resistance.

ABSTRACT

Resistance among mycobacteria leading to multidrug-resistant and extensively drug-resistant tuberculosis is a major threat. However, nanotechnology has provided new insights in drug delivery and medicine development. This is the first comparative report to determine the activity of chemically and biologically synthesised silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) against mycobacteria. Screening data revealed the high mycobactericidal efficiency of AgNPs, with minimum inhibitory concentrations (MICs) of <3 μg/mL, whereas no such activity was exhibited by AuNPs at concentrations up to 100 μg/mL. Moreover, in vitro and ex vivo THP-1 infection model assays showed greater efficacy of chemical AgNPs compared with biogenic AgNPs to

inhibit active and dormant stage mycobacterial growth. Up to 40% cytotoxicity against human cell lines was observed at a AgNP concentration of 10× MIC (30 μg/mL) after 48 h. AgNPs were shown to have more specificity towards mycobacteria than towards other Gram-negative and Gram-positive pathogenic bacteria. The selectivity index was found to be in the range of 11–23, indicating the potential of these nanoparticles for use in developing new therapeutics for tuberculosis.

1. Introduction

Tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis*, is a major global health issue ranked as the second leading cause of death from an infectious disease worldwide. Although most people recover from primary TB infection without further evidence of the disease, the infection may stay dormant for years and in some cases it can reactivate [1]. A combination of four first-line drugs (isoniazid, rifampicin, ethambutol and pyrazinamide) is included in the treatment regimen [2]. However, multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB require second-line anti-TB drugs, which are more expensive and have greater toxicity and side effects. Indiscriminate use of antibiotics, improper treatment regimens and failure to ensure completeness of the treatment course in TB patients causes the development of antibiotic-resistant TB strains [3]. Resistance to conventional antibiotics threatens the progress made in TB control worldwide, prompting the need to seek alternative strategies, one of which may be nanomaterials.

Recently, metal nanoparticles have introduced new paradigms in medicine, where easy synthesis, controlled morphology, upscale production and reduced cost with increased sensitivity and specificity constitute an interesting alternative. These nanoparticles exhibit unique physical, chemical, electrical, optical, mechanical, magnetic, thermal and dielectric properties compared with the bulk counterparts, depending on their composition and morphology [4]. Metal nanoparticles are also shown to possess catalytic, free radical scavenging and tumour suppression activities [5,6]. Silver

nanoparticles (AgNPs) and gold nanoparticles (AuNPs) have been reported for temperature- and morphology-dependent antimicrobial activities against bacterial and fungal pathogens [7,8]. These nanoparticles can be synthesised using either chemical reducing agents such as glycerol, sodium borohydride etc., or through biological systems involving micro-organisms and plants [9–11]. It is often emphasised that bio-nanoparticles are safe, less toxic, biocompatible and eco-friendly compared with chemically synthesised nanoparticles [12]. However, green nanoparticle preparation employing non-toxic biocompatible chemical reducing and stabilising agents, such as trisodium citrate, starch, β-D-glucose, aminocellulose etc., may eliminate the toxicity risks. Therefore, in pursuit of developing alternate and novel antitubercular agents, we have directed our efforts to screen these nanoparticles, synthesised through biological and chemical approaches, for their feasibility as antimycobacterial agents.

2. Materials and methods

2.1. Nanoparticles

Two AgNPs (S1 and S11) and two AuNPs (G1 and G11) were screened for antimycobacterial activity (Table 1). S1 and G1 were bacteriogenic metal nanoparticles synthesised using the environmental bacterium *Acinetobacter* sp. [4,13]. S11 and G11 samples, chemically synthesised through reduction using 1% trisodium citrate, were provided by Gayatri Salunkhe (Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, Pune, India) [11].

2.2. Mycobacterial cultures and growth conditions

Standard cultures of *M. tuberculosis* H37Ra (ATCC 25177), denoted further as MTB, and *Mycobacterium bovis* BCG (ATCC 35743) were procured from the American Type Culture Collection (Manassas, VA). MTB and *M. bovis* BCG were grown in a defined *Mycobacterium phlei* medium [14] and in 50 mM sodium nitrate-containing Dubos medium (Difco, Detroit, MI), respectively. These were maintained as glycerol stocks at –70 °C. Prior to inoculation for experiments, 50 μL of glycerol stock was pre-inoculated in the corresponding medium to obtain metabolically active mycobacteria. For each experiment, cultures were grown to log phase [optical density at 595 nm (OD_{595}) = 1] under aerobic conditions at 37 °C and 150 rpm. As mycobacteria grow in visibly aggregated clumps in the culture medium, cultures were sonicated for 2 min using a water-bath sonicator (Ultrasonic, Freeport, IL) to obtain viable dispersed cells. This step was introduced to reproducibly inoculate mycobacterial bacilli in fresh medium for carrying out the experiments.

