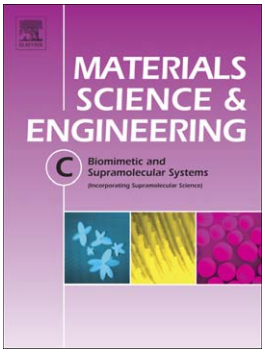

Surface chemical and biological characterization of flax fabrics modified with silver nanoparticles for biomedical applications

F. Paladini, R.A. Picca, M.C. Sportelli, N. Cioffi, A. Sannino, M. Pollini

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Surface chemical and biological characterization of flax fabrics modified with silver nanoparticles for biomedical applications

Paladini F*, Picca RA°, Sportelli MC°, Cioffi N°, Sannino A*, Pollini M*.

*Department of Engineering for Innovation, University of Salento, Via per Monteroni, 73100 Lecce, Italy
°Department of Chemistry, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy

Corresponding author:

Federica Paladini, PhD
Department of Engineering for Innovation
University of Salento, 73100 Lecce, Italy
e-mail: federica.paladini@unisalento.it
Fax: +39 0832297340

Abstract

Silver nanophases are increasingly used as effective antibacterial agent for biomedical applications and wound healing. This work aims to investigate the surface chemical composition and biological properties of silver nanoparticle-modified flax substrates. Silver coatings were deposited on textiles through the *in situ* photo-reduction of a silver solution, by means of a large-scale apparatus. The silver-coated materials were characterized through X-ray Photoelectron Spectroscopy (XPS), to assess the surface elemental composition of the coatings, and the chemical speciation of both the substrate and the antibacterial nanophases. A detailed investigation of XPS high resolution regions outlined that silver is mainly present on nanophases' surface as Ag_2O. Scanning electron microscopy and energy dispersive X-ray spectroscopy were also carried out, in order to visualize the distribution of silver particles on the fibres. The materials were also characterized from a biological point of view in terms of antibacterial capability and cytotoxicity. Agar diffusion tests and bacterial enumeration tests were performed on Gram positive and Gram negative bacteria, namely *Staphylococcus aureus* and *Escherichia coli*. *In vitro* cytotoxicity tests were performed through the extract method on murine fibroblasts in order to verify if the presence of the silver coating affected the cellular viability and proliferation. Durability of the coating was also assessed, thus confirming the successful scaling up of the process, which will be therefore available for large-scale production.

Keywords: XPS, Ag nanoparticle, textile, antibacterial.

1. Introduction

Over the past years, the increasing phenomenon of the bacterial resistance to conventional antibiotics has strongly encouraged the interest in defining new routes for the formulation of novel safe and cost-effective biocidal materials [1-3]. Silver nanoparticles (AgNPs), in particular, have received great attention due to their unique bactericidal properties and have been widely applied to many consumer products and medical purposes [4-10].

Some authors have recently reported on the high degree of commercialization of nanosilver-related products for applications in electronics, disease diagnosis, imaging, treatment of infections etc. [11-14]. Particularly in the treatment of infected burns, wounds and chronic ulcers, AgNPs have received great attention for the formulation of topical dressings [15-18], as the risk of wound infection still remains the most common reason for impaired wound healing [19-23].

Traditional dressing cannot ensure protection against bacteria [24, 25], thus motivating the design of novel products for antibiotics or antimicrobials delivery in the wound site [26-28] and novel treatments of biofilm-associated infections [29]. Silver-containing dressings have been demonstrated to be effective in killing bacteria in mono and polymicrobial biofilms, thus providing evidence of an effect on biofilm in recalcitrant chronic wounds [30-33]. Silver alginate dressings were found to be effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria and yeast isolated and cultured from wounds [34]. A wide array of silver-based dressings is available on the market for application in acute and chronic wound care and in the treatment of diabetic ulcers [29-30]. Local application of several types of dressings including hydrocolloid, alginate, hydrogel, membrane and silver/alginate composites have also been proposed [35-36].

