J Mater Sci: Mater Med (2011) 22:2721–2726
DOI 10.1007/s10856-011-4462-9

Fabrication of novel biofibers by coating silk fibroin with chitosan impregnated with silver nanoparticles

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Received: 10 May 2011 / Accepted: 17 October 2011 / Published online: 1 November 2011

Abstract Nanoparticle based agents often applied as coatings on biomaterials have shown promise in delivering the improved sterility against variety of microbes. In the present study, silk fibers (SF) were coated with chitosan impregnated with silver nanoparticles (Ag–C–SF). These Ag–C–SF fibers were characterized using scanning electron microscopy (SEM) with energy dispersive X-ray analysis, atomic force microscopy (AFM), Infra Red spectroscopy, Thermogravimetric Analysis and Microbiological assay techniques. AFM studies have confirmed the nano sized silver particles in chitosan solution; SEM pictures have exhibited the coating of chitosan along with silver nanoparticles on the silk fibroin. The modified fibers have also shown anti-microbial activity and improved thermal stability. The Ag–C–SF fibers may be explored as wound dressing and tendon reconstruction material in future.

1 Introduction

Modification, manipulation and fabrication of the materials at nano scale are important for their use in medical and technological applications [1]. Particularly, the materials used in medical applications should be non toxic, biocompatible and non-immunogenic. Silk fibroin from Bombyx mori (silk worm) is an FDA approved product for many medical applications such as surgical, drug delivery and tissue engineering. The applications include burn wound dressings, enzyme immobilization matrices, vascular prostheses and structural implants [2]. Silk fibers are preferred as a raw material for the above applications because of its biocompatibility, good tensile properties, flexibility and slow proteolytic biodegradation [3]. Silk is also useful as a smart structural fabric for a range of applications and a variety of composites can be made from it that imparts magnetic, electrical and semiconductor properties [4]. Silk sutures have also been used for tendon tissue engineering [5]. Silk films have been used for improved cell attachment and bone formation, especially when they are coupled with Bone morphogenetic protein-2 (BMP-2) [6].

Chitosan [poly-β-(1→4)-D-glucosamine] is a sustainable, biocompatible, biodegradable and antimicrobial polysaccharide of great relevance in many fields of application [7]. As chitosan is a polycation it can be employed as a nano or micro system in a wide range of biomedical applications such as drug [8–10] or gene delivery systems [11, 12]. As chitosan offers pH responsive solubility it forms films and hydrogels and can be directed to assemble in response to locally applied electrical signals; its backbone provides sites that can be employed for the assembly of proteins, nucleic acids and virus particles [13]. Drug delivery system consisting of chitosan grafted nanoparticles can be developed for various pharmaceutical applications [14]. Biodegradable, anti-microbial bionanocomposite films were developed using chitosan and silver nanoparticles for application in food packaging industry [15].

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The bactericidal activity of silver nanoparticles is attributed to the presence of electronic effects, which are a result of the changes in the local electronic structure of the surfaces of the smaller-sized particles. These effects contribute to the enhancement of the reactivity of silver-nanoparticle surfaces. It has been reported that ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them [16]. Silver nanoparticles have also been recognized as antimicrobial agents because they inhibit ATP Synthesis in the microorganism, denature the DNA and block the respiratory chain [17, 18]. In this report, we describe the fabrication of functional biofibers containing silk fibers coated with chitosan loaded with silver nanoparticles and their characterization. These biofibers may be useful in the management of infected wounds and reconstruction of ligaments or tendons.

2 Experimental sections

2.1 Materials

Cocoons (pupae removed) were kindly supplied by department of sericulture, Government of Andrapradesh, India. Chitosan was prepared in our laboratory from prawn shells as described in our previous study [19], the degree of deacetylation was found to be 75%. Ag_2SO_4, $NaBH_4$ were purchased from sigma-Aldrich, St. Louis, MO, USA. Bacterial strain (*Escherichia coli—E. coli*) was kindly supplied by the microbiology department of Central Leather Research Institute, Chennai. Muller Hinton medium was purchased from Hi-media laboratories pvt. Ltd, Mumbai, India. All other reagents used were of analytical grade.

