

Journal of Coastal Life Medicine

journal homepage: www.jclmm.com



Document heading doi: 10.12980/JCLM.3.201514B324

©2015 by the Journal of Coastal Life Medicine. All rights reserved.

Antibacterial and anticancerous biocompatible silver nanoparticles synthesised from the cold-tolerant strain of *Spirulina platensis*

Selvaraj Karthick Raja Namasivayam*, Duraisamy Jayakumar, Ramesh Kumar, Rajan Sowri Arvind Bharani

Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India

PEER REVIEW

Peer reviewer

Dr. Shima M. A. El Shafay, Lecture of Phycology, Botany Department, Tanta University, Egypt.

Tel: 040 334435; 0100 9570818

Fax: 040 3305804

E-mail: sh.shfa3y@yahoo.com

Comments

In this research the authors studied the silver nanoparticles synthesized from *S. platensis* cold tolerant strain against Gram-negative organism *Pseudomonas aeruginosa* and Gram-positive organism *S. aureus*. Antibacterial and anticancerous silver nanoparticles synthesized from cold resistance strain of *S. platensis* would suggest the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocides.

Details on Page 271

ABSTRACT

Objective: To synthesize silver nanoparticles from the biomass of cold tolerant strain of *Spirulina platensis* and evaluate the synthesized nanoparticles against antibacterial and anticancer activity.

Methods: Silver nanoparticles were synthesized by the algal culture and characterized by UV-vis spectroscopy, Fourier transform infrared spectroscopy, field emission scanning electron microscopy and X ray diffraction studies. Antibacterial activity has been studied with free nanoparticles adopting agar diffusion assay, biofilm inhibition assay and nanoparticles fabricated wound dressing against representative Gram-negative organism *Pseudomonas aeruginosa* and Gram-positive organism *Staphylococcus aureus* respectively. The *in vitro* anticancer activity of silver nanoparticles were screened against human Hep2 cell lines by means of MTT assay.

Results: Reduction of silver ions by the algal culture was observed during 72 h of incubation and the synthesized nanoparticles were further characterized. Antibacterial study reveals both the strains were susceptible to free nanoparticles and fabricated wound dressing treatment. The *in vitro* anticancer activity of silver nanoparticles were screened against human Hep 2 cell lines by means of MTT assay which reveals that cell viability has been reduced as dose dependent manner.

Conclusions: The observed results imply that silver nanoparticles synthesized from *Spirulina platensis* cold tolerant strain can be used as potential antibacterial and anticancerous agent.

KEYWORDS

Spirulina platensis, Silver nanoparticles, Antibacterial, Anticancerous

1. Introduction

The development of antibiotics or other chemotherapeutics revolutionized human health, providing a simple cure for once dreaded diseases. However, widespread production, use, and misuse of antibiotics have contributed to the next-generation concern for global public health: the emergence of multiple drug-resistant

infectious organisms and cancer cells[1,2]. Nanotechnology offers tremendous potential for medical diagnosis and therapy. Various types of nanoparticles have been explored for biomedical applications having been widely employed in biological systems[3]. Nanoparticles have been suggested for imaging, screening and biosensing not using only gold nanoparticle optical properties, but also electrical and furthermore are known to carry higher payloads of drugs than other

*Corresponding author: Selvaraj Karthick Raja Namasivayam, Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India.

Tel: 91-44-24501644

Fax: 91-44-24512344

E-mail: biologiask@gmail.com

Article history:

Received 4 Aug 2014

Received in revised form 11 Aug 2014

Accepted 2 Jan 2015

Available online 3 Mar 2015

vehicles and are currently used in gene and drug delivery as well as in cancer diagnostics and therapeutic applications[4].

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics[5,6]. Silver nanoparticles commonly used for nanomedicine production, are reported to be nontoxic to human, but most effective against bacteria, viruses, and other eukaryotic microorganisms at very low concentration[7]. They are also effective against tumors with anti-proliferative activity[8]. The antimicrobial property allows them to be suitably employed in numerous products such as textiles, food storage containers, home appliances and especially in medical devices[9]. Use of silver nanoparticle is in medicine industry as tropical ointments to prevent infection against burn and open wounds is quite effective. Silver nanoparticles play important role as pesticide filter also[10]. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology[11,12]. Biologically, synthesized silver nanoparticles could have many applications: they might be used as spectrally-selective coatings for solar energy absorption and intercalation material for electrical batteries; they also be found to use as optical receptors and as catalysts in chemical reactions. Concerning the biological application of nanoparticles it has been emphasized that methods of synthesis through biological systems viz, microorganisms including bacteria, yeasts, fungi and diatoms synthesizing inorganic materials either intra or extracellularly would make the nanoparticles more biocompatible. Different plant extracts have been used and reported for synthesis of gold, silver and bimetallic nanoparticles[13,14].

Silver nanoparticles are synthesized by different physical and chemical methods like sol-gel technique, solvo-thermal synthesis, chemical reduction, laser ablation, inert gas condensation, etc. The time consuming physical methods are often difficult to achieve and in chemical methods different toxic reagents are used as capping agent like, cetyltrimethyl ammonium bromide leading to undesirable functional aberrations in target cells. Both physical and chemical procedures are very expensive. Therefore, biosynthesis of silver nanoparticles are becoming popular day by day using microorganisms like bacteria[15-18], fungi[19-21]. Algal mediated synthesis of silver nanoparticles is now being extensively carried out by researchers because of the high rate of synthesis, efficacy and best compatibility[22]. Synthesis of biocompatible potential bioactive silver nanoparticles from various algal crude extracts of *Sargassum wightii*, *Kappophycus alvarezii*, *Gelididella acerosa*[23-25], *Spirulina platensis* (*S. platensis*) [26], *Pterocladia capillacea*, *Jania rubinis*, *Ulva fasciata*, and *Colpomenia sinusa*[27]. Potential antimicrobial silver nanoparticles synthesized from marine microalgae against human pathogenic bacteria has been reported[28]. In the present study, antibacterial and anticancerous silver nanoparticles synthesized from cold resistance strain of *S. platensis* has been studied.