However, only few products involve the use of silver in form of nanoparticles. Moreover, among natural textile fibres used as wound dressing, cotton has received the greatest attention in literature for the preparation of antibacterial fabrics. AgNPs immobilized on cotton fabrics were obtained by γ-irradiation [37], by sonochemical methods [38] or through the preparation of colloidal solutions [36, 39] and physical deposition of AgNPs‑alginate composites [40], with or without the use of binders.

This work aims to propose a different natural substrate, such as flax, in combination with silver in form of nanoparticles. Flax has been used since ancient times for the production of linen cloth widely used in humid climates. Flax is an attractive material for wound dressing, as it may be useful in keeping the wound at the optimal moisture level [6, 41-44]. In a previous work, wound-dressing biomaterials have been obtained by depositing flax substrates with a hydrogel embedding silver particles [45]; in this paper, flax has been directly treated with silver by translating the technology reported in previous works about cotton [46-48] to a different substrate and in a different production

scale. In the previous cited works, the excellent adhesion of the particles to cellulosic fibres and the efficacy of silver-coated gauzes against fungi and bacteria were demonstrated even after conditioning in artificial exudate [47, 48]. However, the novel technological approach to translate the process on large scale has not yet been investigated. In this work, the silver deposition technique was successfully scaled up through the design of a roll-to-roll apparatus allowing the treatment of large volumes of fabrics. Moreover, surface characterization such as X-ray Photoelectron Spectroscopy (XPS) of cellulosic fibres alone has already been proposed [49-51], but the investigation of modified fibres has not yet been deeply explored. Additionally, the XPS analysis of high resolution $Ag3d_{5/2}$ and Ag_{MNN} regions was carried out, to evaluate silver surface chemical speciation [52-53]. The surface chemical characterization of neat and treated flax samples, considering also the influence of the washing cycles and the nominal silver content, was also accomplished by XPS, corroborating results from SEM analysis performed to evaluate the stability of the silver coating and the strong adhesion of the particles to the fibres after laundries.

Cytotoxicity tests were performed on murine fibroblasts 3T3 through the extracts method, in order to evaluate the influence of the silver coating on cellular viability and proliferation.

The antibacterial efficacy of nanosilver treated materials was demonstrated on Gram positive and Gram negative bacteria through qualitative and quantitative microbiological tests, even after the washing cycles.

2. Experimental details

2.1 Surface modification of flax fabrics

Textile materials kindly provided by Silvertech Ltd (Lecce, Italy) were flax substrates with density 1.5 g/cm^3, surface mass 0.02 Kg/m^2, breaking tenacity 30 cN/Tex, produced without any chemical agent. They were treated with silver nanoparticles through the *in situ* photo-reduction of silver nitrate [46]. While previous studies described the successful application of the technology to different materials on a laboratory scale, this work aimed to translate the process on a larger scale in order to evaluate the effectiveness of the silver deposition technique in providing durable antibacterial coatings on mass production. The technology consists of the deposition of a silver solution on the surface of the materials by dip or spray coating, and of the following exposure to UV irradiation (λ=365 nm; 500 Watt) to induce the photo-reduction of the silver salt and the *in situ* synthesis of silver particles. Firstly, the silver solution is prepared by mixing silver nitrate, methanol and deionized water under magnetic stirring at room temperature until complete dissolution of the silver salt. Silver nitrate represents the precursor for metal silver, while methanol is used as both

solvent and reducing agent in the photo-chemical reaction.

Then, the silver solution is deposited on the surface of the material through a proper method defined as function of the nature of the material, the application and the process scale-up. Finally, the wet materials are exposed to the UV source by defining the most appropriate distance from the lamp and minimum exposure time ensuring the complete reaction.

The conversion from silver salt to silver nanoparticles occurs through the following photo-chemical reaction

$$Ag_n + 2Ag^+ + CH_3OH + h\upsilon \rightarrow Ag_{n+2} + 2H^+ + CH_2O \qquad (2.1)$$

The process parameters are defined experimentally, by evaluating the loss of silver after washing cycles through thermo-gravimetric analysis (TGA) and energy dispersive X-Ray spectroscopy (EDX). In this work, $0.1\%_{w/v}$ of silver nitrate in $10\%_{v/v}$ methanol/water have been selected for the preparation of the silver solution.

