Journal of
Nanomedicine & Nanotechnology
ISSN: 2157-7439

Harinatha Reddy and Venkatappa, J Nanomed Nanotechnol 2017, 8:4
DOI: 10.4172/2157-7439.1000453

Research Article Open Access

Synthesis of Silk Silver Nanoparticles form Silkworm Cocoons and Their Antibacterial Activity on Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*

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Abstract

In this study, silk protein was used for the synthesis of silver nanoparticles (AgNPs). The freshly prepared silk colloid solution is color less, after 24 h incubation with $AgNO_3$, the color change was observed from colourless to brown colour. The AgNPs were characterized by UV-visible spectrophotometer. The colloid solution of silk showed an absorption peak between 250-280 nm, after 24 h of incubation with $AgNO_3$ the absorbance peak was centered near 420 nm indicating the reduction of silver nitrate into silver nanoparticles by silk protein. Bacterial sensitivity to silver nanoparticles is commonly tested using a well diffusion assay. MRSA and *E. coli* were isolated from hospital soil samples. *S. aureus* and *E. coli* identified by with by microscopic and biochemical tests. MRSA identified in the selected isolates by the Kirby-Bauer disk diffusion test with vancomycin (30 µg), ampicillin (10 µg), oxacillin (1 µg) and cefoxitin (30 µg) antibiotics using Mueller-Hinton agar plates. The diameter of inhibition zone reflects the magnitude of susceptibility of microbes. The strains susceptible to silk silver nanoparticles exhibited larger zone of inhibition, whereas resistant strains exhibit smaller zone of inhibition. According to zone of inhibition Methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* exhibited sensitivity towards sericin silver nanoparticles.

Keywords: Silk; Silver nano particles (AgNPs); Silk worms; Methicillin-resistant Staphylococcus aureus (MRSA); *E. coli*

Introduction

Silk is a protein produced by *Bombyx mori* during spinning of cocoon; it consists mainly of two proteins, sericin and fibroin. Silk filament is a double strand of fibroin, held together by a protein called silk sericin [1]. Fibroin being the structural center of the silk consists of glycoproteins expressed in the middle of the silk gland. Fibroin protein used in textile industry and sericin protein considered as a waste product from the textile industry. Fibroin and sericin has medical and pharmaceutical applications. Sericin-containing food relives constipation, suppresses development of colon tumors and bowel cancer [2]. Sericin inhibits lipid peroxidation in *in vitro* rat brain homogenate [3] and inhibits UV-induced apoptosis in human skin keratinocytes [4].

The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibers. When the silkworms secrete the liquid silk during the spinning, it passes through the anterior gland and expelled out through the spinneret opening [5]. Sericin is insoluble in cold water, however, it is easily hydrolyzed, where by the long protein molecules break down to smaller fractions, which are easily dispersed or solubilised in hot water [6]. Sericin protein is useful because of its special properties viz., resists oxidation, anti-bacterial, UV resistant and absorbs and releases moisture easily, inhibits activity of tyrosine and kinase etc. Silk proteins are natural polymers and are biodegradable with reactive functional groups that open up possibility to be cross linked with other polymers to be used in controlled delivery [7]. Recently, Silk sericin has been widely used in biomaterial applications due to its biocompatibility, biodegradability and anti-oxidative and bioactive activities [8].

S. aureus is a gram positive, facultative anaerobic bacteria and common cause of skin infections, respiratory infections, bone infections, blood infections and pneumonia on humans. *S. aureus* opportunistic pathogen found in the skin and nose as part of human normal flora. There are five species to *S. aureus, S. epidermidis, S. saprophyticus, S. haemolyticus,* and *S. hominis* consider as potential human pathogens in this genus, but among this pathogenic bacteria *S. aureus* is the most problematic, causes skin, joint and blood infections in humans. Methicillin antibiotic was first used to treat *S. aureus* in 1959 and just after 2 years of use, methicillin-resistant *Staphylococcus aureus* (MRSA) strains had be isolated [9]. In MRSA stains mecA gene encodes a protein, penicillin-binding protein (PBP2a), which cannot allow the binding of β-lactam antibiotics such as such as penicillin, vancomycin, ampicillin, oxacillin and cefoxitin and in prevents the disruption of cell wall formation by these antibiotics, there by MRSA strains have resistance to many commonly used β-lactam antibiotics. Vancomycin has been the standard hospital treatment for the past 40 years, but vancomycin resistant isolates of *S. aureus* have emerged [10]. In the present study we report that activity of silk silver nanoparticles against MRSA and *E. coli* bacteria. Now a days nanoscience and nanotechnology used to treat, and prevent various bacterial, fungal and protozoa diseases in human life.