2.3. Primary screening

Nanoparticles were screened for their inhibitory activity against active (8 days incubation) and dormant (12 days incubation) mycobacteria at concentrations of 0.1, 0.3, 1, 3, 10, 30 and 100 μg/mL. Activity against MTB was determined through the XTT reduction menadione assay (XRMA) reading absorbance at 470 nm as per the protocol described by Singh et al. [15]. The nitrate reductase (NR) assay was performed to estimate inhibition of *M. bovis* BCG by nanoparticles [14]. Absorbance for the NR assay

was measured at 540 nm. Percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(\text{control}–\text{NP})/(\text{control}–\text{blank})] \times 100$$

where 'control' is the activity of mycobacteria without nanoparticles, 'NP' is the activity of mycobacteria in the presence of nanoparticles and 'blank' is the activity of the culture medium without mycobacteria.

The experiment was performed in triplicate and the quantitative value was expressed as the mean ± standard deviation (S.D.).

2.4. Determination of the minimum inhibitory concentration (MIC) and 50% inhibitory concentration (IC_{50})

Depending upon primary screening, active nanoparticles were further evaluated to estimate their MIC and IC_{50} against mycobacteria through a dose–response assay over the concentration range of 0.02–2.56 μg/mL. The dose–response curve was plotted using OriginPro software (OriginLab Corp., Northampton, MA). The MIC and IC_{50} were taken as the lowest concentration of nanoparticle exhibiting growth inhibition of ≥90% and 50%, respectively, relative to the growth control without nanoparticles. The standard antitubercular drug rifampicin was used as a positive control. All experiments were performed in triplicate.

2.4.1 In vitro assay

In vitro activity against MTB and *M. bovis* BCG at active (8 days) and dormant (12 days) stages was performed using the XRMA and NR assay, respectively, as described in Section 2.3.

2.4.2. Ex vivo infection model assay

The human acute monocytic cell line THP-1 was purchased from the National Centre for Cell Science (NCCS) (Pune, India). THP-1 cells were cultured in RPMI 1640 medium (HiMedia, Mumbai, India) supplemented with 10% fetal bovine serum (FBS) (Gibco, Bangalore, India), 1 mM sodium pyruvate (HiMedia), 1% non-essential amino acids (HiMedia), 1% glutamine (HiMedia), 50 mg/mL ampicillin (Sigma Chemical Co., St Louis, MO) and 50 mg/mL gentamicin (Sigma Chemical Co.), and were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator (Thermo Fisher Scientific, Waltham, MA). For the infection model study, 3×10^5 THP-1 cells/mL were passaged in complete RPMI medium containing 100 nM/mL phorbol myristate acetate (Sigma Chemical Co.) in 96-well microtitre plates (Tarsons, Kolkata, India) and were plated for differentiation to macrophages. After 24 h, these were infected with log-phase MTB (OD_{595} = 1) at a multiplicity of infection (MOI) of 100 for 12 h. Fresh Minimum Essential Medium (MEM) (HiMedia) containing 50 mM sodium nitrate (HiMedia) was added to the plate after thorough washing with phosphate-buffered saline (PBS) (pH 7.2). Infected cells were then treated with nanoparticles at different doses. Plates were further incubated for 8 days (active) and 12 days (dormant) followed by estimation of nanoparticle activity through NR assay as described in Section 2.3. The

experiment was performed in triplicate. IC_{50} and MIC values were calculated from their dose–response curves plotted using Origin8 software (OriginLab Corp.).