In order to allow the treatment of higher volumes of products, a roll-to-roll prototype apparatus designed for textile substrates has been adopted. The apparatus, consisting of an impregnation bath, a roll-to-roll transport system and a UV station, has been designed for the treatment of different textile materials, such as natural and synthetic yarns and fabrics. It is however well known that the treatment of natural and synthetic substrates with a silver-based antibacterial coating can cause the darkening of the substrate. As a result, one possible limitation of the work may be associated to the definition of the most appropriate process parameters in order to minimize the change in colour of the fabric. For this reason, some parameters such as the impregnation time, the speed of the roll-to-roll system, the UV exposure time and the distance from UV lamp were properly set to contain the colour variation and to ensure the absence of discoloration areas in the final product. In Figure 1, pictures of the apparatus during the treatment of textile substrates are reported. In this work, flax fabrics (width 1 meter) were impregnated in the bath containing the silver solution; then, the wet substrates were moved by the roll-to-roll system toward the UV station of the apparatus. The substrates were exposed to UV for a minimum time of 5 minutes at a distance of 30 cm from the UV lamps. Then, the substrates were washed with water to remove any presence of unreacted salt and samples were obtained for characterization. In order to test the durability of the coating and the resistance to laundries, the fabrics underwent ten washing cycles by adopting an Electrolux washing machine W4180H and a commercial soap, according to European Standard EN 26330:1993.

Then, the samples were again characterized.

2.2 Surface characterizations

Silver treated samples and untreated samples as control were analysed through scanning electron microscopy SEM (Zeiss EVO) equipped with Energy Dispersive X-Ray spectroscopy detector EDX (Bruker). The efficacy of the technology in providing a homogeneous coating on the fibres and the presence of silver were verified through the elemental analysis even after ten washing cycles.

XPS characterization was carried out on a Theta Probe Thermo VG Scientific spectrometer equipped with a 300 μm-spot monochromatized AlKα source and a 180° spherical sector analyzer with a two-dimensional electron detector. All the spectra were recorded in Constant Analyzer Energy mode using a pass energy of 150 eV for survey and 100 eV for high-resolution (HR) regions (Ag3d, C1s, O1s, N1s, and $AgM_{4,5}N_{45}N_{45}$). Surface sample charging was compensated by means of a flood gun. Data analysis and curve-fitting were performed by means of Avantage 4.75 commercial software. Acquisition time was always kept within 30 minutes in order to prevent artefacts related to sample damage/reduction under the analysis conditions. Unfortunately, for low-content Ag-modified fibres (e.g. $0.1\%_{w/v}$), the intensity of the MNN Auger signal was very low and high number of signal acquisitions could be needed to gather spectra with a sufficiently high signal to noise ratio. The Kinetic Energy (KE) relevant to $AgM_4N_{45}N_{45}$ position was always estimated by keeping low the spectra acquisition time and the estimated values were then confirmed by analysing samples with higher Ag loadings.

2.3 Evaluation of the antibacterial activity

The efficacy of the silver treated textile in preventing the bacterial proliferation was assessed through qualitative and quantitative antibacterial tests, respectively through agar diffusion tests and bacterial enumeration on Gram positive and Gram negative bacteria. The agar diffusion tests were performed according to standard 'SNV 195920-1992'. The procedure consists of placing the samples in contact with bacteria on an agar plate and evaluating the width of the free-bacteria zone around and under the sample after 24 hours of incubation at 37°C. The antibacterial activity of the material can be labelled as "good" if an inhibition zone to bacterial proliferation larger than 1 mm can be observed around the sample. On the other hand, the antibacterial activity of the sample can be labelled as "insufficient" if the material is totally colonized by bacteria. A "sufficient" antibacterial capability is associated to samples showing free bacteria zone just under their surface.

