Chapter-2

Cellulose–polymer–Ag nanocomposite fibres for antibacterial fabrics/skin Scaffolds

Development of CSNCFs
2.1 Introduction

In medical field, medical fabrics are gaining significant importance as health care materials especially in the past two decades. Essentially, these public health materials should provide adequate barriers against highly communicable bacteria and viruses. The spread of HIV, hepatitis viruses, severe acute respiratory syndrome (SARS) etc., by contacting with contaminated materials, has created increased pressure for protection of personnel with functional clothing. In the surgical zone, a lot of priority has been given to the protection of the surgical team from the patient’s infectious blood and other body fluids and vice versa. Therefore, surgical fabrics should possess more antimicrobial properties [1,2]. The cellulose fibre skin scaffolds contains cellulose, a homopolymer of β-D-glucopyranose units linked together by (1→4)-glycosidic bonds [3]. Cellulose molecules are linear in nature and are aggregated through van der waals forces, intra- and intermolecular hydrogen bonds. The reactive sites in cellulose molecules are the three-hydroxyl groups which are widely responsible for metal nano particles bonding characteristics [3].

Silver, inherently possessing the antibacterial properties, has been used to accelerate healing of skin wounds [4,5] and also for treating a variety of diseases including pleurodesis and cauterisation. Few researchers have reported on the possible pro-healing properties of silver [6]. Recently, silver nano particles have been demonstrated to exhibit cytoprotective activities toward HIV-1-infected cells [7]. The acceleration of delayed wound healing and faster diabetic wound healing [4,8] are the additional characteristics of silver nano particles (AgNPs) as reported by Tian et al [4] and Mishra et al [8].

Silver nano particles are produced by reducing the silver salts with chemical agents which are usually associated with environmental toxicity or biological hazards. Therefore, the
development of AgNPs based on naturally occurring carbohydrates is considered to be one of the most appropriate methods, for obvious environmental reasons. In that category, gum acacia (GA) (Gum arabic) and guar gum (GG) (Guaran), are well-known polysaccharides, derived from acacia tree and guar beans respectively and are used for the formation and stabilization of AgNPs [9,10]. These natural polymers are available not only at low cost, but also more abundant and have excellent emulsifying and surface-active properties [11,12], which are beneficial for the design of metal nanoparticles in nanotechnology.

Of late, the incorporation of AgNPs in cotton fibres has received great attention due to their high resistance to microbes. Vigneshwaran et al. [13] have developed the in-situ synthesis of silver nano particles on cotton fabrics. Perelshtein et al. [14] developed a sonochemical method for coating AgNPs, using ultra sound waves [14]. Duran et al. [15] incorporated silver nano particles into cotton fabrics by fungal process. Yu et al. [16] also reported the incorporation of silver nano particles into ultrafine fibres by electro spinning.

Basing on the above discussion, the present investigation involves the development of an efficient, non-toxic, durable and cost effective antimicrobial cellulose fibres with increased applications in medical field. The key feature of this method is that the use of synthetic reducing agents was completely avoided. The effective antimicrobial cellulose fibres were developed by green process by employing naturally available carbohydrates: GA (composed of glycoproteins, arabinose and ribose) and GG (composed of galactose and mannose) for the reduction of silver ions. The developed AgNPs were incorporated into cellulose fibres to use as antibacterial fabrics. The advantages of using the green process are: (a) There is no need to have extra reducing agent(s), (b) The process can be conducted at ambient temperature and (c) Environmentally friendly process. The details of this investigation is presented in this chapter.
2.2 Experimental

2.2.1 Materials

Gum acacia (GA), gaur gum (GG) and silver nitrate (AgNO₃) were purchased from S D fine-Chem Ltd. (Mumbai, India) and used as received without further purification. Cellulose cotton fibres (1mm thickness) were purchased from SIMCO thread mills (Salem, Chennai, India). Twice distilled water was used in all the experiments.

2.2.2 Preparation of polymer Ag-nano particles (Poly-AgNPs) solutions

Initially, 100 mL of 0.3%, 0.5% and 0.7% (w/v) GA and GG solutions were prepared separately by stirring respective amounts of GA and GG (g) in distilled water at 300 rpm for 24 h in an orbital shaking incubator, maintained at ambient temperature. Later, 5 mL of 0.588 mM of AgNO₃ solution was introduced into each of the above solutions and stirred at 300 rpm for 80 h by maintaining constant room temperature 27 °C. The natural polymers, GA and GG solutions reduce the Ag⁺ ions of AgNO₃ present in GA and GG solutions into Ag⁰ nano particles to form poly-AgNPs solutions of GA and GG respectively.