2. Materials and methods

2.1. Algal strain and growth condition

Laboratory stock culture of *S. platensis* was cultured in BG-11 (Blue-Green algae) medium for cyanobacteria[29]. The growth potential of alga was maintained through regular sub-culturing techniques, under laboratory conditions at 28 °C, in a 16/8 h light/dark cycle, under cool fluorescent light (20-30 $\mu\text{mol photons/m}^2/\text{s}$), in BG-11 medium (pH 9). Cold-tolerant strain of *S. platensis* has been raised from the subculture by the modified method of Kim *et al*[30]. *S. platensis* was grown at 28 °C to an OD₆₀₀ of 0.4, and aliquots were transferred to temperature-equilibrated flasks in water baths at 15 °C. Samples were taken at 30 min after transfer. Then, 5 mL of the cold stressed culture was transferred to the conventional BG-11 media and the culture thus obtained was used for biosynthesis of silver nanoparticles.

2.2. Synthesis of silver nanoparticles

Synthesis of silver nanoparticles by cold-resistant strain of *S. platensis* was carried out by the modified method of Mahdich *et al*[31]. A total of 5 mL of exponential growth phase culture of *S. platensis* was transferred to the 250 mL Erlenmeyer flask with 100 mL of 1 mmol/L aqueous AgNO₃ solution (pH 7) for 24 h under shaking condition at 28 °C.

2.3. Characterization

UV-vis spectral analysis was performed on a Shimadzu-1800 spectrophotometer. The biosynthesized silver nanoparticles solution was centrifuged at 10000 r/min for 15 min and the suspension was redispersed in sterile distilled water. Finally, dried samples were palletized with KBr for Fourier transform infrared spectroscopy (FTIR) measurements. The spectrum was recorded in the range of 4000-500 cm^{-1} using Bruker Optic GmbH Tensor 27. X-ray diffraction (XRD) measurement of the silver nanoparticles was carried out using Rigaku smart lab instrument operated at a voltage of 40 kV and a current of 30 mA with Cu K α 1 radiations Field emission scanning electron microscopy-energy dispersive X-ray analysis (FESEM-EDAX) was performed by Supra 55-Carl Zeiss, Germany.

2.4. Evaluation of biological activities

2.4.1. Antibacterial activity

The antibacterial activity of silver nanoparticles was studied against *Pseudomonas aeruginosa* (ATCC 10145) (*P. aeruginosa*) and *Staphylococcus aureus* (ATCC) (*S. aureus*) adopting well diffusion assay. Both the strains were obtained from American type culture collection (ATCC) and maintained on tryptic soy agar slants. A loopful of slant culture was inoculated into tryptic soy broth and incubated at 37 °C for 12-16 h to reach mild log phase. The respective broth culture was uniformly spread with sterile cotton swabs on sterile Mueller Hinton

agar media (Hi-media, India). The wells were made using cork borer and aliquots of silver nanoparticles (aliquots of 25, 50, 75 µg/mL were prepared from concentrated silver nanoparticles) was loaded into the wells. The plates were incubated at 37 °C for 24 h.

2.4.2. Determination of minimum inhibitory concentration (MIC)

Modified method of microdilution calorimetric assay using the chromogenic reagent MTT was used to study the MIC^[31]. Respective bacterial strains were grown in Luria-Bertani medium (yeast extract 5 g, peptone 10 g/L, sodium chloride 5 g/L, and pH 7.0) overnight at 28 °C. A total of 10 µL of different concentration of nanoparticles (10-100 µg/mL) and prepared bacterial suspension (90 µL) containing 1×10⁶ CFU/mL were added into each well of the 96 well microplate. The microtitre plates were incubated in the dark at 28 °C for 24 h. Then 10 µL of MTT (5 mg/mL in 0.2 mol/L, pH 7.2, phosphate buffer saline) was added into each well and the plates were incubated another 4 h. The MIC value was defined as the lowest sample concentration that inhibited visible growth of the test bacterium, as indicated by MTT staining. Only living microorganisms can convert MTT to formaldehyde and a blue colour appeared in the well^[32](Abe *et al*, 2000).

2.5. Biofilm inhibition study

2.5.1. Inocula preparation

Respective bacterial culture was inoculated from fresh slopes of tryptic soy agar into tryptic soy broth and incubated with shaking at 37 °C for 24 h. Cells were collected by centrifugation and the collected cell debris washed twice in phosphate buffer saline and suspended to OD₅₂₀ prior to use in biofilm experiments^[33].

2.5.2. Biofilm inhibition assay

Biofilm inhibition was studied by the microtitre plate spectrophotometric assay. A total of 100 µL of respective bacterial cell suspension and the respective concentration of silver nanoparticles was added into the wells of a 96-well polyvinyl chloride microtiter plate. The microtiter plates were covered and sealed before incubation under stationary conditions at 37 °C for 24 h. After the incubation time, the content was discarded and the plates thoroughly washed with water. Then 100 µL of 0.1% aqueous solution of crystal violet was added and incubated at room temperature for 30 min followed by washing with water the remaining stain was solubilized with 200 µL of 95% ethanol. Biofilm inhibition was studied by determination of the absorbance of the ethanol solubilised mixture at 540 nm in an UV spectrophotometer. Control (without bacteria only crystal violet), three replicates were maintained for each treatment^[34,35].