2.5. Cytotoxicity assay

2.5.1. Cell lines (THP-1, A549, PANC-1 and HeLa)

The effect of nanoparticles on cell growth was determined in a panel of human tumour cells including the acute monocytic leukaemia cell line THP-1 (NCCS, Pune, India) as well as lung adenocarcinoma A549, cervix adenocarcinoma HeLa and pancreas adenocarcinoma PANC-1 obtained from The European Collection of Cell Cultures (ECACC) (Salisbury, UK). THP-1 cells were maintained in RPMI 1640 without phenol red; A549 and PANC-1 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (HiMedia); and HeLa cells were cultured in Eagle's Minimum Essential Medium (EMEM) (HiMedia). All media used were supplemented with 10% FBS and 0.68 mg/mL gentamicin. Cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humidified environment.

2.5.2. MTT assay

In vitro cytotoxicity against these four human cell lines was determined through reduction of tetrazolium (MTT) dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich) as described previously [16]. Briefly, log-phase cells were harvested using trypsin [0.05% trypsin and 0.02% ethylene diamine tetra-acetic acid (EDTA) in PBS] from tissue culture flasks and the suspension was diluted with

appropriate culture medium to obtain a cell density of 10^5 cells/mL as determined by haemocytometry. An aliquot of 100 μL of each suspension was seeded in 96-well cell culture plates and was incubated at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity in a CO_2 incubator. After 24 h, nanoparticles (1 μL/well) at varying concentrations of 3, 10 and 30 μg/mL were added to the wells containing cells. Paclitaxel, an anti-cancer drug, was used as a positive control. Suitable controls with an equivalent concentration of dimethyl sulphoxide (DMSO) (Sigma-Aldrich) were also included. The plates were further incubated for 48 h. At the end of the incubation period, the solution containing the unattached cells was discarded and the wells were washed three times with 1 mL of PBS followed by addition of 10 μL of MTT (5 mg/mL in PBS) to adherent cells in growth medium. After 4 h at 37 °C for MTT cleavage, the formazan product was solubilised by addition of 100 μL of 0.04 N HCl in isopropanol. Absorbance was measured on a SpectraMax® PLUS 384 plate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 570 nm. Percentage cytotoxicity was calculated using the following formula:

$$\% \text{ cytotoxicity} = [(\text{control}–\text{NP})/(\text{control}–\text{blank})] \times 100$$

where ‘control’ is cell growth in medium without nanoparticles, ‘NP’ is cell growth in the presence of nanoparticles and ‘blank’ is culture medium without cells.

The experiment was performed in triplicate and the quantitative value was expressed as the mean ± S.D.

2.6. Specificity of silver nanoparticles

To determine specificity, nanoparticles at concentrations of 0.1, 0.3, 1, 3, 10, 30 and 100 μg/mL were tested against bacteria. Bacterial strains, namely *Escherichia coli* (NCIM 2931), *Staphylococcus aureus* (MTCC 3160) and *Streptococcus mutans* (MTCC 497), were obtained from the National Collection of Industrial Microorganisms (NCIM) (Pune, India) and the Microbial Type Culture Collection (MTCC) (Chandigarh, India). *Acinetobacter baumannii* AIIMS 7 (GenBank **EU779829**) isolated and identified in our laboratory was also used in the study. These cultures were grown in Luria–Bertani (LB) medium (HiMedia) at 37 °C and 150 rpm. For the antibacterial assay, the OD-adjusted (OD_{620} = 1) culture was inoculated in LB broth (1% v/v). Then, 5 μL of nanoparticles and 245 μL of culture were dispensed in a 96-well microtitre plate and were incubated for 18 h at 37 °C before reading the absorbance at 620 nm. Ampicillin was used as a positive control. Wells for growth and sterility control were also included. MIC and IC_{50} values were calculated from the dose–response curve.

2.7. Selectivity index

The selectivity index was calculated by dividing the 50% lethal concentration (LC_{50}) for cell lines (THP-1, A549, PANC-1 and HeLa) by the MIC for in vitro activity against active/dormant MTB [17]. The LC_{50} was taken as the lowest concentration of nanoparticles killing 50% of the cells as obtained from the percentage cytotoxicity curve plotted using Origin8.