2.2.3 Preparation of cellulose-silver nanocomposites fibres (CSNCFs)

CSNCFs were produced by rotating the pre-weighed and washed cellulose fibres (5 g), immersed in the Poly-AgNPs solutions in an orbital shaking incubator at 300 rpm for 24 h at an ambient temperature. Rotation allows the AgNPs to impregnate into the cellulose fibres. Finally, the CSNCFs developed were taken out, dried and kept in a desiccator prior to characterization. Different CSNCFs having various formulations were developed by using different concentrations (0.3%, 0.5% and 0.7%) of GA and GG.

2.2.4 Characterizations
Formation of AgNPs was monitored for all the Poly-AgNPs solutions, using UV–Vis spectrophotometer (Elico-SL 164, Hyderabad, India). Fourier transform infrared (FTIR) measurement was done using Perkin Elmer (Model Impact 410, Wisconsin, MI, USA) spectrophotometer in order to determine the shifting of functional groups of GA and GG in pure solution and in the various Poly-AgNP solutions. Thermal properties were determined from the TGA data, using SDT Q 600 thermal analyzer (T. A. Instruments-water LLC, Newcastle, DE, USA), at a heating rate of 20 °C/min and passing nitrogen gas at a flow rate of 100 mL/min. Mechanical properties (tensile strength, modulus and % elongation-at-break) were determined using INSTRON 3369 Universal Testing Machine (Buckinghamshire, England) set at a crosshead speed of 5 mm/min and at 23 °C. In each case, four samples were analyzed and the average value was reported. For SEM analysis all the samples were carbon coated, prior to examination with a field emission scanning electron microscope. Scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis of cellulose fibre and CSNCFs were performed using a JEOL JEM-7500F (Tokyo, Japan) operated at an accelerating voltage of 15 V. Transmission electron microscope (TEM) (JEM-1200EX, JEOL, Tokyo, Japan), was used for the morphological observation. TEM sample was prepared by dispersing two to three drops of (1 mg/1 mL) polymer-silver nano particles solution on a 3 mm copper grid and dried at ambient temperature.

2.2.5 Antibacterial activity

The antibacterial activity was tested for all the CSNCFs, following the inhibition zone method by taking *E. coli* as the model bacteria, using modified agar diffusion assay (disc test) maintained at 37 °C for 1 day as the incubation period. Nutrient agar medium was prepared by mixing peptone (5.0 g), beef extract (3.0 g) and sodium chloride (5.0 g) in 1000 mL of distilled
water and the pH was adjusted to 7.0. Finally, agar (15.0 g) was added to the solution. Sterilization of agar medium was done in a conical flask in an autoclave (MAC, MACRO scientific works Pvt. LTD., Delhi) at a pressure of 6.8 kg (15 lbs) for 30 min. This medium was transferred into sterilized petri dishes in a laminar air flow chamber (Microfilt Laminar Flow Ultra Clean Air Unit, Mumbai, India). After solidification of the media, E. coli (50µL) culture was spread on the solid surface of the media. Over this inoculated petri dish, small pieces of AgNPs cellulose fibres and pure cellulose fibre (C) were distributed and incubated for 2 days at 37 °C in an incubation chamber. After this period, the inhibition zones were observed and photographed.

2.3 Results and discussions

Development of CSNCFs involves the initial synthesis of AgNPs using solutions of Natural Polymers and impregnation of the developed AgNPs into cellulose fibres. This approach involves the use of 0.3, 0.5 and 0.7% (w/v) of GA and GG solutions as reducing media for the preparation of AgNPs from 0.588 mM AgNO₃ solutions. The conversion of Ag⁺ ions into silver nano particles was due to the presence of functional groups in GA and GG [17] where the pendent hydroxyl groups of GA and GG were assumed to be actively involved in the reduction process. Further, the high molecular chains present in GA and GG provide stabilization of the formed AgNPs. The impregnation of AgNPs in to the cellulose fibers was done by immersing and rotating the cellulose fibers in the poly-AgNPs solutions of GA and GG at 300 rpm for 24 h at ambient temperature of 27 °C in an orbital shaking incubator, and subsequently taken out and dried. Silver nano particles incorporated on the cellulose fibers were considered to be attached due to physical interactions with the surface hydroxyl groups of cellulose [3]. The schematic
representation of the incorporation of silver nano particles on the cellulose fibers is illustrated in Scheme 1.