2.6. Effect of nanoparticles on the biochemical composition of biofilm matrix

2.6.1. Isolation of biofilm matrix

Effect of silver nanoparticles on the biochemical composition of

biofilm matrix material was isolated from the microtitre plate was studied by the method of Azeredo and Sutherland^[36]. Adherent biofilms were transferred to screw cap bottles containing 10 mL distilled water. The bottles were sonicated for 5 min in an ultrasonic water bath and vortexes vigorously for 1 min to disrupt the biofilms. Cell suspensions were then pooled and centrifuged. The collected supernatant used as source for studying biochemical composition mainly protein by Lowry *et al.* and total carbohydrate by Dubois *et al*^[37].

2.6.2. Nano fabrication of silver nanoparticles on the wound dressing

Fabrication of silver nanoparticles on the wound dressing material was carried out by the modified method of Shuangyun *et al*^[38]. Fabrication was achieved by submerging the dressing pieces (10×10 mm) in 5 mL of silver nano suspension (1 mg/mL) and then the dressing pieces have been extemporaneously dried at room temperature, sterilized by ultraviolet irradiation for 30 min.

2.6.3. Characterization of wound dressing

The surface topography of nanoparticles coated dressing was characterized by scanning electron microscopy.

2.6.4. Antibacterial activity

The antibacterial activity of coated dressing was tested against *P. aeruginosa* and *S. aureus* adopting agar diffusion assay. Sterilized nano fabricated wound dressing was placed on the Mueller Hinton agar plates swabbed with respective bacterial culture incubated at 37 °C for 24 h and the plates were observed for zone of inhibition. After the incubation period the diameter of the zone was recorded.

2.7. Anticancer activity

2.7.1. Chemicals

RPMI1640, fetal bovine serum, trypsin, methylthiazolyldiphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide were purchased from Hi media & Sigma Aldrich Mumbai.

2.7.2. Cytotoxicity assay

Cytotoxicity of silver nanoparticles was determined by inhibition of cell growth of Hep2 cell line using a tetrazolium dye (MTT) assay and percentage of cell viability was determined by spectrophotometric determination of accumulated formazan derivative in treated cells at 570 nm in comparison with the untreated ones. Hep2 cell line was obtained from National centre for cell sciences, Pune, India. RPMI1640 was used as the source of cell growth medium and a humidified atmosphere (d 5% CO₂) was maintained for cell culture. Hep2 cells were harvested in a logarithmic growth phase, then seeded on 96 wells at a cellular density of 5×10³ cells/mL followed by the addition of 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8 µg/mL concentrations of nanoparticles, incubated for 24 h at 5 % CO₂ incubator. After removal of the sample solution and

washing with phosphate-buffered saline (pH 7.4), 20 μ L/well (5 mg/mL) of 0.5% MTT in phosphate buffered saline solution was added. After 4 h incubation, 1 mL of dimethyl sulfoxide was added. Viable cells were determined by the absorbance at 540 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The effect of the nanoparticles on the proliferation of Hep 2 cells was expressed as the % cell viability, using the following formula:

$$\text{Percentage of cytotoxicity} = \frac{\text{Mean OD of individual test group}}{P \text{ Mean OD of control group}} \times 100$$

3. Results

3.1. Synthesis and characterization of silver nanoparticles

In the present study, silver nanoparticles were synthesized from cold-resistance strain of *S. platensis* and evaluation of antibacterial and anticancerous activities has been made. Reduction of silver ions into silver nanoparticles by the algal culture was visually identified by colour change from yellow to brown in the aqueous solution of reaction mixture at 1 h incubation time and the intensity of the colour increased during the increased incubation time (72 h). UV-vis absorption spectroscopy analysis reveals a broad surface plasmon absorption maxima at 430 nm (Figure 1). Moreover a surface plasmon peak remain in the range of 430 nm at increasing incubation period (72 h) suggesting nanodispersive particles in the aqueous solution. Characterization of synthesized silver nanoparticles by scanning electron microscopy reveals spherical particles with the size of 27 nm (Figure 2). Further, characterization was carried out by FTIR. FTIR spectrum of the synthesized nanoparticles shows strong peak at 3748 cm^{-1} reveals free alcoholic and carboxylic acid groups present (Figure 3). The peak at 2367 cm^{-1} designates the asymmetric -CH bending, thus indicating its role in reduction of silver ions. Further, the presence of peak at 1762 cm^{-1} confirms the amide I stretching group. Peak at 1384 cm^{-1} revealed C@C responsible for the reduction of silver salt. The formation of synthesized silver nanoparticles was further supported by XRD measurements. This method is used to elucidate crystallinity and the lattice properties of the silver nanoparticles. Presence of distinct high diffraction peaks at 21.6°, 35.5° and 43.5° respectively, indexing the Bragg's reflection planes (111), (200), (220) and (311) confirmed the face centered cubic structure of crystalline silver nanoparticles (Figure 4).