2.2 Isolation of silk fibers from the cocoons

The cocoons were boiled in water for one hour to remove sericin [20]. Later the cocoons were dried at room temperature (30°C), individual fibers were removed manually and wound around a wooden spindle.

2.3 Preparation of chitosan solution

2% chitosan solution was prepared by dissolving chitosan in 0.1 N HCl.

2.4 Preparation of silver nanoparticles using chitosan solution

Silver nanoparticles were prepared using chitosan solution by modifying the procedure described earlier [21]. Briefly, to 1 ml of chitosan solution, 1 ml of aqueous Ag_2SO_4 solution (1×10^{-4} M) was added followed by stirring for one hour. To this solution, 0.2 ml of 1 M $NaBH_4$ (dissolved in 0.3 M NaOH) was added drop wise followed by stirring till the solution attained golden brown color. At this stage addition of $NaBH_4$ was stopped, the stirring was continued for another 15 min; formation of silver nanoparticles was observed. This solution containing silver particles was stored at 10°C till further use.

2.5 Coating of chitosan solution containing silver nanoparticles onto silk fibers

The chitosan solution containing silver nanoparticles was filled in a petridish and the silk fibers were passed through the solution at a speed of 1 cm/min and the fibers were immersed into the solution for 10 min. Later these fibers were dried at room temperature (30°C) for 48 h and stored under vacuum until further use/analysis. Circular discs with a diameter of 5 mm were punched from the cocoon surface, dipped into the chitosan solution containing silver nanoparticles for 10 min, dried at 30°C for 48 h and stored under vacuum and utilized for conducting antimicrobial assay (Fig. 1).

3 Characterization

3.1 Scanning electron microscopy and energy dispersive X-ray analysis

SEM measurements were carried out on a Leica stereo scan-440 scanning electron microscope equipped with phoenix EDX attachment. Prior to the analysis, the samples

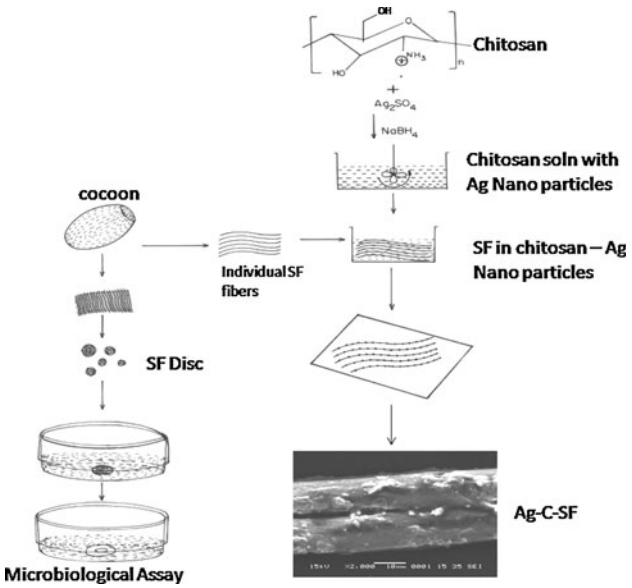

Fig. 1 Schematic diagram of preparation of Ag–C–SF and its antimicrobial screening

were gold sputter coated under argon atmosphere to render them electrically conductive. The EDX spectrum was recorded in the spot profile mode by focusing the electron beam onto the specific regions of the biofibers. Pictures were then taken at an excitation voltage of 10 kV.

3.2 Atomic force microscopy

Atomic force microscopy was used to study the size and shape of the silver nanoparticles formed in the chitosan solution. The samples were measured using AFM (Agilent Technologies, Scanning probe microscope, Picole, USA). The chitosan solution containing silver nanoparticles was added to silicon wafer drop wise using micropipette. The silicon wafer was spin coated at an rpm of 12,000 to form uniform layer. The spin coated silicon substrate was dried for two hours prior to AFM imaging. AFM analysis was done by using silicon cantilevers of spring constant 0.2–0.77 nm and the tip height of 10–15 nm.