In order to quantify the bacterial reduction induced by the presence of silver on textiles, bacterial counts on *Escherichia coli* (DH5(α), inoculating cell density $9.1*10^6$ CFU/ml) and *Staphylococcus aures* (SA1, inoculating cell density $6.5*10^6$ CFU/ml) were performed through the serial dilution method. In triplicate, samples of silver treated and untreated textiles (average weight 25 mg) were UV sterilized for one hour and incubated in 5 ml of Luria Broth inoculated with 100 microliters of bacterial suspension for 24 hours at 37°C. After incubation, samples were extracted from the broth and serial dilutions were performed in 0.85% sterile saline. One hundred microliters of each dilution was plated on agar plates and the dishes were incubated for 24 hours at 37°C. The results were expressed as percentage of bacteria reduction.

2.4 *In vitro* cytotoxicity tests

Murine fibroblasts (3T3, ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS PAA) in a cell culture incubator (Heraeus, Hera Cell) set at 37°C, 95% relative humidity and 5% $^{v}/_{v}$ of CO_2. In triplicate, samples of untreated and silver treated flax were UV sterilized and incubated in DMEM for 24 hours. *In vitro* cytotoxicity tests were performed by seeding and incubating 10,000 cells with the extracts of the materials for 24, 48 and 72 hours, according to Standard 10993-5. Then, the nuclei were stained by Hoerscht-propidium iodide (HPI) dye. The number of viable cells was scored by epi-fluorescent microscope (Zeiss Axiovert 25) at 32x magnifications. Six different fields per sample were counted through Image-Pro Insight software by distinguishing the living cells by their intense blue colour and the dead cells by a pink colour. The results were expressed as mean viable cells number±standard deviation.

3. Results and Discussion

3.1 Surface modification of flax fabrics

Flax substrates were modified by different amounts of silver nanoparticles through the *in situ* photo-reduction of silver nitrate described in the Experimental section. While previous studies described the applications of this process on a laboratory scale, this work for the first time provides insights into the extension of the photo-deposition on a larger scale. The percentage of silver adopted in this work was selected by considering the results obtained in previous works, where it resulted sufficient to confer antibacterial capability to the product. At this purpose, the biological

characterization was performed on flax deposited with $0.1\%_{w/v}$ silver solution. The presence of methanol is necessary because it absolves to the function of photo-reducing agent. However, in this work the mixture with water was preferred in order to contain the costs of the large-scale production. The chemical and biological properties of the materials were analysed and the process parameters were defined in function of the specific nature and application of the material, in terms of antibacterial capability required and cost-effectiveness ratio.

3.2 Surface characterizations

Samples of untreated flax and silver treated flax were analysed through SEM microscopy in order to determine the efficacy of the deposition treatment in providing a homogenous coating and a good distribution of the silver particles. Moreover, the treated sample was also characterized after ten washing cycles in order to verify the stability of the treatment and its resistance to laundries. SEM images (3000x) reported in Figure 2 show neat flax fibres (Fig. 2a), silver treated flax fibres (Fig. 2b) and silver treated flax fibres after the washing cycles (Fig. 2c). As clearly visible, the large-scale deposition process ensured a good distribution of the particles and the uniformity of the coating. Moreover, the strong adhesion of the silver particles to the substrate and the durability of the coating were confirmed. Moreover, the distribution of the silver particles appeared even to be slightly better on the samples subjected to multiple washings. Indeed, the main difference between the two samples was the number of particle aggregates, which decreased after washing. On the other side, the mean particle size did not change significantly upon washing, and remained equal to 150±50 nm for both samples, as per SEM direct measurements, carried out over more than 200 particles. This finding was in agreement with the results reported in previous works, where the same silver deposition technology was applied to cellulosic fibres [48, 54].

These results were also confirmed by the EDX analysis reported in Figure 3, where similar intensities of the peak of silver can be observed in the samples before and after the washings.

The adhesion of our photo-deposited silver nanoparticles is in good agreement with what found by other authors investigating the *in situ* deposition of Ag nanophases on fabrics [55-56]. Although possible, direct complexation of Ag^+ species with functional moieties from the textile fibres is scarcely mentioned as the main reason of such a stable deposition. van der Walls forces and inter-particle aggregation, leading to particle clusters which can be more strongly bonded to the fabric surface have been hypothesized by Perera et al. [56], while Jooyoung and Jyongsik have hypothesized that polymers such as poly-alcohols may act as a *gelator and stabilizer* during the *in situ* synthesis of Ag nanophases onto fibres [57].

