Sunlight induced synthesis of silver nanoparticles on cellulose for the preparation of antimicrobial textiles

Gulnur Assylbekova, Aliya Suigenbayeva, Malik Sataev, Shayzada Koshtkarbaeva, Perizat Abdurazova, Saule Sakibayeva, Polina Prokopovich

ABSTRACT

Antibiotic resistance is a major threat to health with the use of silver nanoparticles as a possible approach; however, the synthetic routes of such nanomaterials are not environmentally friendly because of the chemicals required. In this work we approach both problems with a single solution that employs sunlight in the visible spectrum to prepare nanosilver on the surface of cotton fabrics soaked in a solution of silver nitrate. Photoactivation leads to the activation of aldehyde groups providing reducing ability to cellulose and enabling the formation of elemental silver without the use of any chemical reducing agents. Concentrations of silver nitrate solution of 30 g/L reached the saturation of the content of elemental silver on fabric at 52.8 mg/dm². SEM images showed that silver particles were evenly distributed in fabric in the form of spherical particles 100–600 nm in diameter. The materials exhibited antimicrobial properties against E. coli and S. aureus retaining such properties after repeated washings. Moreover, no adverse effects were observed on fibroblast cells exposed to the prepared textiles.

Introduction

The well-known antimicrobial [1,2] and antiviral [3,4] properties of silver have been widely exploited in the preparation of medical materials through the formation of silver films onto: implants [5,6], drug delivery systems [7], antibacterial coatings for biomedical devices [8,9] and antimicrobial packaging materials [10,11]. Silver use is not restricted to medical items by has also found applications in numerous household items: curtains, napkins, bandages, bactericidal inserts in various appliances (refrigerators, fans, air conditioners) and clothes (socks, soles, etc.) [12-15]. The synthesis of silver films can be achieved in a variety of physical and chemical methods [16]. Examples of physical methods are magnetron sputtering of silver particles in a vacuum chamber and its subsequent deposition on fabric surface [17,18]. This method is based on the use of glow discharge in inert gas, where positively charged ions, formed in the discharge, bombard the surface of the cathode in the area of erosion causing the release of metal particles that precipitate as a thin layer on the surface of fabric being treated; the high kinetic energy of particles leaving the surface of the cathode ensures a solid adhesion of the film to the substrate. γ-radiation has also been used to produce antimicrobial silk fibers modified with nanoparticles of silver exposing to γ-radiation silk fibers treated with a silver nitrate solution. These materials showed 96% antimicrobial activity that remained above 85% after 10 cycles of washing [19]. The main obstacles hindering the use of physical methods are the need of costly equipment, the need for preliminarily obtaining metal nanoparticles, difficulties related to metallization of inner surfaces and complexity of controlling the coating thickness. Therefore, chemical methods are predominantly used to apply silver-containing films to fabrics [16]. In these processes, metal silver is obtained from aqueous solution of silver nitrate using a chemical reducing agents (glucose, ascorbic acid, hydrazine or hydrazine
The penetration of electromagnetic waves of sunlight into both the surface and deep layers of cellulose activates the terminal molecules of all these layers. This leads to a simultaneous silver formation reaction in these areas. At the same time, Ag particles located in deep areas are washed out after drying in squares 1.0 × 1.0 cm.

All procedures were carried out in a laboratory room at 25–30 °C. The solar beam flux was determined using solar radiation meter SM 206-SOLAR and it was equal to 880 ± 30 W/m² on average. This flux is typical to Central Asian during autumn-winter season [26].

To obtain a sorption layer on the surface, the sample was soaked (about 3 ml/dm²) in silver nitrate (AgNO₃) solution for 30 min. The samples were then exposed to sunlight for up to 30 min. The drying process was combined with photochemical reactions that occur during sun exposure.

Cellulose is a natural polymer consisting of a number of separate chains. Ends of separate chains form links that differ from other links of the chain. End links of chains can exist in two tautomeric forms: cyclic (semi-acetal) and open (aldehyde). When exposed to electromagnetic waves of sun rays, equilibrium is shifted towards aldehyde groups and molecule acquires reducing properties. When cotton fabrics containing a layer of aqueous solution AgNO₃ are exposed to solar beams, the following reactions occur [Fig. 1]:

\[ \text{Ag}^+ + e^- = \text{Ag} E^0 = 0.799 \] (1)

The reaction of oxidation of aldehyde group of open tautomeric form can be described by the following equation:

\[ R. \text{CHO} + H_2O = R.\text{COOH} + 2H^+ + 2e^- \] (2)

where R is the part of non-exposed open-end molecule of cellulose.