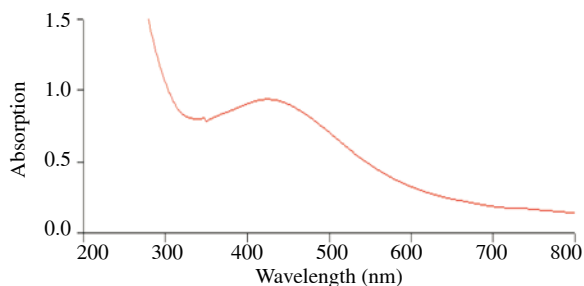


Figure 1. UV-vis absorption spectra of silver nanoparticles synthesized from *S. platensis*.

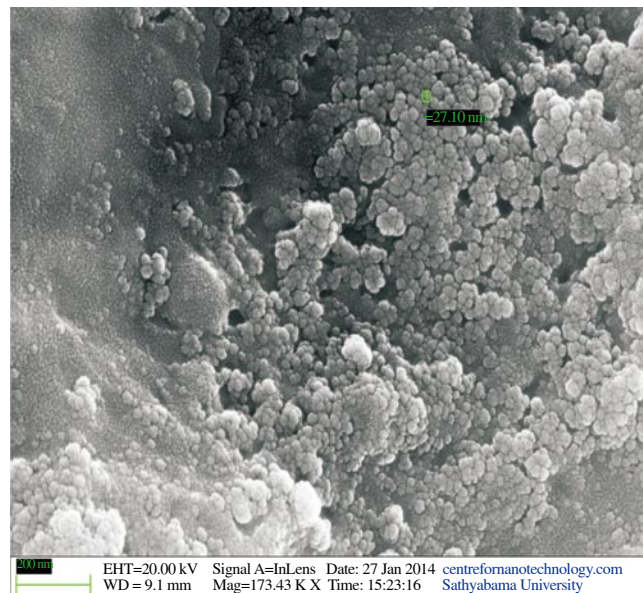


Figure 2. SEM of nanoparticles synthesized from *S. platensis*.

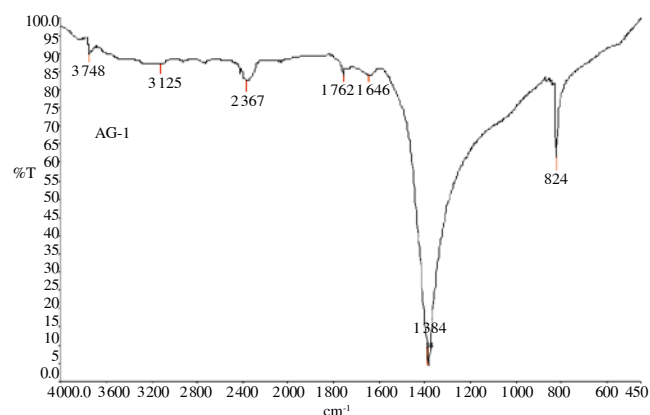


Figure 3. FTIR spectra of synthesized nanoparticles.

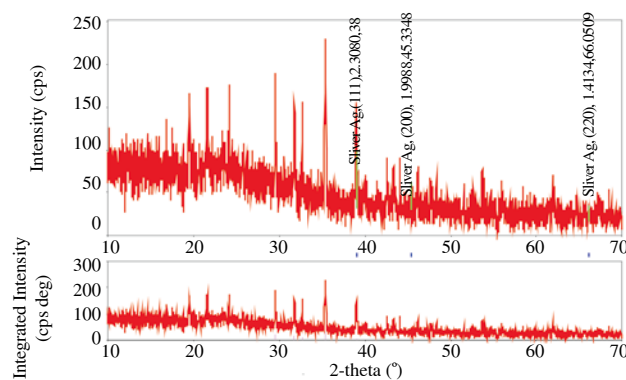


Figure 4. XRD spectra of synthesized silver nanoparticles.

3.2. Antibacterial activity

In the present study, antibacterial activity was studied against *P. aeruginosa* and *S. aureus*. It can be seen that both the tested strains were susceptible to the silver nanoparticles as dose dependent manner (Table 1). An increase in inhibitory zone was recorded in high concentration (Figures 5a and 5b). In the case of *P. aeruginosa*, maximum zone of inhibition was recorded at 75 μ g/mL with 29.0 mm followed by 50 μ g/mL with 24.2 mm, 25 μ g/mL with 22.0 mm

of zone of inhibition (Figure 5a) *S. aureus* shows high sensitivity to high concentration of nanoparticles (75 µg/mL) as in *P. aeruginosa*. Zone of inhibition against *S. aureus* (32.1, 28.0 and 22.0 mm) has been observed at the respective concentration (Figure 5b). MIC of the nanoparticles against both the tested bacterial strains was studied by broth dilution method. The MIC values of nanoparticles against *P. aeruginosa* and *S. aureus* was found to be 31.2 and 29.2. It can be seen that nanoparticles showed high antibacterial efficacy.

Table 1

Zone of inhibition of silver nanoparticles against pathogenic bacteria.

Concentration (µg/mL)	Zone of inhibition (mm)	
	<i>S. aureus</i>	<i>P. aeruginosa</i>
25	22.0	22.0
50	28.0	24.2
75	32.1	29.0

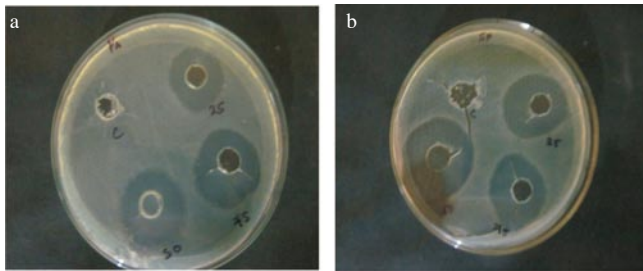


Figure 5. Zone of inhibition of nanoparticles against *P. aeruginosa* (a) and *S. aureus* (b).