The materials (gum acacia, guar gum and cellulose fibre) used in the present investigation to develop CSNCFs are naturally available.

Gum acacia (GA), a natural polysaccharide derived from exudates of Acacia senegal and Acacia seyal trees. It is commonly used as food hydrocolloid. Chemically, it consists $\beta$-(1→3) galactose units in the backbone with branches of arabinose and rhamnose [18].

Guar gum (GG) is primarily the ground endosperm of guar beans which consists, mainly (1→4)-$\beta$-D-mannopyranosyl backbone with branch-points from the six-position linked to single $\alpha$-D-galactopyranosyl residues [19]. Guar gum is an effective hypocholesterolemic agent and prevents hypercholesteromia and reduces body weight.

Cellulose fibre consists majorly cellulose, a homopolymer of $\alpha$-D-glucopyranose units linked together by (1→4)-glycosidic bonds [3]. Cellulose is the most abundant organic polymer on the Earth and present in the primary cell wall of the plants. Cellulose fibres that are
naturally present in cotton consists 90% of cellulose. These cellulose fibres are used in textile industries and also to design wound dressing materials/scaffolds. The cellulose fibres used in the present investigation are processed under normal conditions.

Diagram of formation of cellulose–polymer silver nanocomposites fibers: A) UV–Visible spectra of (a) GA-AgNPs solution (b) GG-AgNPs solution; B) TEM image of (a) GG 0.3% GG-AgNPs solution (b) 0.7% GG-AgNPs solution; C) TEM image of (a) 0.3% GA-AgNPs solution (b) 0.7% GA-AgNPs solution; D) SEM images of (a) 0.3% GA CSNCF (b) 0.7% GA CSNCF (c) 0.3% GG CSNCF (d) 0.7% GG CSNCF.
2.3.1 UV-Visible spectral analysis

Formation of silver nano particles was predicted by color change from colourless to ruby red colour of Poly-AgNPs solutions and was confirmed with the UV–Vis spectra by observing an intense absorbance band exactly between 430–450 nm. This is due to surface Plasmon resonance excitation vibrations of the silver nano particles [15,20]. Fig 1a and Fig 1b shows the UV-Vis spectra of the different GA and GG AgNPs solutions respectively. From the figure, it is clear that there is an increase in the intensity of the peak as observed with corresponding increase of GA and GG concentrations. Furthermore, for the same concentrations, GG shows high intense peaks than GA. Absorption peaks at 432.51, 434.65, 435.71, 434.74, 437.95 and 443.16 nm are observed for GA 0.3% , GA 0.5% , GA 0.7% , GG 0.3% , GG 0.5% and GG 0.7% respectively.

Also for the same concentration, wavelength than Poly-AgNPs solution of GA. This red shift indicates either an increase in the size or aggregation of AgNPs in GG solutions than in GA solutions [21]. The same can be confirmed by TEM analysis, where the GG-AgNPs were identified to be larger in size than GA-AgNPs (Fig 3). The increase in absorption with time up to 80 h was shown in Fig 2.
Fig 1: UV–Visible spectra of (a) GA-AgNPs solution (b) GG-AgNPs solution

Fig 2: UV absorption and time dependent spectra of polymers AgNPs (GG, GA) solutions

2.3.2 Transmission electron microscopic (TEM) analysis

The formation of AgNPs in polymer solutions (GA, GG) was conformed from TEM analysis. From the shown TEM images as shown in Fig 3A and Fig 4B, it is clear that the formed AgNPs were spherical in nature with an average particle size of 5±3 nm in poly-AgNPs solution of GG (Fig 3A(a) and Fig 3A(b)) and 4±2 nm in poly-AgNPs solution of GA (Fig 3B(a) and Fig 3B(b)). From the TEM analysis, it is concluded that the size of the formed AgNPs was in between 2 and 8 nm, which was about 62% smaller in size compared to our earlier work [22], where the size of AgNPs was around 21 nm. Furthermore, the size of AgNPs in poly-AgNPs solution of GG was larger than the size of AgNPs in poly-AgNPs solution of GA, which further supported by UV–Vis data where the surface plasmon resonance peak of the formed AgNPs from GG reduction was observed at higher wave length (red shift) than from GA reduction. It was expected that both GA and GG can stabilize the AgNPs by well-established chemical bonding between the functional groups [23,9]. This can be technically concluded that, as the aggregation of AgNPs was not found as per the TEM images, which may be attributed due to the effective passivation of the surfaces and the suppression of the growth of the nano particles.
through strong interactions via the functional molecular groups of GG (OH) and GA (OH, COOH).