Assuming that the potential of reaction 2 does not vary significantly from the oxidation potential of a separate molecule of formaldehyde (HCHO + H₂O = HCOOH + 2H⁺ + 2e⁻, E⁰ = −0.01), then oxidation of the end molecule of cellulose is thermodynamically possible.

The total reaction will look as follows:

\[ R. \text{CHO} + 2\text{AgNO}_3 + H_2O = R.\text{COOH} + 2\text{Ag} + 2\text{HNO}_3 \] (3)

Materials and methods

Samples preparation

White cotton gauze fabric (article AA010278, 97–98% made of cellulose) widely used in medicine were employed in the study. Pretreatment of fabric samples involved soaking in hot (40 °C) distilled water for 30 min to remove water-soluble substances. The samples were then cut out after drying in squares 1.0 × 1.0 cm.

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10 ml of fresh sterile BHI broth in a 15 ml tube were inoculated with a loopful of cells from a single colony on a BHI plate. 1 ml of the cell suspension of either bacteria was deposited on the coated and control samples (1.0 × 1.0 cm) previously placed at the bottom of a 24 wells plate. After, the plates were incubated statically for 1 h at 37 °C, the bacterial suspension was removed and the samples were rinsed with fresh sterile Phosphate Buffer Solution (PBS) three times. 1 mL of a diluted solution of sterile BHI broth in PBS (1/10 BHI) was added to each well and the plates incubated at 37 °C statically for 24 h. Then, 50 μl from each well were transferred to a 100 well plate (Bioscreen C, Labsystems, Helsinki, Finland) containing 100 μl of fresh sterile BHI broth. Bacterial growth curves at 37 °C were recorded every 15 min through optical density (OD) at 600 nm (OD_{600}) using a plate reader (Bioscreen C analyzer; Labsystems, Helsinki, Finland) [27–29].

All tests were performed in triplicates and on three independent cultures resulting in 9 growth curves for each bacterium on each bone cement sample. Each growth curve was fitted using the Gompertz growth model to extract values of lag phase and growth rate [30]. Results are presented as mean ± 1 standard deviation (SD).

**Laundry durability of antimicrobial activity**

Laundry durability of silver coated fabric was assessed according to American Association of Textile Chemist and Colorists test method 61–2020 [31] using detergent, without optical brightener, at concentration of 0.15% w/w and 50 stainless steel balls in warm water at 80 °C. Accelerated laundering rounds were used with 1 round equivalent to 5 rounds. Samples were exposed to several laundry cycles and the holding antimicrobial capacity tested only once per sample (no repeated measurements).

**In vitro cytocompatibility**

The biocompatibility of the textile after silver deposition on mammalian cells was assessed by MTT assay using untreated cotton fabric as control. MTT assess mitochondrial activity as proxy for cell viability.

Human dermal fibroblast cells were kindly supplied by Prof. Stephens [32] from Cardiff University and grown in Minimum Essential Medium Eagle (MEM) supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 1% penicillin-streptomycin (PS). Cells were incubated at 37 °C in a humidified air atmosphere with 5% CO₂; medium were changed twice per week.

Sample (1 cm²) were sterilized by immersing in 70% (v:v) ethanol for 5 min and air dried for 12 h. the samples were then immersed in 1 mL of culture medium at 37 °C for 24 h to obtained extract solutions.

When cells reached about 70% confluence, the medium was removed and cells washed with sterile PBS three times. 100 μL of fresh medium, for control samples, or 100 μL of extract solution were added into each well, and the cells were incubated at 37 °C in 95% air and 5%CO₂. After 24 h the medium was replaced with 100 μL of fresh medium and 50 μL MTT after rinsing the cells were rinsed with sterile fresh PSB three times.