Materials and Methods

Collection of silkworm cocoons

The silkworm CSR-4 cocoons were collected form Sericulture department at Sri Krishnadevaraya University, Anantapur.

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Received: July 23, 2017; **Accepted:** August 14, 2017; **Published:** August 21, 2017

Citation: Harinatha Reddy A, Venkatappa B (2017) Synthesis of Silk Silver Nanoparticles form Silkworm Cocoons and Their Antibacterial Activity on Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. J Nanomed Nanotechnol 8: 453. doi: 10.4172/2157-7439.1000453

Preparation of sericin colloidal solution

The silkworm cocoons were cut into small pieces. The solution of silk was prepared by boiling 20 gm of the cocoon pieces in 200 ml of distilled water for 20 min. After cooling, the solution was filtered using whatman filter paper. The freshly prepared silk colloidal solution showed absorption peak between 250-280 nm [11].

Estimation of protein in sericin colloidal solution

The total protein content was determined with Folin Ciocalteau's reagent according to the Lowry et al. [12].

Biosynthesis of sericin silver nanoparticles

The crude extract of silk was used for experiments. Five ml of the crude extracts of silk was added to a conical ask containing 5 ml of 3 mM aqueous $AgNO_3$ solution heated at 65°C with continuous stirring. The silver ions were reduced to silver nanoparticles (AgNPs) within few minutes by silk extract. The conversion of solution color showed the formation of silver nanoparticles by observing color change from colorless to brown color. The synthesized silver nano particles were analyzed for UV-Visible spectroscopic studies after time duration of 24 hrs. The synthesized silver nano particles colloidal solution showed strong absorption between 400 and 420 nm.

Collection of microorganisms

MRSA and *E. coli* were isolated from hospital soil samples. The soil samples were cultured on selective and differential media (Mannitol salt agar media and Eosin methylene Blue agar) and incubated at 37°C for 48 hours. *S. aureus* and *E. coli* identified by microscopic and biochemical tests. MRSA identified in the selected isolates by the Kirby Bauer disk diffusion test with vancomycin (30 µg), ampicillin (10 µg), oxacillin (1 µg) and cefoxitin (30 µg) antibiotics using Mueller-Hinton agar plates.

Inhibition zone assay

In vitro antibacterial activity was evaluated by agar well diffusion method using Mueller Hinton Agar (MHA) [13]. Working stock was prepared as 1ml of each bacterial strain was initially inoculated in 100 ml of sterile Mueller Hinton broth and incubated for 37° ± 1°C for 24 hr respectively. Then 0.2 ml of the each test organisms from the working stock were seeded into 100 ml sterile MHA medium and cooled to 48°C to 50°C in a sterile Petri dish respectively. When the MHA solidifies, six holes of uniform diameter (7 mm) were made using sterile aluminium borer. Then, 70 µl of standard silk silver nanoparticles solution (10, 20, 30, 40, 50 µg/ml) respectively and control (Ciprofloxacin 25 mg/ml) were placed in each hole separately under aseptic condition. All the bacterial plates were then incubated at 37° ± 1°C for 18 hr and the zone of inhibition was measured (including the diameter of the bore (7 mm)) and the results were recorded.

Results and Discussion

The freshly prepared silk colloid solution is color less, after 24 h incubation with $AgNO_3$, the color change was observed from colorless to brown color (Figure 1). The AgNPs were characterized by UV-visible spectrophotometer. The colloid solution of silk showed an absorption peak at 250-280 nm, after 24 h of incubation with $AgNO_3$ the absorbance peak was centered near 420 nm indicating the reduction of silver nitrate into silver nanoparticles by fibroin and sericin proteins (Figure 2).