Antibiofilm effect of nanoparticles against *P. aeruginosa* and *S. aureus* has been studied. Biofilm inhibition study revealed nanoparticles with all the tested concentration inhibited biofilm of both the tested strains. Results were represented as inhibition percentage of biofilm development (Figure 6). Nanoparticles showed distinct effect on biofilm formation with a dose dependent manner. Concentration at 75 µg/mL recorded maximum antibiofilm effect against both the tested strains. As presented in Figure 6, nanoparticles with 75 µg/mL recorded 84.5% and 88.2% of biofilm inhibition against *P. aeruginosa* and *S. aureus* respectively. Further concentration of nanoparticles reveals 71.0%, 50.1% and 73.2%, 58.2% of biofilm inhibition against *P. aeruginosa* and *S. aureus* respectively. Isolation of biofilm matrix material of both the bacterial strains has been carried out to study the effect of nanoparticles on the biochemical composition mainly total carbohydrates and protein (Table 2).

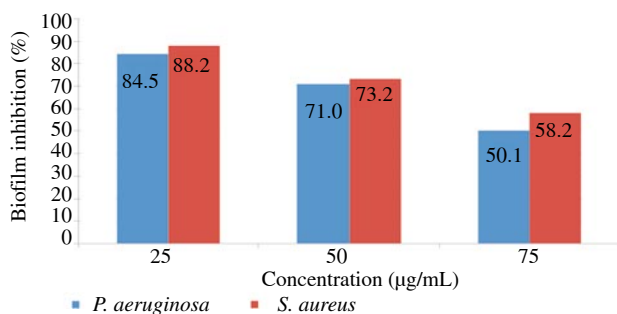


Figure 6. Biofilm inhibition (%) of pathogenic bacteria treated with nanoparticles.

Table 2

Effect of silver nanoparticles on the biochemical composition of biofilm matrix of pathogenic bacteria.

Concentration (µg/mL)	Total protein		Total carbohydrates	
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
25	203.4	105.0	231.4	198.0
50	121.5	54.0	78.0	86.7
75	51.0	12.0	11.0	20.4

Changes in reduction of total carbohydrates and protein have been recorded in nanoparticles treatment as dose dependent manner. Antibacterial activity of nanoparticles fabricated wound dressing has been studied. Surface topography of wound dressing with SEM reveals complete dispersion of nanoparticles on the fiber surface and the size of the embedded particles was 60-70 nm (Figure 7). Antibacterial activity of nanoparticles fabricated wound dressing was studied by solid plate agar diffusion assay. Both the bacterial strains were found to be susceptible. Zone of inhibition 17.0 and 16.5 mm was recorded against *P. aeruginosa* and *S. aureus* respectively (Figures 8a and 8b).

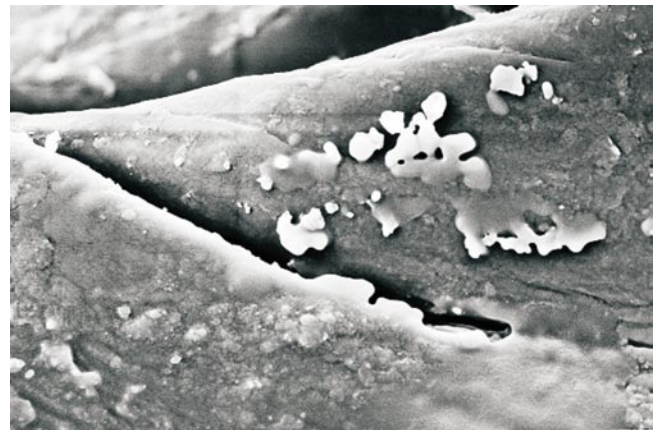


Figure 7. SEM of wound dressing coated with nanoparticles.

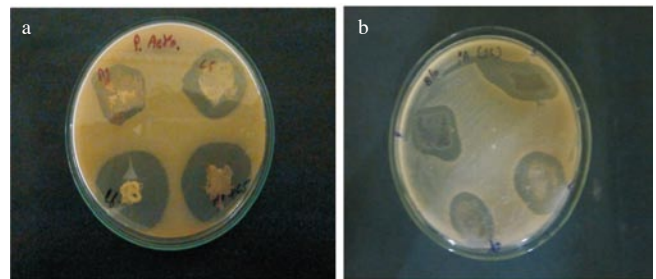


Figure 8. Zone of inhibition of nanoparticles against *P. aeruginosa* (a) and *S. aureus* (b).