3.3 Infrared spectroscopy

The IR spectroscopy of the biofibers prepared was taken using Nicolet impact 400 FTIR spectroscope by preparing a 500 mg KBr pellet containing 2–6 mg of sample.

3.4 Thermogravimetric analysis

The thermal stability of the fibers was determined with a thermogravimetric analyzer NETZSCH Instruments, Burlington, MA over a temperature range of 37–1,000°C at a heating rate of 20°C per minute under Nitrogen atmosphere.

3.5 Anti-microbial screening [22]

To establish the antibacterial properties of the Ag–C–SF, the bacterial cells were cultured aerobically in 25 ml of nutrient broth at 37°C for 24 h. SF was treated as control, C–SF and AG–C–SF samples were treated as experimental. Disc diffusion method with some modifications was used for screening the anti bacterial activities of SF, C–SF and Ag–C–SF. Nutrient agar (for bacteria) plates were inoculated with 0.1 ml of an appropriate dilution of the tested culture (*E. coli*). SF, C–SF and Ag–C–SF discs were placed on the surface of the inoculated plates and incubated at 37°C for 24 h. The diameter of inhibition zone (mm) including the disc diameter was measured.

4 Results and discussion

Normally patients pick up infection from the hospital associated bacteria on the surfaces of medical devices and biomaterials. Hence, biomaterials with their surfaces having anti bacterial agents are highly sought after. Nanoparticle based agents often applied as coatings have shown promise in delivering the improved sterility against variety of microbes [23]. Present study is an effort in that direction to fabricate bio fibers having silver nanoparticles on their surface.

4.1 Morphology

SEM images of SF and Ag–C–SF are shown in Fig. 2a and b, respectively. Smooth surface can be observed in the SEM images of SF; in Fig. 2b we can clearly see the coating of chitosan on the fiber impregnated with silver particles. The chitosan coating on the fiber is continuous. Some of the silver particles are in nano size (75 nm) and some are aggregated to micron size (1,200 nm). The EDX spectrum of Ag–C–SF (Fig. 3) clearly showed the presence of silver. This confirms that the particles appeared in the SEM picture are silver nanoparticles.

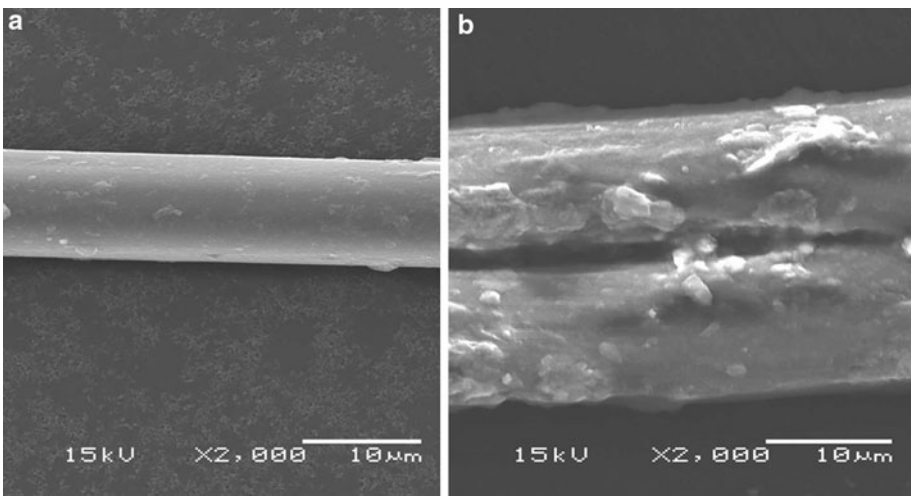

Fig. 2 **a** SEM image of SF showing smooth surface of SF. **b** SEM image of Ag–C–SF exhibits the coating of chitosan on SF, silver nanoparticles can be seen on the surface