Bacterial sensitivity to silver nanoparticles tested using a well diffusion assay. The diameter of inhibition zone reflects the magnitude of susceptibility of microbes. The strains susceptible to silk silver nanoparticles exhibited larger zone of inhibition, whereas resistant strains exhibit smaller zone of inhibition. According to zone of inhibition *S. aureus and E. coli* exhibited the highest sensitivity toward silk silver nanoparticles (Figure 3). The microbial property of silver nanoparticles was analyzed by measuring the inhibition zone (Table 1). The synthesized silk silver nanoparticles show an effective antibacterial activity against pathogens of Gram positive and Gram negative bacteria. The result suggests that silver nanoparticles undergo an interaction with bacterial cell and displayed the strong action against MRSA *and E. coli*. The antimicrobial properties of silver compounds and silver ions had been historically recognized and applied in a wide range of applications from disinfecting medical devices and for the treatment of

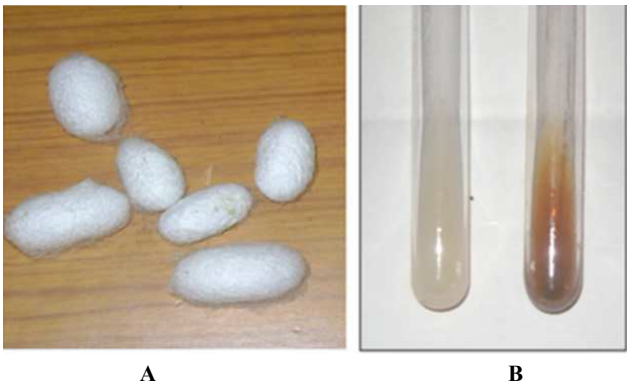

Figure 1: (A) Silkworm cocoons (B) The silk colloid solution colour change from colourless to brown colour after 24 h of incubation with $AgNO_3$.

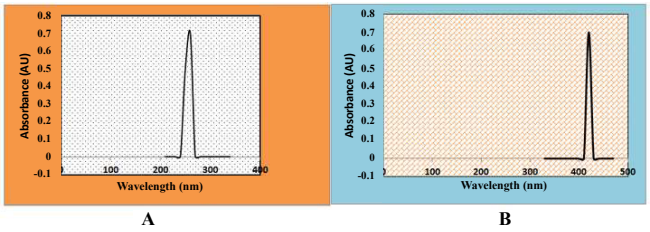

Figure 2: (A) UV-Visible spectroscopic absorption peak of silk colloid solution at 276 nm. (B) UV-Visible spectroscopic absorption peak of silk silver nanoparticles at 420 nm.

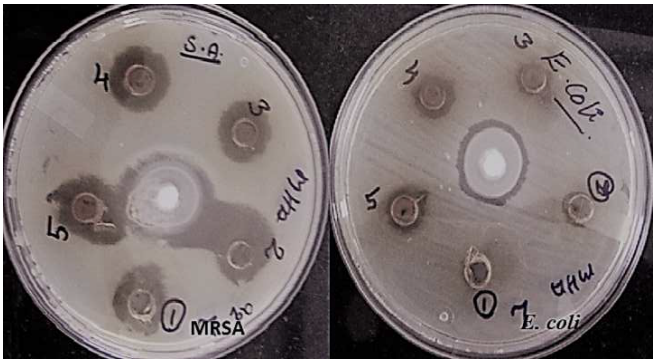

Figure 3: Antibacterial activity of silk silver nanoparticles on (A) *Methicillin-resistant Staphylococcus aureus* (MRSA) *(B) E. coli.*

S. No	Microorganisms	Zone of inhibition measured in mm (µg/ml)					
		Ciprofloxacin (25 mg/ml)	10	20	30	40	50
1	MRSA	26	11	16	18	20	24
2	*E. coli*	23	10	13	16	18	21

Table 1: Antibacterial activity of silk silver nanoparticles on *Methicillin-resistant Staphylococcus aureus* (MRSA) and *E. coli*.

water. AgNPs is currently used to control bacterial growth in a variety of applications, including dental work and burn wounds. In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microbes, showing strong biocidal effects [14].

In this experiment AgNPs were showed high antibacterial activity on Gram-positive methicillin-resistant *Staphylococcus aureus* bacteria and Gram-negative *E. coli* bacteria. AgNPs seem to be alternative antibacterial agents to antibiotics and have the ability to overcome the bacterial resistance against antibiotics. When *E. coli* cells treated with AgNPs, the AgNPs were accumulated in the cell wall of bacteria and induce formation pores in the bacterial cell walls, eventually leading to cell death [15]. The positively charged silver nanoparticles bind to the negatively charged polyanionic backbones of teichoic acids and cell wall glycopolymers, leading to structural strain and permeability of the bacterial cell wall [16].

Acknowledgement

The authors express their sincere thanks to all the authors in reference list for support to this research.

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