Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 8, 2015

Original Article

FABRICATION OF SILVER NANOPARTICLES SYNTHESIZED FROM *GANODERMA LUCIDUM* INTO THE COTTON FABRIC AND ITS ANTIMICROBIAL PROPERTY

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Received: 04 Mar 2015 Revised and Accepted: 15 Jun 2015

ABSTRACT

Objective: To synthesize and characterize the silver nanoparticles from the mushroom, *Ganoderma lucidum* and application of this nanoparicle into the cotton fabric to impart sterility.

Methods: SNPs synthesised by using the fruiting body of *Ganoderma lucidum,* Resulted AgNPs fabricated cotton fabric was characterized using SEM (Scanning Electron Microscope) and silver nanoparticles produced from *G. lucidum* were characterized using UV-VIS Spectrophotometer, SEM, FE-SEM (Field Emission Scanning Electron Microscope), EDAX (Energy dispersion X-RAY spectroscopy)and XRD (X-ray diffraction spectroscpy). Then the incorporated cotton fabric was tested against 5 different strains.

Results: The results showed that cotton fabric incorporated with AgNPs displayed significant antibacterial activity against all the selected strains.

Conclusion: It was demonstrated that application of biologically synthesized AgNPs production and fabrication of cotton fabric, indicates sterile properties. Further it can be modified as perfect wound dressing material in the health care market. Moreover to avoid the damage to the environment the fabric containing silver nanoparticles can be treated with cyanogenic bacterial strains.

Keywords: Antimicrobial, Cyanogenic, Fabrication/incorporated, *Ganoderma lucidum*, Silver nanoparticles, Synthesis.

INTRODUCTION

The emergent development of nanotechnology has lead into the enlarged production and application of nanoparticles. They have incorporated nanoparticles into various categories of consumer products, including cosmetics, textiles, electronics and medicines [1, 2]. Silver nanoparticles are potent and broad-spectrum of antibacterial agents [3] Silver nanoparticles have been useful in the fabrication of different products, such as clothes, Socks, laboratory gowns, surgical gowns and dressing bandages, which are proved to inhibit bacterial growth [4,5] The silver compounds have been used in the medical field to treat various conditions of burns and infections. It has been commercially employed in the market as antimicrobial agent [6].

Estimable efforts have been done to explore their size dependent interaction of silver nanoparticles with bacteria with the help of electron microscopy. Researchers have used nano-silver as the basic medium for antibiotic delivery and for synthesis of composites, used as a disinfecting filters and coating materials [7].

Nowadays, the development of multi-resistant microorganisms have become a major problem, for example *Staphylococcus aureus* is resistant to methicillin and *Candida albicans* is resistant to fluconazole [8]. Thus, a newly revised wound dressing material in the market would be the great breakthrough in management of wounds and infections. In order to prevent or reduce infection a new generation of dressing material incorporated with antimicrobial agents like silver has been developed [9]. Silver ions and silver based products shows highly toxic to the microorganism, thus various combination of silver ions have been formulated and recently, it is revealed that hybrids of silver nanoparticles with amphiphilic hyper branched macromolecules expose effective antimicrobial surface coating [10, 11].

In the last few decades, there has been the vast reduction of commercial availability of textile containing antibacterial agents as it was causing environmental pollution. Silver is a good antibacterial agent, non-toxic and natural inorganic metal, it materialize as an interesting matter to be used in various kinds of textile. Silver nanoparticles coated with polyurethane foams are stable without any loss of silver nanoparticles after several washes, and this can be used in diverse forms. [12] Many synthetic procedures are available for synthesis of silver nanoparticles but these methods uses toxic chemicals, which can rise into great concern in part of an environment. Subsequently researchers have turned their path into eco-friendly method that is by biological way to synthesis silver nanoparticles. The nanoparticles synthesised could be stabilized by the proteins or other bioactive compounds [13].

Our aim in this respect, the biosynthesis of inorganic nanomaterial's using medicinal mushroom, *G. lucidum* and to fabricate silver nanoparticles into cotton fabric as the source of antimicrobial dressing material.