**Results and discussion**

Dispersed particles of silver were formed on the surface of fabric as a result of photochemical reactions producing the black coloured film. The photochemical nature of formation of elemental silver was confirmed by the observation that soaked in silver nitrate and dried in the dark place did not exhibit any blackening as shown when screening a part of sample surface with lightproof polymer washer (Fig. 2).

Electromagnetic solar beams can penetrate not only through translucent bodies, but also partially through color solid-state bodies and liquid media, this contributes to the photochemical reaction in inner areas of fabrics, which is a positive feature for the adhesion between particles and fabrics. Because of the small thickness of the fabric samples (0.3 mm), the film of silver particles formed on both the front reverse sides that had an even degree of blackness. Therefore, it is expected an even distribution of silver in inner areas of the fabric as well.

The main factor determining the content of silver in the fabric was the concentration of silver ions in the sorption layer. Photochemical formation of silver particles occurs almost immediately after the start of exposure to sunlight and the degree of blackness reached a plateau after 7–10 min. Regardless of the silver ions concentration tested the maximum blackness was reach after 10–12 min of exposure to sunlight.
Thereafter, the degree of blackness did not change further and the sample dried. Concentration of silver nitrate of 30 g/L or greater did not increase the final amount of silver deposited; and does not change at a further increase in concentration (Fig. 3). Hence, the photochemical process was limited by the reducing agents—aldehyde forms of end cellulose molecules. Therefore, silver nitrate solutions in concentration less than 30 g/L can be used to obtain silver films. Additionally, an important parameter of the photochemical process is the degree of conversion of silver into fabric. Values of this parameter at different concentration of silver in the sorption layer are given in Table 1. From this data it follows that the degree of conversion was 100% for lowest concentration of AgNO$_3$ employed (1 g/L). With increasing AgNO$_3$ concentration up to 30 g/L, the conversion remained at about 90%. This can be explained by silver nitrate acting as an oxidizing agent; therefore, an increase in its concentration has a positive effect on the process of photo-assisted reduction of end-cellulose molecules. At concentration above 30 g/L, end cellulose molecules were fully saturated with silver; therefore, any AgNO$_3$ excess it was not reacted (reducing conversion efficiency). Further confirmation of the limiting nature of the cellulose end molecules was provided by the quantification of the silver deposited on to the textile samples; with silver nitrate concentration ranging from 1 to 30 g/L, the content of silver varied from 1.92 to 52.81 mg/dm$^2$ but no additional deposited was detected with AgNO$_3$ greater than 30 g/L (Table 1). From the SEM image of the fabric (Fig. 4), it can clearly be seen that many surface filaments were white, which is typical to metal-containing films. The elemental composition of silver on the surface layer reached 2.54% and spherical particles 100–600 nm in diameter constituted the deposited silver as result of the photoreaction.

Growth curves of both E. coli and S. aureus inoculated with survival cell after contact with textile samples prepared with lowest AgNO$_3$ concentration tested (1 g/L) exhibited a lag phase of a few hours similarly to untreated samples (controls). With increasing AgNO$_3$ concentrations in the solution employed in the sample preparation, the lag phase duration decreased (Table 2). This is likely due to the increased concentration of oxidants in the solution, which can facilitate the reduction of silver ions to metallic silver. The quantification of the silver deposited on to the textile samples; with silver nitrate concentration ranging from 1 to 30 g/L, the content of silver varied from 1.92 to 52.81 mg/dm$^2$ but no additional deposited was detected with AgNO$_3$ greater than 30 g/L (Table 1). Further confirmation of the limiting nature of the cellulose end molecules was provided by the quantification of the silver deposited on to the textile samples; with silver nitrate concentration ranging from 1 to 30 g/L, the content of silver varied from 1.92 to 52.81 mg/dm$^2$ but no additional deposited was detected with AgNO$_3$ greater than 30 g/L (Table 1). From the SEM image of the fabric (Fig. 4), it can clearly be seen that many surface filaments were white, which is typical to metal-containing films. The elemental composition of silver on the surface layer reached 2.54% and spherical particles 100–600 nm in diameter constituted the deposited silver as result of the photoreaction.

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**Table 1**
The degree of photochemical transition of silver into fabrics at various concentrations of AgNO$_3$ in the sorption layer (sample surface 1 dm$^2$).