3. Results and discussion

3.1. Preliminary screening

In the present study, metal nanoparticles synthesised by chemical and biological routes were evaluated for their antimycobacterial activity. Although very few reports are available on the antimycobacterial activity of nanoparticles [18–20], there are no comparative reports concomitantly describing antitubercular and cytotoxic effects of chemically and biologically originated AgNPs and AuNPs. In our previous studies, we have synthesised these metal nanoparticles of various morphologies employing bacteria and trisodium citrate as a reducing agent [4,11,13]. The morphology and mode of synthesis of these AgNPs and AuNPs is given in Table 1. In preliminary screening, nanoparticles S1, S11, G1 and G11 were tested at concentrations of 0.1, 0.3, 1, 3, 10, 30 and 100 μg/mL. Percentage inhibition at these concentrations was calculated. Fig. 1 shows the percentage inhibition obtained against active and dormant MTB and *M. bovis* BCG at a 3 μg/mL concentration of nanoparticles. AgNPs (S1 and S11) exhibited higher antimycobacterial potency, inhibiting >90% of mycobacterial growth at 3 μg/mL, which strengthens the fact of the antimicrobial nature of AgNPs. This is also in accordance with the study of Banu and Rathod reporting the potential of AgNPs synthesised from the fungus *Rhizopus stolonifer* to inhibit mycobacteria [18]. Unlike the report on the antimicrobial activity of AuNPs [21], in the current study AuNPs G1 and G11 showed very little activity against mycobacteria at the same concentration. Size- and shape-dependent antimicrobial activity of metal nanoparticles has already been reported,

which could explain this difference in action of AgNPs and AuNPs against mycobacteria [8,22]. AuNPs employed in this study were polydispersed and had a larger size, which may not be able to penetrate through the cell to exert a killing effect. Dormancy is the reversible state of metabolic shut down and it was observed during screening that mycobacteria offer greater resistance in the dormant stage than the active stage. Further experiments were carried out with the more effective S1 and S11 AgNPs.

3.2. MIC and IC_{50}

S1 and S11 AgNPs were further tested for their antitubercular activity against dormant and active mycobacteria both in vitro and within THP-1 host macrophages using the first-line antitubercular drug rifampicin as a reference standard (Table 2). In vitro studies against *M. bovis* BCG and MTB revealed the strong antitubercular activity of S11 to inhibit mycobacteria both at the active and dormant stages. The MIC of S11 against mycobacteria ranged from 1.31–2.14 μg/mL and the IC_{50} ranged from 0.05–0.33 μg/mL. Potency of S1 as an antimycobacterial agent was seen against BCG (IC_{50} < 0.2 μg/mL), whereas the MIC and IC_{50} against MTB remained >2.56 μg/mL. In comparison with AgNPs from *R. stolonifer* having an MIC of 12.5 μg/mL against MTB [18], the AgNPs in the current study showed much better antitubercular activity. Furthermore, complete inhibition of *M. tuberculosis* H37Rv and *Mycobacterium smegmatis* mc^2155 was reported on exposure to chemical AgNPs after treatment with chloroform [19]. However, no such chloroform treatment was required in the current study for mycobacteria inhibition.

A similar pattern was observed in the ex vivo THP-1 infection model assay with MTB, with S11 having greater efficiency compared with S1 to inhibit MTB. Size-dependent activity may also be plausible for S1 and S11 AgNPs. Owing to the smaller size of 1–5 nm, chemically synthesised S11 AgNPs exhibited higher in vitro and ex vivo activities both against MTB and *M. bovis* BCG in comparison with 8–12 nm sized S1 bio-AgNPs. It was again observed that the MIC of dormant stage mycobacteria was greater than its active stage. According to Kuete, antimicrobial activity can be classified as significant if the MIC is <100 μg/mL, moderate if the MIC is 100–625 μg/mL and weak if the MIC is >625 μg/mL [23]. In view of this, the overall antitubercular activity exhibited by S1 and S11 AgNPs is significant. Despite having higher antimycobacterial activity among AgNPs, S11 exhibited lower potencies compared with that of rifampicin, a standard anti-TB drug.

Multiple mechanisms have been proposed to elucidate the mechanism of action of AgNPs on bacteria. These include disruption of cellular morphology, enzyme inactivation, inhibition of DNA replication, and generation of reactive oxygen species (ROS) and oxidative stress [24,25]. AgNPs are also suggested to change the three-dimensional structure of proteins present in the cell wall by interfering with disulphide bonds and facilitate their penetration to cause respiration arrest inside the cell [8,19]. However, this aspect needs further investigation to elucidate the exact mechanism of action of AgNPs against mycobacteria.