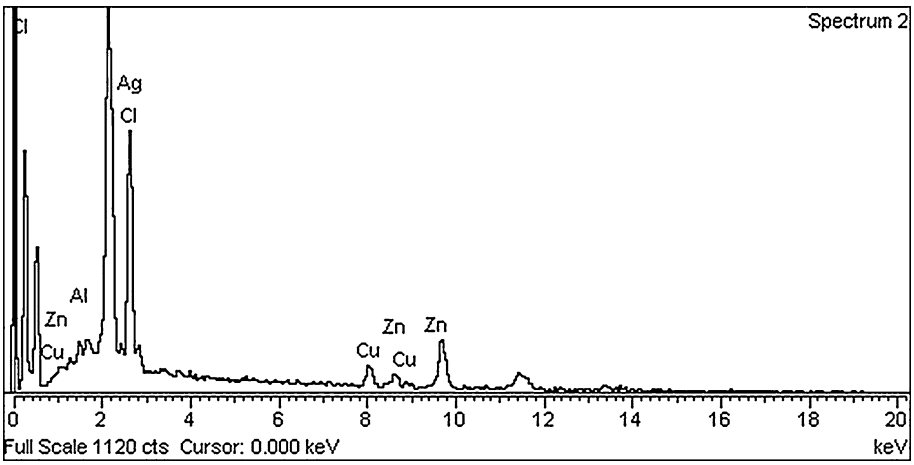

Fig. 3 EDX spectrum of Ag–C–SF shows the presence of Ag

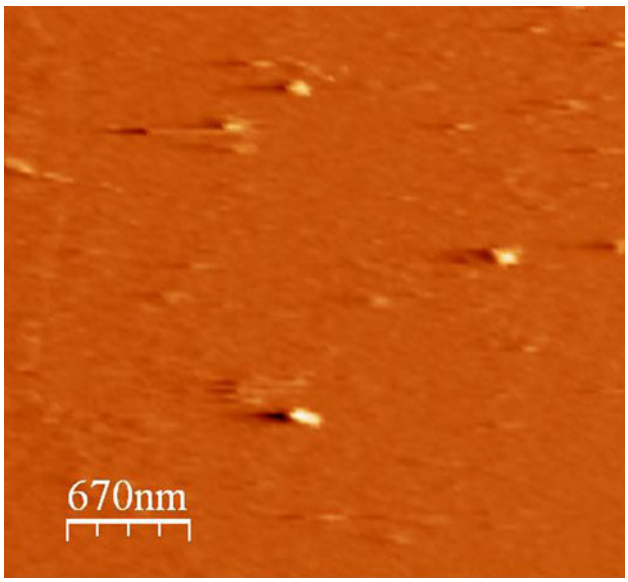

Fig. 4 AFM picture of silver nanoparticles in chitosan solution

4.2 AFM studies

The AFM pictures of silver nanoparticle loaded chitosan solution are shown in Fig. 4a and b. Live profiles of the silver nanoparticles were taken and the particle size distribution of the particles was found to be 75–150 nm. From the AFM pictures it was found that the shape of the silver nanoparticles was irregular.

4.3 Infrared spectroscopy

The IR spectra of SF and Ag–C–SF are shown in Fig. 5a and b. The IR spectrum of SF shows amide absorption bands at 1,655 cm^{-1} (amide I), 1,564 cm^{-1} (amide II) and 1,237 cm^{-1} (amide III) along with hydroxyl groups as a broad band between 3,200 and 3,500 cm^{-1} with a peak at 3,406 cm^{-1}. –C–OH vibration band was observed from 1,000 to 1,060 cm^{-1} as a broad band. In the IR spectrum of Ag–C–SF we can observe the absence of amide III band;