Flax fibres, either bare and Ag-modified, were characterized by XPS spectroscopy. Considering the

typical detection limit of approximately $0.1\%_{at}$ for this technique, textile samples for XPS analyses were prepared by using two metal loadings, including a higher percentage of silver ($0.5\%_{w/v}$), along with the aforementioned $0.1\%_{w/v}$ Ag loading. Very similar surface chemical composition of the organic part of the fibre was found in all the investigated samples (Table 1). Reasonably, the highly-loaded samples showed a higher Ag surface concentration, although the error associated to the XPS quantifications did not allow to assess if the Ag concentration in the surface layer sampled by XPS is linearly correlated to the overall silver content in the fibre. C and O percentages were found to be in agreement with literature values, indicating that fibres consist of cellulose, hemicellulose, lignin and waxy materials [50]. The O/C elemental ratio = 0.43 ± 0.03 was well below the theoretical value of 0.83 for pure cellulose, again suggesting the presence of other components in the fibre [50]. XP spectra relevant to C1s region for untreated and treated fabrics (before and after washing) are reported in Figure 4. The carbon moieties expected for cellulose-based natural fibres could be identified on the samples surface and their attribution is summarized in Table 2. Interestingly, no shake-up peak above 291 eV (visible when unsaturated or aromatic carbons are present in high amounts) was observed, thus suggesting that the found O/C ratio is due to a surface more enriched with waxy species than with lignin. No significant variation in the carbon chemical environments was observed as a function of the Ag loading. The presence of nitrogen is compatible with the presence of wax and/or protein traces. Other impurities (e.g. Si, Cl, Na, Ca) could be barely detected at the limit of quantification of the technique and are most probably due to fibres treatment and manipulation.

Applying the washing cycles to the treated fibres (especially in the case of $0.1\%_{w/v}$ Ag-treated fabrics) did not greatly affect the Ag surface content, thus further corroborating the SEM-EDX results. XPS was particularly important for excluding the presence of unreacted silver precursor ($AgNO_3$) as no nitrogen signal attributable to nitrate ion (falling at BE = 406.6 ± 0.2 eV [57]) could be identified in the N1s region. Regarding silver speciation, $Ag3d_{5/2}$ signal presented generally a single peak at BE = (368.2 ± 0.2) eV (as shown in Fig. 5), whose position is compatible either with Ag(0) and Ag_2O [58]. In fact, slight chemical shifts are associated to silver main photoelectronic signal. As a result, the study of Ag_{MNN} region [53, 59] was essential to evaluate the most probable chemical state of surface silver. The modified Auger parameter (α') [60], calculated as the sum of $BE(Ag_{3d5/2})$ and the kinetic energy of the $M_4N_{45}N_{45}$ highest peak, was used to unambiguously assess the Ag surface chemical speciation [52, 53]. Moreover, the use of α' prevented the occurrence of errors or artefacts due to sample charging effects. Both low- and high- content Ag-modified fibres were investigated for the Ag chemical speciation. In all cases, the Kinetic Energy (KE) value of the $AgM_4N_{45}N_{45}$ region was found to be 356.1 ± 0.2 eV. The overall analysis of

$M_4N_{45}N_{45}$ and $M_5N_{45}N_{45}$ silver Auger transitions was performed by curve-fitting this region with six components (a-f in Fig. 6), as defined in previous studies [52-53, 59, 61]. The found values differed significantly from those of $AgNO_3$ and fell in the range between those recorded for bulk metallic Ag and Ag_2O powders [53], thus evidencing that size-effects or confinement effects phenomena, typical of ultrafine (<10 nm) $Ag^{(0)}$ nanoparticles, are absent in these samples. Such result could be hypothesized considering that the typical size of the as-prepared silver clusters was well above 10 nm, ruling out the occurrence of size effects. Another important feature is an independent parameter proposed by Bera et al. [52] and defined as the area ratio *R* between components 'd' and 'e'. In the present work, a value $R = 0.31 \pm 0.05$ was obtained, which is in agreement with the presence of Ag_2O on the samples surface (for a standard Ag(I) oxide, $R = 0.26 \pm 0.03$).