MATERIALS AND METHODS

Extraction of aqueous extract

Mushroom (*G. lucidum*) sample obtained was washed several times with deionized water. 68 g of finely blended sample were boiled for 2 min in 300 ml water and filtered using whatmann's filter paper (0.2 mm). The filtrate is cooled to room temperature and used as reducing agent and stabilizer.

Synthesis of AgNPs

35 mg of $AgNO_3$ was dissolved in 250 ml water. To obtain silver nanoparticles, 6 ml of mushroom was added into 30 ml of $AgNO_3$ solution. The formation and development of silver nanoparticles is indicated by light yellow colour and the reduction is completed in 30 min. The formation of nanoparticles was examined under UV-visible spectrophotometer [14].

Characterization of the nanoparticles

The nanoparticles was characterized by UV-visible studies, and the particles were then subjected to SEM and FE-SEM studies for their size determination, EDAX to determine the percentage of metals present and XRD was done for determination of size and conformation of the reduction.

Silver nanoparticles loading on cotton fabric

Cotton fabrics were washed, sterilized and dried before use. Experiments were performed on samples with maximum dimensions of 5 cm × 5 cm. The final filtrate (100 ml, 240 ppm) obtained above was treated by centrifugation for 5 minutes and half of the filtrate (superior part) was eliminated to concentrate the silver nano particles. In order to impregnate cotton fabrics (5 cm × 5 cm), these were submersed in an Erlenmeyer flask (50 ml) and kept in shaker at 600 rotations per minute for 24 h and dried at 70 °C [15].

Antibacterial activity

The antibacterial behaviour of the fabrics was evaluated against 5 different cultures. The cotton fabric was placed on agar plates inoculated with five different ATCC cultures. The inoculum was prepared in the dilution of 1.3–1.6 10^5/ml. After 24 h, the plates were sterilized and the cotton fabrics were analyzed by light microscope, scanning electron microscope and Energy Dispersive Spectroscopy (EDS) at a voltage of 20 KV after coated with Au under vacuum.

In order to study the antimicrobial activity of the fabrics, squares of 1 cm of each fabric such as fabricated silver nanoparticles-FSn and Non-fabricated silver nanoparticles-Non-FSn were prepared in aseptic condition. Each square was placed in a sterile vial and the fabrics subjected to pre treated with 800 μl distilled waters for 10 min. Nutrient broth (2.2 ml) was then added to each vial and make up to total volume of 3 ml. An aliquot (10 µl) of bacterial culture suspension was added to each vial (1.6×10^5/ml) containing the fabrics, control broths with and without bacterial inoculation were also included. The vials were then incubated with agitation at 35 °C, 220 rotations per minute. Aliquots of 10 µl broth were sampled at 24 h and serial dilution for the aliquots was prepared in broth. Duplicate aliquots (50 µl) of the serially diluted samples were spread on the plates. The plates were incubated at 35 °C and bacterial counts were performed. The bacteriostatic activity was evaluated after 24 h and percentage reduction of bacteria were calculated using the following equation

$$(\%) = ((A-B)/A) \times 100$$

Where, R=the reduction rate, A = the number of bacterial colonies from untreated fabrics, and B = the numbers of bacterial colonies from treated fabrics. [15].

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

There are several methods to synthesis silver nanoparticles. In this present study, biosynthesis of AgNPs was carried out using *G. lucidum* (G) extract within 30 min. While mushroom extracts incubated with deionized water (positive control) retained its original colour (pale yellow),but silver nitrate treated mushroom extract turned to brown colour after 30 min due to deposition of silver nanoparticles. The colour development of the extract is due to excitation of surface Plasmon resonance (SPR) in the metal nanoparticles [16]. The colour of the solution was changed to intense brown after 24 h of incubation. The extract is also stable for maximum 2 months.