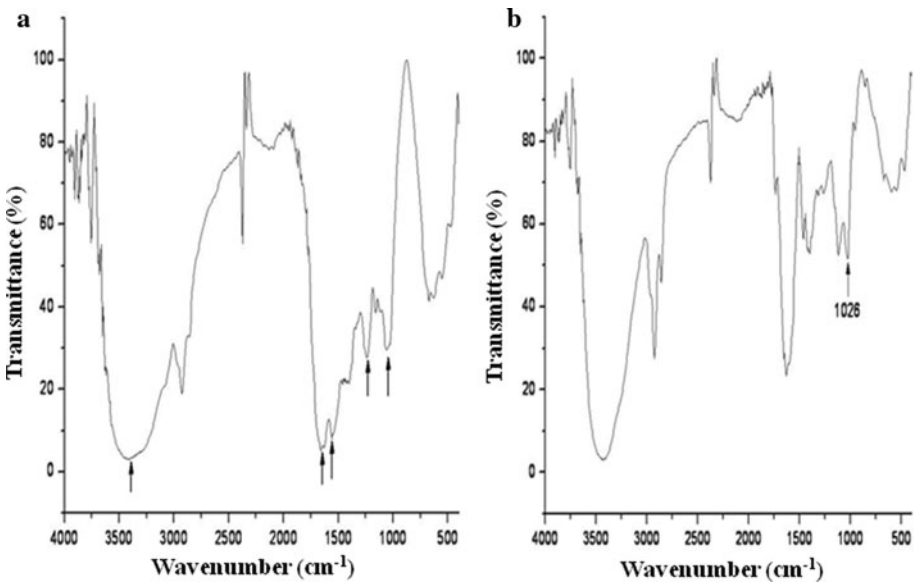

Fig. 5 **a** IR spectrum of SF showing amide absorption bands at 1,655 (amide I), 1,564 (amide II) and 1,237 cm^{-1} (amide III) –C–OH vibration band between 1,000 and 1,060 cm^{-1} is also seen. **b** In IR spectrum of Ag–C–SF, the amide III band is absent, –C–OH vibration band with low intensity is seen at 1,026 cm^{-1}

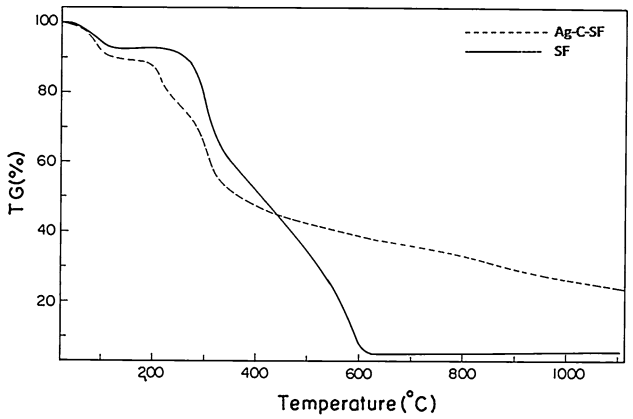

Fig. 6 Thermogram of SF (—) showing a single step weight loss between 260 and 594°C. 88% weight loss was observed at 600°C. Thermogram of Ag–C–SF (——) showing a two step weight loss, first between 185 and 220°C and the second between 220 and 570°C. At 820°C only 79% weight loss was observed

–C–OH– band is shortened and seen at 1,026 cm^{-1} with lower intensity. These observations indicate the hydrogen bond formation between functional groups (amino and hydroxyl groups) of chitosan and SF.

4.4 Thermogravimetric analysis

Figure 6 illustrates the thermal decomposition profile of SF and Ag–C–SF. In thermogravimetry, the losses of weight due to evolution of water, carbon monoxide, carbon dioxide and evaporation of other pyrolysis products are collectively measured as percentage of original weight. In this investigation it was observed that the SF decomposed at single stage from 260 to 594°C with a maximum weight loss at 332°C. The weight loss between 260 and 594°C was 88% and the total mass change between 37 and 600°C was found to be 95%. A two step weight loss was observed in the case of Ag–C–SF, first being from 185 to 220°C and the second from 220 to 570°C. The first weight loss (15%) is attributed to the decomposition of the bound water and second weight loss (33%) was due to the decomposition of SF and chitosan. At 820°C a total mass change of 79% was observed. The coating of the chitosan loaded with silver nanoparticles on to the SF surprisingly increased the thermal stability of composite materials. In the case of SF 95% material was decomposed at 600°C whereas 79% of the material loss was observed at 828°C for Ag–C–SF.