This evidence is also in agreement with the value calculated for α' (724.3 ± 0.4 eV), which is again compatible with the Ag_2O environment [53]. Such results are not surprising, as the silver clusters are not stabilized by any matrix/capping agent leading to partial surface oxidation.

3.3 Antibacterial tests

Although the antimicrobial effect of silver nanoparticles has been extensively studied, their bactericidal mechanism has not been clearly elucidated [62].

Morones *et al*. identified some action mechanisms of silver nanoparticles against Gram-negative bacteria, such as the anchorage of the silver nanoparticles to the surface of the cell membrane, which drastically lead to alterations in cell permeability and respiration. Moreover, the ability of silver nanoparticles to penetrate inside bacteria, the interaction with DNA and the release of silver ions were also proposed to explain the bactericidal effect of nanosilver [63, 64]. The incorporation of silver nanoparticles into the membrane structure has been demonstrated by Sondi *et al*., and significant changes and damages to the membrane were observed because of the formation of "pits" on the surface [65]. Kim *et al*. attributed the antibacterial mechanism of silver nanoparticles to the formation of free radicals, subsequently followed by free radical-induced membrane damages [66].

Silver ions can enter the bacterial cell in contact with silver nanoparticles, thus inhibiting respiratory enzymes and inducing the generation of reactive oxygen species with consequent damage of the cell. According to the studies performed by Pal *et al.*, silver nanoparticles demonstrated a shape-dependent interaction with the gram-negative bacterium *E. coli* [67].

Other parameters influencing the bactericidal effect of silver nanoparticles are size, concentration

and dose. Silver nanoparticles having size ranging between 10 and 100 nm showed strong bactericidal potential against both Gram-positive and Gram-negative bacteria [62].

As example of Gram positive and Gram negative bacteria, in this work the efficacy of silver-treated samples in inhibiting the bacterial colonization of textile was tested through agar diffusion tests and bacterial counts on *S. aureus* and *E. coli*. The results of the agar diffusion tests are reported in Figure 7, where a complete colonization of the untreated samples by *Escherichia coli* and *Staphylococcus aureus* is clearly visible, as expected (Figs. 7 a-a'). On the other hand, silver-treated samples exhibited a "good" antibacterial capability against both the bacterial strains, the width of the inhibition area to bacterial growth being larger than 1 mm (Figs. 7 b-b'). The results were again confirmed also after the washing cycles (Figs. 7 c-c'), thus demonstrating the durable antibacterial properties of the silver coatings. The bacteria-free area calculated resulted 1.5 mm for both bacterial strains before washings (Fig. 7b,b') and 1.25 mm after washings (Fig. 7c,c'). The bacterial counts confirmed the data obtained by the agar diffusion test, as the bacterial reduction obtained resulted 100% for both *E. coli* and *S. aureus*. Representative pictures of agar plates obtained by the serial dilution method are reported in Figure 8, where the strong antibacterial efficacy of the silver treated samples can be observed. These results suggested that the percentage of silver adopted successfully inhibited bacterial growth and proliferation and can be proposed for biomedical application.

3.4 *In vitro* cytotoxicity tests

The viability, proliferation and morphology of fibroblasts in presence of the silver coating obtained through the presented technology were studied in a previous work on different substrates [68]. In that work, TUNEL, BrdU assay and actin staining were performed on silver-treated catheters for haemodialysis, in order to evaluate the cytotoxic effects of the silver particles deposited [68]. In particular, fibroblasts apoptosis was evaluated by TUNEL after 24 hours of cell culture with catheters coated with different percentages of silver. In the same experimental conditions, fibroblasts proliferation was evaluated through the incorporation of BrdU into culture medium and following determination of the BrdU-positive nuclei in response of the different silver concentrations. Actin staining was performed to evaluate any cell shape abnormality and shrinkage in the cell structure associated to the presence of silver [68]. All the results obtained indicated that the percentages of silver tested and the technology adopted did not affect the cellular response, so it has been selected also in the present research. The samples treated with $0.1\%_{w/v}$ silver solution were tested *in vitro* through extract tests and HPI staining, in order to determine if the presence of silver affected the cell viability and proliferation. For the specific application, the extracts from the