The formation of silver nano particles inside the polymer network (GA and GG) was also investigated by FTIR analysis. The FTIR spectra of pure GA, pure GG, aqueous solutions of GA, aqueous solution of GG and aqueous polymer/silver nano particles (Ag⁰/GA and Ag⁰/GG) solutions were recorded in order to identify the functional groups involved in the synthesis of AgNPs. The complete FTIR data is presented in Fig 4.
**Guar gum (GG):** The FTIR spectrum of pure GG was presented in Fig. 4A. In case of GG, a broad band at 3304 cm⁻¹ due to the presence of OH stretching was observed, a sharp absorption band located at 2925 cm⁻¹ can be attributed to the CH group stretching and a band at 1635 cm⁻¹ (due to the ring stretching) were observed [24]. Other important peaks observed (Fig. 4A) are at 1249 and 1012 cm⁻¹ were due to the C-O-C stretching from the glycosidic linkages and OH bending from alcohols [25]. A considerable modification can be noticed (Fig 4B) in the well-defined spectrum of aqueous solution of guar gum and aqueous GG-AgNPs.

**Gum acacia (GA):** The spectrum of pure GA showed many strong peaks (Fig 4A) at 3299 (OH stretching) 2976, 2897 (asymmetric and symmetric CH stretching) and 1602 cm⁻¹ (C=O stretching of the carbonyl group, typical of saccharide absorption) [26]. It was noticed that when acacia molecules anchor to the distilled water and silver nanoparticles surfaces, their functional group frequencies were shifted (Fig 4C).

Over all, the shifting of the peaks confirms the encapsulation of AgNPs by interacting with the functional groups present in GG or GA molecules, providing stabilization for the formed AgNPs [23].
Fig 4: FTIR spectra of A) Pure GA and Pure GG powder; B) aqueous GG solution and GG-AgNPs (0.5%) solution; C) aqueous GA solution and GA-AgNPs (0.5%) solution.
2.3.4 Scanning electron microscopy-energy dispersive spectroscopic (SEM-EDS) analysis

To evaluate the dispersion of poly-AgNPs (GA-AgNPs/GG-AgNPs) on the cellulose fibers, SEM observations were carried out for CSNCFs (Fig 5B (b) and Fig 5B (c)) and the results were compared with pure cellulose fiber (Fig 5A (a) and Fig 5B (a)) and polymer-coated cellulose fibers (Fig 5A (b) and Fig 5A (c)). The images are clearly presented, which demonstrates that the dispersed poly-AgNPs were smaller in size without any aggregations. For a better view, the fibers were scanned at higher magnifications. Overall, the surface topography indicated that poly-AgNPs were intensely deposited on the fibers by the current approach. EDS spectrum was used to find out the type of particles that were absorbed on to the surface of cellulose fibers. Fig 6 shows the EDS spectrum of pure cellulose fibers and CSNCFs samples. The EDS spectrum of the pure cellulose fibers (Fig 6 (a)) did not show any characteristic peak of silver, while the EDS spectra of CSNCFs (Fig 6 (b) and Fig 6(c)) clearly showed the characteristic peak for silver confirming the existence of Ag$^0$ element on the CSNCFs surfaces. It is observed that silver is a major element present in the CSNCFs.

![SEM images of Pure Cotton cellulose fibre (b) 0.7% coated cellulose fibre (c) 0.7% GG coated cellulose fibre; B) (a) Pure Cotton cellulose fiber (b) 0.7% GA-AgNPs coated cellulose fibre (c) 0.7% GG-AgNPs coated cellulose fibre.](image-url)
Mechanical properties

The data pertaining to the tensile stress–strain curves of CSNCFs fibers of GA, GG and pure cellulose fibers are tabulated (Table 1) and depicted in Fig. 7. The data illustrates that the mechanical properties such as: maximum stress (Fig 7(a)), Young’s modulus (Fig 7(b)) and % elongation-at-break (Fig 7(c)) of all the cellulose fibers. The results indicates that the CSNCFs can be utilized for longer duration of use without any significant damage or breakage.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Maximum stress (MPa)</th>
<th>Young’s modulus (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Cotton fibre with polymers</td>
<td>Cotton fibre with polymers</td>
<td>Cotton fibre with polymers and Ag⁰</td>
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<td>0.3 GA</td>
<td>21.14</td>
<td>25.12</td>
<td>176.30</td>
</tr>
<tr>
<td>0.7 GA</td>
<td>37.35</td>
<td>39.86</td>
<td>305.45</td>
</tr>
<tr>
<td>0.3 GG</td>
<td>14.83</td>
<td>17.30</td>
<td>128.17</td>
</tr>
<tr>
<td>0.7 GG</td>
<td>33.64</td>
<td>38.70</td>
<td>274.45</td>
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Table 1: Mechanical properties of cellulose fibres with polymers and cellulose-silver nanocomposites fibres.
Uniaxial stress–strain curves of polymer cellulose fibres and cellulose-silver nanocomposites fibres (a) Maximum stress (b) Young’s modulus (c) % Elongation at break.