3.3. Anticancer activity

The *in vitro* cytotoxicity effects of silver nanoparticles synthesized from *S. platensis* were screened against human Hep 2 cell lines by means of MTT assay. A serial 10 fold dilution of silver nanoparticles was prepared. Hep 2 cells grown in 96 well plates were incubated and the viability in respective dilution was determined by MTT assay which reveals that nanoparticles reduced the viability as dose dependent manner. Effective high cytotoxic effect was recorded at 1000 µg/mL followed by 500, 250, 125, 62.5, 31.2, 15.6, 7.8 µg/mL

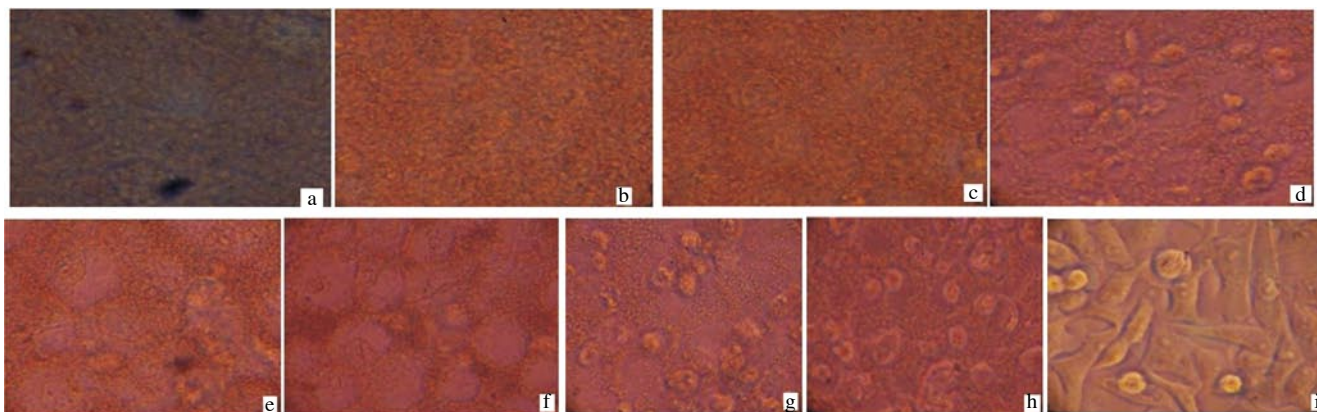


Figure 10. Microscopic examination of Hep2 cell line treated with different concentrations of silver nanoparticles.

a: 1000 µg/mL; b: 500 µg/mL; c: 250 µg/mL; d: 125 µg/mL; e: 62.5 µg/mL; f: 31.2 µg/mL; g: 15.6 µg/mL; h: 7.8 µg/mL; i: control Hep2 cell line.

and the percentage of viability at respective concentration was 8.7%, 13.5%, 15.5%, 20.3%, 26.2%, 29.1%, 45.6% and 59.2% respectively (Figure 9).

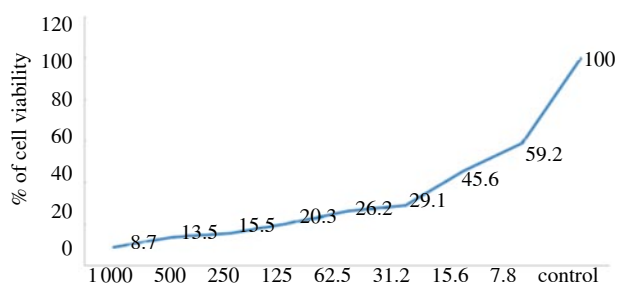


Figure 9. Effect of silver nanoparticles on viability (%) of Hep2 cell lines.

Morphological characteristics of silver nanoparticles treated cells using an inverted microscope revealed that changes in the cell morphology were observed at 1000 and 500 µg/mL when compared to the cells treated at least concentrations (Figure 10). It can be clearly observed that the cells treated with silver nanoparticles with 1000 µg/mL showed distinct changes in the morphological features whereas cells treated with 7.8 µg/mL have a well-developed nucleus, devoid of peripheral cellular distribution as in control cells. The cytotoxic effect of silver nanoparticles on cell viability has a major role in antitumor activity, thereby reducing disease progression. The cytotoxic effects of silver are the result of active physiochemical interaction of silver atoms with functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA[39].

4. Discussion

Nowadays, biological method of nanoparticles synthesis is a vast growing technique in the field of nanotechnology. The biological sources had the more quantity of trouble-free protocols and when applied for the human health associated field, it is easy to approach for maintain aseptic environment during the synthesis process of nanoparticles. Algal mediated synthesis of silver nanoparticles is now being extensively carried out by researchers because of the high rate of synthesis, efficacy and best compatibility[22]. Synthesis of silver nanoparticles from cold resistance strain of *S. platensis* was primarily confirmed. The formation of brown colour suggests the

presence of silver nanoparticles[19] due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field[40]. Mechanism of synthesis of silver nanoparticles by the algal culture is due to the production of metabolites that reduces silver ions into silver nanoparticles and these metabolites mainly enzymes such as NADH dependent nitrate reductase[41]. Size distribution analysis of the capped silver nano conjugates confirmed that the particles were well dispersed. Bio-organic materials of the algal cells bound to the nanoparticles surface which is responsible for the stability of nanoparticles[42,43]. It is evident through the FTIR spectra that the presence of different functional groups in the algal biomass might serve as the reducing and capping agents of the silver nanoparticles. Further characterization was carried out by XRD. All the peaks in the XRD pattern can be indexed as a standard silver crystal[44] which also shows a high purity as synthesized silver crystal.