<table>
<thead>
<tr>
<th>Concentration of AgNO$_3$ in the sorption layer (g/L)</th>
<th>Content of Ag in the sorption layer (mg)</th>
<th>Content of Ag in sample (mg)</th>
<th>Degree of transition to fabric (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.92</td>
<td>1.92</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>9.55</td>
<td>8.50</td>
<td>89.0</td>
</tr>
<tr>
<td>15</td>
<td>29.37</td>
<td>26.20</td>
<td>89.2</td>
</tr>
<tr>
<td>30</td>
<td>58.49</td>
<td>52.81</td>
<td>90.3</td>
</tr>
<tr>
<td>35</td>
<td>68.07</td>
<td>52.78</td>
<td>77.5</td>
</tr>
<tr>
<td>40</td>
<td>76.33</td>
<td>52.80</td>
<td>69.2</td>
</tr>
</tbody>
</table>

**Table 2**
The elemental composition of a fabric sample soaked with a solution of silver nitrate (30 g/L) and dried in sunlight.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mass%</th>
<th>Atomic%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>47.43</td>
<td>55.84</td>
</tr>
<tr>
<td>O</td>
<td>48.97</td>
<td>43.29</td>
</tr>
<tr>
<td>Al</td>
<td>0.24</td>
<td>0.13</td>
</tr>
<tr>
<td>Si</td>
<td>0.82</td>
<td>0.41</td>
</tr>
<tr>
<td>Ag</td>
<td>2.54</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Table 3**
Growth curve parameters of E. coli and S. aureus cells after contact with textile samples prepared with different AgNO$_3$ solutions (mean ± SE, n = 3).

<table>
<thead>
<tr>
<th>AgNO$_3$ concentration (g/L)</th>
<th>E. coli $\mu$ lag (h)</th>
<th>S. aureus $\mu$ lag (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.17 ± 0.2</td>
<td>18.1 ± 0.4</td>
</tr>
<tr>
<td>15</td>
<td>0.19 ± 0.2</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.20 ± 0.2</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>0.21 ± 0.3</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>0.21 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
</tbody>
</table>
Phase increase monotonically (Fig. 5). As the impact of microbial cells growth on the OD600 of the media is detectable only above a threshold level, the duration of this “apparent lag phase” can be directly correlated to the initial concentration of viable cells [29]. Thus, the longer the apparent lag phase the lowest the number of microbial cells surviving the initial contact with the silver coated samples. The results showed an increase of antimicrobial activity of the samples with increase concentration of AgNO3 in the dipping solution (Table 3) consistent with the data related to the amount of resulting silver on the surface (Table 2), moreover, the higher susceptibility of S. aureus to the deposited silver compared to E. coli is in agreement with previous findings that have attributed such variation to the structure differences between the two species (mainly, E. coli been a Gram^- bacteria and S. aureus a Gram+) [34, 35].

Depending on the concentration, silver can have toxic effects on eukaryotes, hence any application aspiring to use this element must also satisfy the validation of cytocompatibility on relevant cell lines selected based on the expected application. The mitochondrial activity, a proxy for viability, of fibroblasts exposed to the release medium of the coated textiles was not statistically different (p > 0.05) than samples exposed to the medium in contact with uncoated samples at all times tested (Fig. 6). The amount of silver released from the coatings, therefore, did not impact the viability of fibroblast cells that were chosen because of their biological relevance as the main component of connective tissue [36].

The antimicrobial fabrics developed in this work are not single-use, consequently the possible waning of the antimicrobial activity following washing was also investigated (Fig. 7). The samples exhibited antimicrobial activity against both bacteria tested monotonically decreasing with increasing washing. This can be attributed to the progressive release of silver from the deposited nanoparticles during the washing process. However even after 10 washings (equivalent to ~50 washing in a normal home cycle), the materials still provided antimicrobial activity showing that products containing these films can be washed repeatedly retaining most of the initial activity.

Conclusions

When cotton fabrics presoaked in a silver nitrate solution are exposed to sunlight, the end-links of cellulose become activated. The resulting aldehyde groups can be photo-oxidized and reduce silver ions inducing the formation of silver nanoparticles on the surface.

This photochemical process can be used to modify cellulose-containing materials with silver to confer antimicrobial properties without the use of chemical reducing agents.

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