2.3.6 Thermal analysis

To verify the thermal stability of CSNCFs fibers, thermal properties of these fibers were examined by thermogravimetric analysis (TGA). The primary thermograms of CSNCFs of GA and GG with reference to pure cellulose fiber (C) are shown in Fig 8(a) and Fig 8(b) respectively. The results indicated that, in case of AgNPs-loaded CSNCFs fibers, an initial weight loss at a temperature below 100 °C was observed and this was due to the loss of moisture present on the AgNPs and cotton fibers. Also, the maximum decomposition of all CSNCFs occurred at slightly higher temperature at around 398.68 °C when compared to that of pure cellulose fiber. Thermal studies led to the conclusion that with the increase in the percentage (%) of GA and GG, the stability also increased. This might be due to the rise in the nano particles number on the cellulose fibres’ surface, which occurred due to the strong bonding characteristics developed among AgNPs and cellulose fiber with increase in GA and GG concentrations. Overall, the thermal analysis indicated that the CSNCFs designed in this investigation are thermally stable.
2.3.7 Antibacterial properties

AgNPs inherently possess bacteria killing property but more effective action of AgNPs on the bacteria always plays a major role in deciding its well functioning. In the current investigation, much smaller AgNPs are developed by green process. These smaller AgNPs enter into the bacteria cell more effectively, causing damage to the nuclei and resulting bacterial death at a faster rate. Also, the smaller AgNPs kill bacteria more effectively than the bigger AgNPs as supported from the various investigations [27-30]. The antimicrobial efficacy of the developed CSNCFs from AgNPs was tested against gram negative bacterium *E. coli*. The inhibition zone for all the fibers was found to be higher than 1.7 mm, as shown in Fig. 9. According to the Standard Antibacterial test “SNV 195920-1992”, specimens showing more than 1 mm microbial zone inhibition can be considered as good antibacterial agents [31]. Hence, the developed CSNCFs from the current approach can be considered as good antibacterial agents and effective in killing the microbes. It is also concluded that the developed CSNCFs in the present investigation exhibited higher inhibition zone than the earlier reported work of Ravindra et al. [22]. This achievement is due to synthesizing of much smaller AgNPs in the current approach when compared with the earlier work of Ravindra et al. [22]. Hence, the improved result. Further, it is also noticed that for the same concentration of polymer as reducing media, CSNCFs
developed form GG showed higher inhibition zone than those of CSNCFs developed from GA, which is evident form Fig 9.

![Image of bacterial inhibition zones](image)

**Fig 9:** Antibacterial activity of A) (a) GA polymer coated cellulose fibers (b) 0.3% GA CSNCF and (c) 0.5% GA CSNCF (d) 0.7% CSNCF on *E. coli*; B) (a) GG polymer coated cellulose fibers (b) 0.3% GG CSNCF (c) 0.5% GG CSNCF (d) 0.7% GG CSNCF on *E. coli*.

2.4 Conclusion
Cellulose–silver nanocomposites fibers with excellent antimicrobial properties were successfully developed from a green process where AgNO3 was reduced with natural carbohydrates, GA and GG. The composites were characterized by spectral, thermal and electron microscopy techniques. The results showed that the silver nano particles were greatly dispersed in the cellulose matrix with strong interactions between the cellulose and polymer/silver particles. The CSNCFs exhibited good antibacterial activity against E. coli. Therefore, it is concluded that the AgNPs composite cellulose fibers developed can be suggested for their utilization as effective tissue scaffolding for burn/wound treatments. The fibers alone can be used as higher durable antibacterial finishings in textiles industries and also as potential surgical fabrics in the medical fields.

The work presented in this investigation is an important contribution in the field of development of antimicrobial fibres using natural polymers only without the usage of any chemical compounds for antibacterial fabrics to be used in Medical field.

The work presented in this investigation was published in International cited Journal “Carbohydrate Polymers 93 (2013) 553-560” having an Impact Factor of 3.479 and also cited by other International Research Scientists.

2.5 References