Due to the increasing spread of antibiotics or other conventional chemotherapeutics resistance pathogenic strains and cancer cells, it is necessary to develop effective, less toxic biocides. Nanotechnology principles mainly inorganic nanomaterials has been exploited widely in the field of medicine and healthcare worldwide. The common antimicrobial agents are extremely toxic, poor efficacy and it is necessary to formulate new types of safe and cost-effective biocidal materials. Antimicrobial formulations in the form of nanoparticles could be used as potential bactericidal materials. A reactive metal nanoparticles display excellent biocidal action against Gram-positive and Gram-negative bacteria as demonstrated. Thus, the preparation, characterization, surface modification, and functionalization of nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. The improved antibacterial activity could be due to the amount of silver ions released from the nanoparticles which act as reservoirs for the same. The common antimicrobial agents are extremely toxic, poor efficacy and it is necessary to formulate new types of safe and cost-effective biocidal materials. Antimicrobial formulations in the form of nanoparticles could be used as potential bactericidal materials A reactive metal nanoparticles display excellent biocidal action against Gram-positive and Gram-negative bacteria as demonstrated. Thus, the preparation, characterization, surface modification, and functionalization of

nanosized inorganic particles open the possibility of formulation of a new generation of bactericidal materials. The improved antibacterial activity could be due to the amount of silver ions released from the nanoparticles which act as reservoirs for the same. Antibiofilm effect reveals both the tested strains were found to susceptible and the biochemical composition of the biofilm matrix was highly reduced in nanoparticles treatment. The matrix is one of the most distinctive features of a microbial biofilm. It forms a three dimensional, gel-like, highly hydrated and locally charged environment in which the microorganisms are largely immobilized. Matrix-enclosed micro colonies, sometimes described as stacks or towers, are separated by water channels which provide a mechanism for nutrient circulation within the biofilm. The composition of the matrix varies according to the nature of the organism. Reduction of the biochemical composition of the biofilm matrix leads to weakening of the biofilm thus facilitate entry of the drugs^[33]. In the present study, antibacterial and anticancerous silver nanoparticles synthesized from cold resistance strain of *S. platensis* would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocide.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The application of nanoparticles as delivery vehicles for bactericidal agents represents a new paradigm in the design of antibacterial therapeutics. Nanomaterials based on biological origin are highly appreciable because of less toxicity, high efficacy and biocompatibility.

Research frontiers

Silver nanoparticles were synthesized by the algal culture and characterized by UV-vis spectroscopy, FTIR, field emission scanning electron microscopy and XRD studies.

Related reports

Antibacterial activity has been studied with free nanoparticles adopting agar diffusion assay, biofilm inhibition assay and nanoparticles fabricated wound dressing against representative Gram-negative organism *P. aeruginosa* and Gram-positive organism *S. aureus*.

Innovations and breakthroughs

The *in vitro* anticancer activity of silver nanoparticles were screened against human Hep2 cell lines by means of MTT assay. Reduction of silver ions by the algal culture was observed during 72 h of incubation and the synthesized nanoparticles were further characterized.

Applications

Silver nanoparticles synthesized from *S. platensis* cold-tolerant strain can be used as potential antibacterial and anticancerous agent. Antibacterial and anticancerous silver nanoparticles synthesized from cold-resistance strain of *S. platensis* would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocides.

Peer review

In this research the authors studied the silver nanoparticles synthesized from *S. platensis* cold-tolerant strain against Gram-negative organism *P. aeruginosa* and Gram-positive organism *S. aureus*. Antibacterial and anticancerous silver nanoparticles synthesized from cold-resistance strain of *S. platensis* would suggests the possible utilization of biogenic silver nanoparticles as an effective and biocompatible biocide.

References

- [1] Chen MW, Yang ZW, Wu HM, Pan X, Xie XB, Wu CB. Antimicrobial activity and the mechanism of silver nanoparticle thermosensitive gel. *Int J Nanomedicine* 2011; **6**: 2873-2877.
- [2] Kim SH, Lee HS, Ryu DS, Choi SJ, Lee DS. Antibacterial activity of silver-nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. *Korean J Microbiol Biotechnol* 2011; **39**(1): 77-85.
- [3] Azeredo J, Sutherland IW. The use of phages for the removal of infectious biofilms. *Curr Pharm Biotechnol* 2008; **9**: 261-267.
- [4] Guevara AP, Apilado A, Sakurai H, Kozuka M, Tokuda H. Anti-inflammatory, antimutagenicity and anti-tumor promoting activity of mahogany seeds *Swietenia macrophylla* (Meliceae). *Philip J Sci* 1996; **125**: 271-278.
- [5] Baillie GS, Douglas LJ. Matrix polymers of *Candida* biofilms and their possible role in biofilm resistance to antifungal agents. *J Antimicrob Chemother* 2000; **46**: 397-403.
- [6] Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: advantages and limits. *Curr Med Chem* 2011; **11**: 256-279.
- [7] Bookstaver PB, Williamson JC, Tucker BK, Raad II, Sherertz RJ. Activity of novel antibiotic lock solutions in a model against isolates of catheter-related bloodstream infections. *Ann Pharmacother* 2009; **43**: 210-219.
- [8] Carneiro VA, Santos HS, Arruda FV, Bandeira PN, Albuquerque MR, Pereira MO, et al. Casbane diterpene as a promising natural antimicrobial agent against biofilm associated infection. *Molecules* 2010; **16**: 190-201.
- [9] Donelli G, Francolini I, Romoli D, Guaglianone E, Piozzi A, Ragnunath C, et al. Synergistic activity of dispersin B and cefamandole nafate in inhibition of staphylococcal biofilm growth on polyurethanes. *Antimicrob Agents Chemother* 2007; **51**: 2733-2740.
- [10] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem*