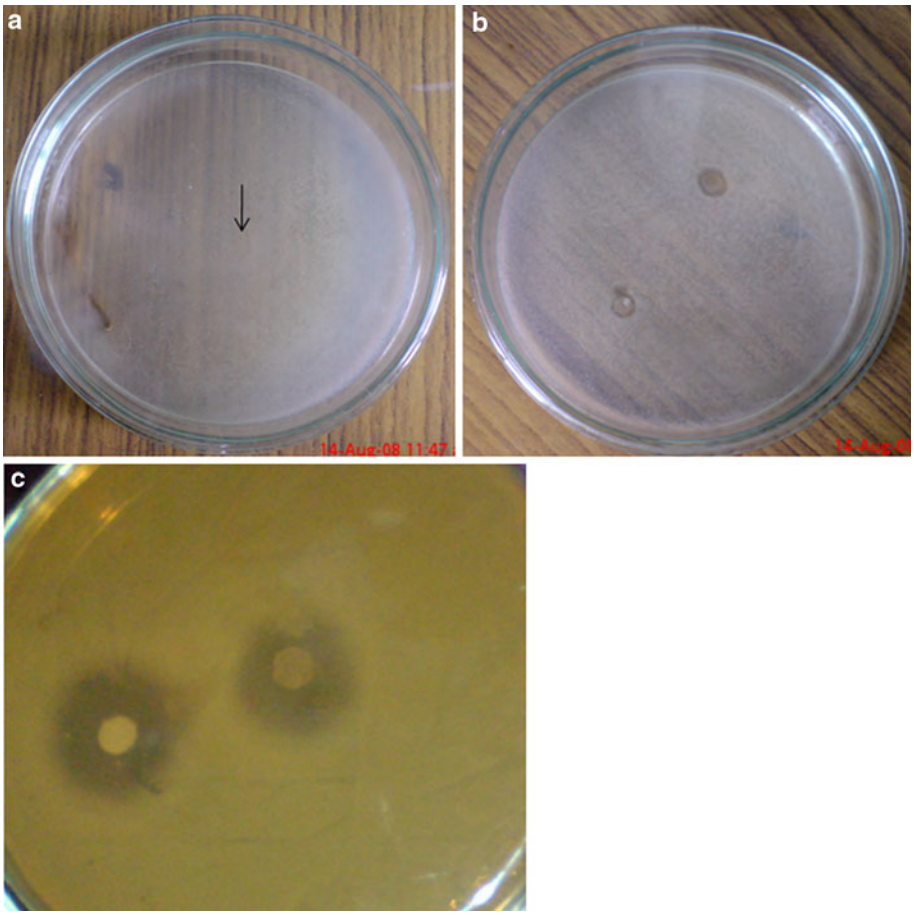

Fig. 7 **a** Culture plate consisting of SF disc (control); bacterial growth is seen in entire culture plate. **b** Culture plate consisting of C–SF disc; 6 mm inhibition zone can be seen. **c** Culture plate consisting of Ag–C–SF disc; 16 mm inhibition zone can be seen

4.5 Microbial assay

In the control plates (SF disc) the bacterial growth was observed in the entire culture plates (Fig. 7a), however, in the experimental plates containing C–SF disc (Fig. 7b), the zone of the bacterial inhibition was found to be 6 ± 1 mm which is around 60% more than the diameter of the disc. This is due to the chitosan's antimicrobial activity. Discs containing Ag–C–SF have shown 16 ± 3 mm of inhibition zone (Fig. 7c). This enhanced antibacterial activity might be due to the silver nanoparticles. The positively charged silver atoms adhere to bacterial cell walls because of the overall charge on the cell surface at biological pH is negative. These oppositely charged electrostatic interactions might be the reason for the bactericidal effect of silver nanoparticles to enter into bacteria, inhibit the ATP synthesis and denature DNA and blocking the respiratory chain.

5 Conclusions

The novel biomaterial Ag–C–SF, prepared in the study has shown antimicrobial activity and increased thermal stability. This material might be a promising material for the use in wound healing, tendon reconstruction etc. Use of Ag–C–SF as a biosensor may be explored in future.

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