The progress of the reaction between metal ions and the extracts were examined by UV–visible spectra of silver nanoparticles in aqueous solution shown in fig. I (A). The intensity of the colour increased with the incubation time. Nanoparticles size can also be determined by the change in the colour of the reactant [17, 18]. The smaller the size of nanoparticles greater the colour shifts towards reddish brown. The synthesis of silver nanoparticles was also confirmed from the UV spectra of AgNPs where the maximum absorbance was found at 418 nm after 30 min of incubation.

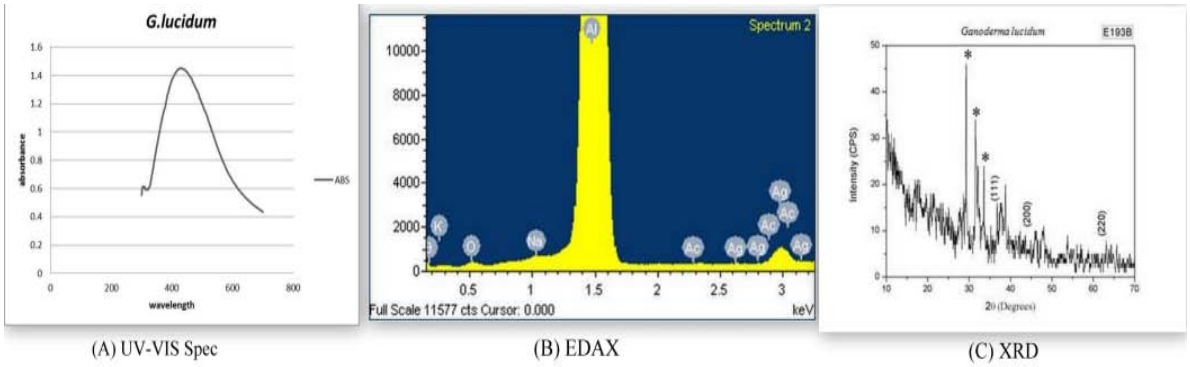


Fig. 1: Analysis of silver nanoparticles
(A)UV-Vis spectrum after 30 min of incubation (B) EDAX analysis of *G. lucidum* AgNPs (C) XRD analysis of *G. lucidum* AgNPs

The SEM pictures of the extracts are shown in three resolutions with scale G2-10.00 µm and G3-2.00 µm in fig. II; the particles are not prudently seen. Thus, for the better resolution of particles, FE-SEM was selected and the particle size was seen below 100 nm fig. III. The Energy Dispersive X-ray (EDX) analysis of the AgNPs confirmed the elemental composition of nanoparticles is silver fig. I (B).

The size of the AgNPs was determined by the Scanning Electron Microscopy (SEM) and elemental composition of the nanoparticles was further confirmed by the Energy Dispersive X-ray (EDX) analysis. The peaks of silver around 3 keV, observed in EDAX of sample, correspond to the binding energies of silver. The result indicates that the synthesized product is composed of purely silver nanoparticles. The reduction of AgNPs was due to the presence of relatively large amount of polyphenols present in the sample. Similar type of studies was done using *Aloe vera* and reported that poly phenolic compounds are responsible for reduction of silver ions [19].

X-ray scattering techniques are a family of non-destructive analytical techniques which reveal information about the crystal structure, chemical composition and physical properties of materials. These techniques are based on observing the scattered intensity of an x-ray beam hitting a sample as a function of incident and scattered angle, polarization, and wavelength or energy.

The synthesized silver nanoparticles were in crystalline form as shown in XRD pattern fig. I (c), which is similar with the literature values of FCC crystal structure [JCPDS file-87-0720] of silver. All prominent peaks at respectively 2θ values known for zero-valent FCC silver representing the (111), (200), (210) crystal planes due to Bragg's reflections are present. It confirms the shape of the sample is face centric cubic.

The size of nanoparticles obtained was approximately 75 nm. In addition to the Bragg peaks, representative of FCC silver nanocrystal and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The morphology and the nanocrystal size were determined from characteristic peaks obtained from the XRD image [20].

Applications

Antibacterial activity of treated fabric

In our study, 1.57 % of silver nanoparticles were incorporated in cotton fabrics which were characterized by EDAX as shown in fig. VI.