- 1956; **28**: 350-356.
- [11] Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *J Ethnopharmacol* 2000; **70**: 343-349.
- [12] Ganesh S, Namasivayam SKR. Biofilm inhibitory effect of chemogenic nano zerovalent iron against biofilm of clinical isolate of *Staphylococcus aureus*. *Asian J Chem* 2012; **24**: 5533-5535.
- [13] Namasivayam SKR, Avimanyu B. Silver nanoparticles synthesis from *Lecanicillium lecanii* and evaluation of treatment on cotton fabrics by measuring their improved antibacterial activity with antibacterial antibiotics against *Staphylococcus aureus* (ATCC 29213) and *Escherichia coli* (ATCC 25922) strains. *Int J Pharm Pharm Sci* 2011; **3**: 190-195.
- [14] Namasivayam SKR, Roy EA. Antibiofilm effect of medicinal plant extracts against clinical isolate of biofilm of *Escherichia coli*. *Int J Pharm Pharm Sci* 2013; **5**: 486-489.
- [15] Raveendran P, Fu J, Wallen SL. A simple and green method for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles. *Green Chem* 2006; **8**: 34-38.
- [16] Shankar SS, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol Prog* 2003; **19**: 1627-1631.
- [17] Dahl JA, Maddux BL, Hutchison JE. Towards greener nanosynthesis. *Chem Rev* 2007; **107**: 2228-2269.
- [18] Hutchison JE. Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology. *ACS Nano* 2008; **2**: 395-402.
- [19] Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci* 2009; **145**: 83-96.
- [20] Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology* 2007; **18**: 225.
- [21] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, et al. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B Biointerfaces* 2003; **28**: 313-318.
- [22] Sudha SS, Karthick R, Rengaramanujam J. Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria. *Indian J Exp Biol* 2013; **51**: 393-399.
- [23] Singaravelu G, Arockiyamari JS, Kumar VG, Govindaraju K. A novel extracellular biosynthesis of monodisperse gold nanoparticles using marine algae, *Sargassum wightii* Greville. *Colloids Surf B: Biointerfaces* 2007; **57**: 97-101.
- [24] Rajasulochana P, Dhamotharan R, Murugakoothan P, Murugesan S, Krishnamoorthy P. Biosynthesis and characterization of gold nanoparticles using the alga *Kappaphycus alvarezii*. *Int J Nanosci* 2010; **9**: 511.
- [25] Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology* 1979; **111**: 1-61.
- [26] Vivek M, Kumar PS, Steffi S, Sudha S. Biogenic silver nanoparticles by *Gelidiella acerosa* extract and their antifungal effects. *Avicenna J Med Biotechnol* 2011; **3**(3): 143-148.
- [27] Govindaraju K, Kiruthiga V, Singaravelu G. Evaluation of biosynthesized silver nanoparticles against fungal pathogens of mulberry *Morus indica*. *J Biopest* 2008; **1**(1): 101-104.
- [28] Merin DD, Prakash S, Bhimba BV. Antibacterial screening of silver nanoparticles synthesized by marine micro algae. *Asian Pac J Trop Med* 2010; **3**: 797-799.
- [29] Gualerzi CO, Giuliadori AM, Pon CL. Transcriptional and post-transcriptional control of cold-shock genes. *J Mol Biol* 2003; **331**: 527-239.
- [30] Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a nine-year period. *Antimicrob Agents Chemother* 2009; **53**: 639-645.
- [31] Mahdieh M, Zolanvari A, Azimeea AS, Mahdieh M. Green biosynthesis of silver nanoparticles by *Spirulina platensis*. *Scientia Iranica* 2012; **19**(3): 926-929.
- [32] Abe K, Matsuki N. Measurement of cellular 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) reduction activity and lactate dehydrogenase release using MTT. *Neurosci Res* 2000; **38**: 325-329.
- [33] Al-Fattani MA, Douglas LJ. Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. *J Med Microbiol* 2006; **55**: 999-1008.
- [34] Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: advantages and limits. *Curr Med Chem* 2011; **18**: 256-279.
- [35] Baillie GS, Douglas LJ. Matrix polymers of *Candida* biofilms and their possible role in biofilm resistance to antifungal agents. *J Antimicrob Chemother* 2000; **46**: 397-403.
- [36] Azeredo J, Sutherland IW. The use of phages for the removal of infectious biofilms. *Curr Pharm Biotechnol* 2008; **9**: 261-266.
- [37] Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956; **28**: 350-356.
- [38] Lu S, Gao W, Gu HY. Construction, application and biosafety of silver nanocrystalline chitosan wound dressing. *Burns* 2008; **34**: 623-628.
- [39] Yoo HS, Lee KH, Oh JE, Park TG. *In vitro* and *in vivo* anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates. *J Control Release* 2000; **68**: 419-431.
- [40] Mulvaney P. Surface plasmon spectroscopy of nanosized metal. *Langmuir* 1996; **12**: 788-800.
- [41] Noaman NH, Fattah A, Khaleafa M, Zaky SH. Factors affecting antimicrobial activity of *Synechococcus leopoliensis*. *Microbiol Res* 2004; **159**: 395-402.
- [42] Amaladhas TP, Sivagami S, Devi TA, Ananthi N, Velammal SP. Biogenic synthesis of silver nanoparticles by leaf extract of *Cassia angustifolia*. *Adv Nat Sci Nanosci Nanotechnol* 2012; **3**: 1-7.
- [43] Dhas TS, Kumar VG, Karthick V, Angel KJ, Govindaraju K. Facile synthesis of silver chloride nanoparticles using marine algae and its antibacterial efficacy. *Spectrochim Acta A Mol Biomol Spectrosc* 2013; **120**: 416-420.
- [44] Xia T, Kovoichich M, Liang M, Mädler L, Gilbert B, Shi H, et al. Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. *ACS Nano* 2008; **2**: 2121-2134.