3.3. Cytotoxicity of silver nanoparticles towards cell lines

Cytotoxicity is one of the major concerns for nanoparticles to act as an antimycobacterial agent. The choice of reducing agent, solvent and reducing environment dictate the greener synthesis of nanoparticles, which is generally considered to be safe and non-toxic [26]. Synthesis of biological nanoparticles is often accentuated considering biocompatibility and use of harsh reducing agents in chemical synthesis [12]. Therefore, S1 and S11 AgNPs were tested against four human cancer cell lines for their antiproliferative activity by the MTT cell proliferation assay. Fig. 2 shows the percentage cytotoxicity obtained against cell lines of different origin at 30 μg/mL concentration of AgNPs. It is important to note that the concentration tested was 10 times higher than the observed MIC for mycobacteria. Up to 40% inhibition was observed at such a high concentration of AgNPs at the end of 48 h of treatment, except for S11 exhibiting 83% cytotoxicity against HeLa cells. This suggested the equivalent biocompatibility of both types of AgNPs, contradicting the reports on the cytotoxic nature of AgNPs against human cell lines [27,28]. This could be due to the non-toxic nature of sodium citrate used as a reducing agent to synthesise S11 AgNPs. In a similar study, no cytotoxicity and DNA damage was seen in a RAW264.7 mouse macrophage cell line on exposure to biogenic AgNPs at ten times the effective mycobactericidal dose of 5 ppm. However, treatment with 10 ppm AgNPs lead to cytotoxicity, DNA damage, micronuclei formation and cell cycle arrest at G1 phase [20].

3.4. Specificity of silver nanoparticles

To check the specificity of AgNPs for mycobacteria, the MIC and IC_{50} values of these nanoparticles against Gram-positive and Gram-negative bacteria were also estimated (Table 3). Gram-positive bacteria (*S. aureus* and *S. mutans*) showed higher resistance, with an MIC > 100 μg/mL for both S1 and S11. However, S11 was found to be more active against Gram-negative bacteria, with an MIC between 20–70 μg/mL; the MIC of S1 against these bacteria was >100 μg/mL. Although the Gram-negative bacterium *A. baumannii* was not inhibited by ampicillin at the highest concentration of 100 μg/mL, S11 served to inhibit bacterial growth at a lesser concentration of 70 μg/mL. This difference in action of AgNPs against Gram-positive and Gram-negative bacteria can be attributed to the difference in their cell wall composition, which acts as barrier against penetration of nanoparticles [29]. It was observed that the MICs of S1 and S11 are up to 33-fold higher against bacteria than mycobacteria, indicating greater specificity of these AgNPs towards mycobacteria. These results are in contradiction with the study of Praba et al. reporting bacterial inhibition at a low AgNP concentration [19].

3.5. Selectivity index

The selectivity of S1 and S11 towards human cell lines against MTB is described via the selectivity index (Table 4). The selectivity index reflects the quantity of compound that is active against mycobacteria but is not toxic towards host cells. A higher selectivity index indicates that the compound can be used as a therapeutic agent. The LC_{50} of S1 and S11 against the tested cell lines was >30 μg/mL. The only exception was S11 having an

LC_{50} of 24 μg/mL against HeLa cells. It was found that both the AgNPs have a selectivity index of >10 at dormant and active stages of MTB. However, S11 showed a comparatively higher selectivity index of up to 22.92, whereas it was 11.72 for S1. Although the selectivity index of rifampicin is very high, it is important to consider the significance of this study with respect to the developing resistance among micro-organisms against available antibiotics. MDR and XDR mycobacterial strains have been reported to exhibit resistance against known anti-TB drugs such as isoniazid, rifampicin, ethambutol and pyrazinamide [3]. Hence, there is a great need to screen new compounds having therapeutic potential. Our efforts in present study were directed towards this. This is the first study to report the specificity and selectivity of metal nanoparticles towards mycobacteria. Also unlike conventional antibiotics, microbial resistance against AgNPs is not known, which makes them an ideal candidate for the development of a drug for TB. Moreover, according to a study of Hartkoorn et al. on the drug susceptibility of TB, antimycobacterial activity was considered to be specific when the selectivity index was >10 [30]. In the current report, both S1 and S11 exhibited a moderate selectivity index of >10, indicating their potential as an antitubercular agent, and thus they should be investigated further.