The bacteriostatic activity of the silver impregnated fabrics against five different stains such as *Streptococcus aureus, E. coli, Proteus species, Pseudomonas species and Klebseilla pneumonia* was studied and this activity was indicated by a zone formation fig. VII.

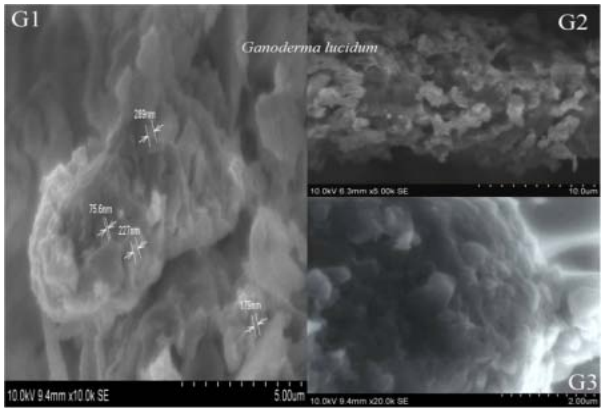


Fig. 2: SEM images of *G. lucidum.* (5.00 µm, G2-10.00 µm andG3-2.00 µm)

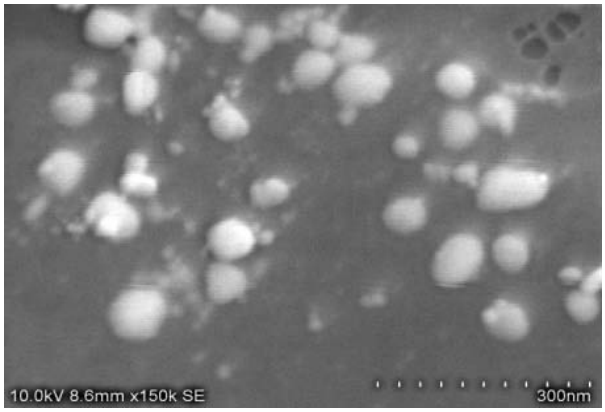


Fig. 3: FE-SEM images of *G. lucidum. (*G1-(300 nm)

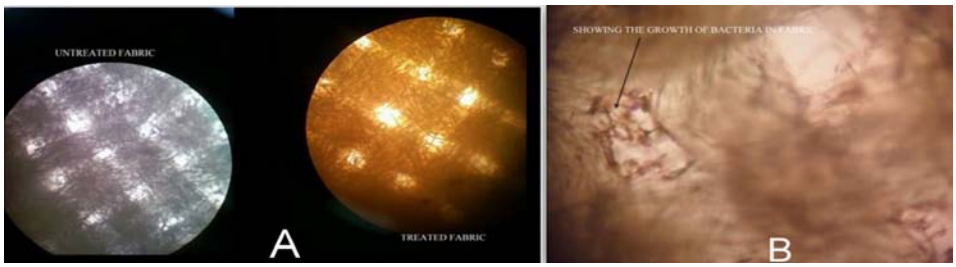


Fig. 4: Light microscope image of fabric. (A) silver nanoparticles fabricated into cotton fabric, (B) Silver nanoparticles untreated fabric-showing bacterial growth

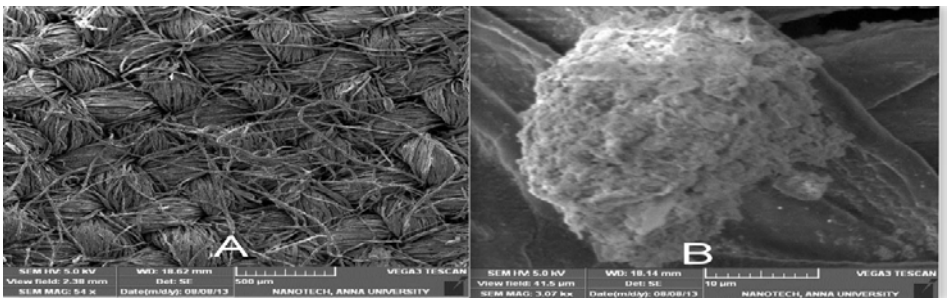
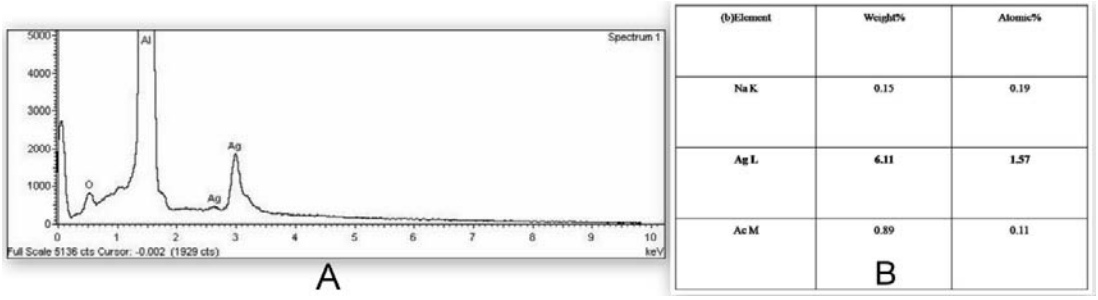


Fig. 5: SEM images of fabric. (A) Silver nanoparticles fabricated fabric, (B) Silver nanoparticles untreated fabric-showing the growth of bacteria



(b)Element	Weight%	Atomic%
Na K	0.15	0.19
Ag L	6.11	1.57
Ac M	0.89	0.11

Fig. 6: EDAX of silver nanoparticles fabricated fabric, (A) shows the graph (B) shows the percentage of silver nanoparticles

The antibacterial activity of cotton fabrics with and without silver nanoparticles was evaluated and the fabrics was analysed by SEM-EDAX. A fabric without silver nanoparticles (control) shows a significant bacterial growth, fig. IV (B) and V (B). However, bacterial growth was not seen in the cotton fabrics impregnated with silver nanoparticles fig. V (A). The results demonstrate that silver nanoparticles are used to maintain the fabrics sterile. The similar results were observed by Nelson Duran *et al.,* 2007 and Aynul Rifaya *et al.*,2014 [21,22]. The fabrics incorporated with and without silver nanoparticles were evaluated by suspending the fabrics in culture broth for estimating the growth of bacteria by plating and the number of colonies was depicted in table I.

Table 1: Showing percentage of bacterial colonies present in antibacterial treatment

ATCC culture	*Control*	*G. lucidum*
Streptococcus aureus	88%	33%
E. coli	91.3%	59%
Proteus sps	66.1%	46%
Pseudomonas sps	77.5%	36.3%
Bacillus subtilis	98%	53%
Klebseilla pneumonia	93%	43.8%

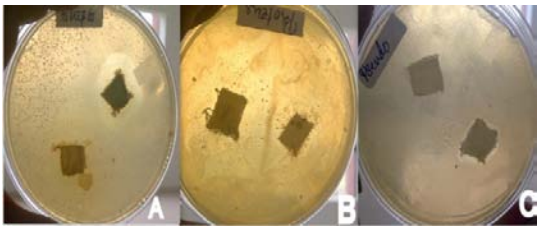


Fig. 7: Viability of cotton fabric against bacteria. (A)Streptococcus aureus (B) Proteus species. (C) Pseudomonas species

CONCLUSION

This study revealed the possibilities of using biologically synthesized silver nanoparticles and their incorporation in materials, providing them sterile properties. The cotton fabrics incorporated with silver nanoparticles exhibited strong antibacterial activity against 3 Pathogens (*Proteus s species, Streptococcus aureus, and Pseudomonas species*). Thus, it is shown that the dressing material incorporated with silver nanoparticles can be utilized as sterile fabric that could be commercially used for wounds and infections.

ACKNOWLEDGEMENT

The authors are grateful to the Centre of Nanoscience and Nanotechnology, University of Madras, Chennai to carry out the instrumentation works. I thank Mr. Joseph Varghese and Mr. Balakumaran for their kind and enthusiastic help during this study.