4. Conclusion

This is the first study to report the comparative antitubercular activity of chemical and biological AgNPs and to establish their specificity and selectivity towards mycobacteria. Chemical AgNPs exhibited greater efficiency in terms of mycobacterial inhibition, specificity and selectivity compared with bio-AgNPs. Moreover, both AgNPs showed

satisfactory biocompatibility against human cancer cell lines. These AgNPs are good candidates for further activity-guided fractionation in the search for new active therapeutic compounds. Although these results support the antimycobacterial nature of these AgNPs, the exact mechanism is still not fully elucidated. Synergistic effects of formulation comprising of AgNPs and TB drugs and their conjugates against mycobacteria are under investigation to combat MDR- and XDR-TB threats.

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Fig.1. Percentage inhibition of mycobacteria at active and dormant stages in the presence of 3 μg/mL nanoparticles. MTB, *Mycobacterium tuberculosis* H37Ra (ATCC 25177); BCG, *Mycobacterium bovis* BCG (ATCC 35743); S1 and S11, silver nanoparticles; G1 and G11, gold nanoparticles.

Fig. 2. Percentage cytotoxicity of silver nanoparticles (S1 and S11) at 30 μg/mL against human cell lines.

Table 1

Morphology of metal nanoparticles used for primary screening

Sample	Metal nanoparticle	Synthesis [a]	Shape	Size (nm)
S1	AgNP	Biological	Spherical	8–12
S11	AgNP	Chemical	Spherical-oval	1–5
G1	AuNP	Biological	Polyhedral	10–30
G11	AuNP	Chemical	Aggregated	5–15

AgNP, silver nanoparticle; AuNP, gold nanoparticle.

[a] Biological synthesis of nanoparticles was mediated through *Acinetobacter* sp., and chemical synthesis was achieved through reduction using 1% trisodium citrate.

Table 2

Antitubercular activity of AgNPs (S1 and S11) by in vitro and ex vivo assay

Sample	In vitro activity								Ex vivo activity against MTB			
	BCG				MTB							
	Dormant		Active		Dormant		Active		Dormant		Active	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
S1	0.19	2.56	0.15	1.80	>2.56	>2.56	>2.56	>2.56	>2.56	>2.56	>2.56	>2.56
S11	0.05	2.14	0.33	1.80	0.22	2.04	0.17	1.31	0.12	2.18	0.12	1.97
Rifampicin [a]	0.0083	0.0023	0.002	0.014	0.0014	0.043	0.0018	0.048	0.0018	0.048	0.0021	0.051

AgNPs, silver nanoparticles; BCG, *Mycobacterium bovis* BCG (ATCC 35743); MTB, *Mycobacterium tuberculosis* H37Ra (ATCC 25177); IC_{50} and MIC, lowest concentration of nanoparticle exhibiting growth inhibition of 50% and ≥90%, respectively, relative to the growth control without nanoparticles.

All values are in μg/mL.

The experiment was performed in triplicate and mean values were plotted in a dose–response curve to obtain IC_{50} and MIC values of AgNPs.

[a] The standard antitubercular drug rifampicin was used as a positive control.

Table 3

Antibacterial activity of AgNPs (S1 and S11) against Gram-positive and Gram-negative bacteria

Sample	*Staphylococcus aureus*		*Streptococcus mutans*		*Escherichia coli*		*Acinetobacter baumannii*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
S1	>100	>100	>100	>100	57.27	>100	>100	>100
S11	83.39	>100	59.51	>100	6.36	23.2	16.95	69.32
Rifampicin	>100	>100	>100	>100	23.81	86.23	87.12	>100
Ampicillin	65.91	>100	26.03	62.24	1.03	2.89	>100	>100

AgNPs, silver nanoparticles; IC_{50} and MIC, lowest concentration of nanoparticle exhibiting growth inhibition of 50% and ≥90%, respectively, relative to the growth control without nanoparticles.

All values are in μg/mL.

The experiment was performed in triplicate and mean values were plotted in a dose–response curve to obtain IC_{50} and MIC values of AgNPs.