CONFLICT OF INTERSETS

We authors have no conflict of interest.

REFERENCES

1. Medina C, Santos-Martinez MJ, Radomski A, Corrigan OI, Radomski MW. Nanoparticles: pharmacological and toxicological significance. Br J Pharmacol 2007;152:552-8.
2. Maynard, Michelson. Woodrow wilson international center for scholars: analysis-consumer products-nanotechnology project. [http://www.Nanotechproject.org/inventories/consumer/analysis_draft/]. Nanotechnology 2005;16:2346-53.
3. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, *et al.* Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3:95-101.
4. Lee HY, Park HK, Lee YM, Kim K, Park SB. A practical procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. Chem Commun 2007;28:2959-61.
5. Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane RP, Balasubramanya RH. Functional finishing of cotton fabrics using silver nanoparticles. J Nanosci Nanotechnol 2007;7:1893-7.
6. R Holladay, W Moeller, D Mehta, J Brooks, R Roy, M Mortenson. Application Number WO2005US47699 20051230 European Patent Office; 2006.
7. Y Li, P Leung, L Yao, QW Song, E Newton. Antimicrobial effect of surgical masks coated with nanoparticles. J Hospital Infectent 2006;62(1):58-63.
8. M Schaller, J Laude, H Bodewaldt, G Hamm, HC Korting. Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an ex vivo model of cutaneous infection. Skin Pharmacol Physiol 2004;17:31-6.
9. HQ Yin, R Langford, RE Burrell. Comparative evaluation of the antimicrobial activity of ACTICOAT antimicrobial barrier dressing. J Burn Care Rehabil 1999;20:195-200.
10. Sondi B, Salopek-Sondi. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 2004;275:177-82.
11. C Aymonier, U Scholotterbeck, L Antonietti, P Zacharias, R Thomann, JC Tiller, *et al.* Hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibiting antimicrobial properties. Chem Commun 2002;24:3018-1.
12. P Jain, T Pradeep. Potential of silver nanoparticles-coated polyurethane foam as an antibacterial water filter. Biotechnol Bioeng 2005;90:59-63.
13. N Durán, PD Marcato, OL Alves, GIH De Souza, E Esposito. Mechanistic aspects of biosynthesis of silver nanoparticles by several fusarium oxysporum strains. J Nanobiotechnol 2005;3:3-8.
14. Daizy Philip. Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract. Spectrochim Acta Part A 2009;73:374–81.
15. Nelson Duran, Priscyla D Marcato, Gabriel IH, De Souza, Oswaldo L. Alves, Elisa Esposito. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Biomed Nanotechnol 2007;7:203–8.
16. SS Shankar, A Raj, B Ankamwar, S Singh, A Ahmad, M Sastry. Biological synthesis of triangular gold nanoprisms. Nat Mater 2004;3:482-8.
17. BJ Wiley, SH Im, J McLellan, A Siekkinen, Y Xia. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. J Phys Chem B 2006; 110(32):15666-75.
18. JH Fendler. editor. John Wiley and Son; 1998;7:299.
19. SP Chandran, M Chaudhary, R Pasricha, A Ahmad, M Sastry. Synthesis of gold nanotriangular and silver nanoparticles using *Aloe vera* plant extract. Biotechnol Prog 2006;22(2):577-83.
20. G Narasimha, B Praveen, K Mallikarjuna, B Deva Prasad Raju. Mushrooms (Agaricus bisporus) mediated biosynthesis of sliver nanoparticles, characterization and their antimicrobial activity. Int J Nano Dim Summer 2011;2(1):29-36.
21. Nelson Duran, Priscyla D Marcato, Gabriel IH, De Souza, Oswaldo L Alves, Elisa Esposito. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Biomed Nanotechnol 2007;3:203–8.
22. Aynul Rifaya M, Meyyappan RM. Use of herbal nano silver for fabrication of antimicrobial cotton fabrics and testing its efficacy against microbes. Int J Pharm Pharm Sci 2014;6(2):342-6.