Table 4

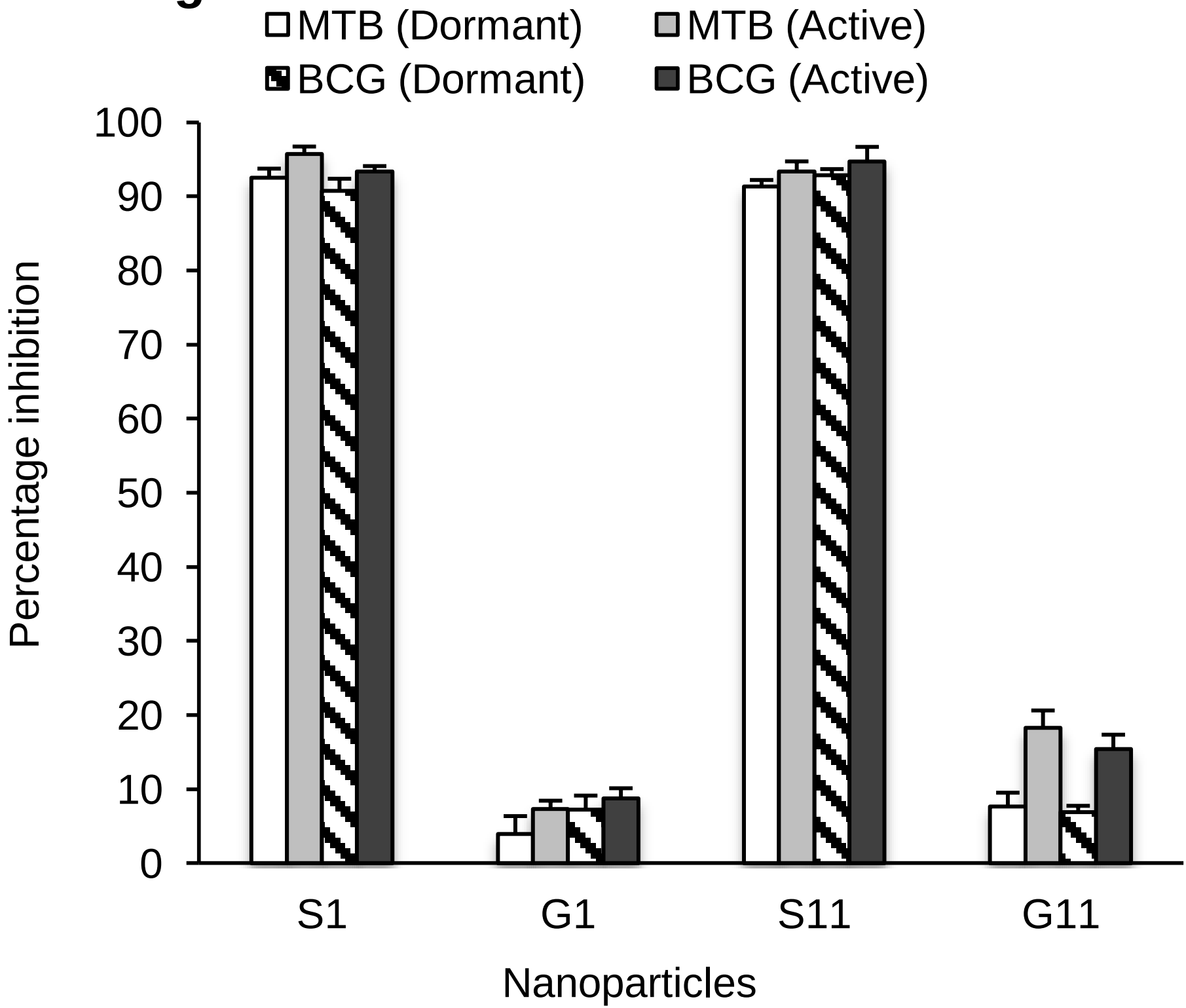
Selectivity index of AgNPs (S1 and S11) on human cell lines against *Mycobacterium tuberculosis* H37Ra

Sample	THP-1	HeLa	A549	PANC-1
Dormant state				
S1	11.72	11.72	11.72	11.72
S11	14.72	11.78	14.72	14.72
Rifampicin	666.67	666.67	666.67	666.67
Active state				
S1	11.72	11.72	11.72	11.72
S11	22.92	18.34	22.92	22.92
Rifampicin	1041.67	1041.67	1041.67	1041.67

AgNPs, silver nanoparticles.

The selectivity index was calculated by dividing the 50% lethal dose (LC_{50}) of AgNPs for cell lines by their in vitro minimum inhibitory concentration (MIC) against active/dormant *M. tuberculosis*.

Edited Figure 1



Edited Figure 2