materials were collected after 24 hours of incubation in DMEM, and the effect of the silver coating was evaluated after 24, 48 and 72 hours from incubation. The results are reported in Figures 9 and 10. In figure 9, representative images of murine fibroblasts 3T3 stained at 24, 48 and 72 hours are reported for each experimental condition. As visible, the presence of the silver coating did not affect the cellular viability and proliferation and the cells demonstrated good proliferation also in case of silver treated sample. In the graph in Figure 10, the average cell number is reported for each experimental condition at the defined time points. The silver-treated sample exhibited an exponential cell growth, in line with the control sample (3T3). In comparison with the untreated flax where the cell proliferation exhibited a linear behaviour, the silver treated samples showed improved proliferation. Future works aim to investigate the effect of silver on the cellular response at extended time points.

3.5 Conclusions

In this work, the surface chemical composition and biological properties of silver nanocoatings deposited on flax textiles via large-scale photo-deposition have been investigated. This work demonstrates that the silver photo-deposition technology can be easily translated from laboratory to large scale, without affecting the properties of the silver coating deposited. The engineering and design of the apparatus for silver deposition, along with the definition of appropriate process parameters, allowed the production of advanced textiles with good antibacterial capability against Gram positive and Gram negative bacteria and with no effect on cellular viability and proliferation. Moreover, the coating of silver particles demonstrated a good distribution along the fibres and high durability even after several washing cycles, as assessed through the surface characterizations. In particular, XPS surface spectroscopy provided the surface chemical composition of the fibres and the evaluation of the modified auger parameter showed that Ag_2O chemical environment is the most abundant silver species on the samples surface. Finally, even if very low, the percentage of silver employed for the deposition treatment provided promising results for biomedical applications as wound dressings.

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Captions to figures and tables

Figure 1. Pictures of the prototype apparatus developed for the silver deposition treatment of textile substrates. a.) Textile substrates moving towards UV station; b.) UV exposure of the textile substrates.

Figure 2. SEM pictures (3000x) showing the homogeneous distribution of the silver particles on the flax fibres before and after ten washing cycles. a.) Untreated sample; b.) Silver-treated sample; c.) Silver-treated sample after 10 washing cycles.

Figure 3. EDX analysis of textiles showing the presence of silver on the surface of the materials and resistance of the silver treatment to washing cycles. a.) Untreated sample; b.) Silver-treated sample; c.) Silver-treated sample after ten washing cycles

Figure 4. C1s XP region relevant to textile samples. Upper panel: neat (a), $0.1\%_{w/v}$ Ag-treated (b), and $0.1\%_{w/v}$ Ag-treated and washed (c) flax fibres; lower panel: $0.5\%_{w/v}$ Ag-treated (d), and $0.5\%_{w/v}$ Ag-treated and washed (e) flax fibres.

Figure 5. $Ag3d_{5/2}$ XP region relevant to $0.1\%_{w/v}$ (a) and $0.5\%_{w/v}$ (b) Ag-treated flax fibres.

Figure 6. Typical $AgM_{4,5}N_{45}N_{45}$ Auger region as acquired onto Ag-treated flax fibres. KE position for calculating α' is highlighted by a dashed line.

Figure 7. Agar diffusion tests on *Escherichia coli* and *Staphylococcus aureus* before and after laundries: a-a') untreated samples tested on *E. coli* and *S. aureus* respectively; b-b') silver treated samples tested before laundries on *E. coli* and *S. aureus* respectively; c-c') silver-treated samples tested after ten laundries on *E. coli* and *S. aureus* respectively. Silver-treated samples exhibited a strong antibacterial capability in comparison with the untreated samples

Figure 8. Representative pictures obtained by bacterial enumeration tests on *Escherichia coli* and *Staphylococcus aureus* before and after laundries: a-a') untreated samples tested on *E. coli* and *S. aureus* respectively; b-b') silver treated samples tested before laundries on *E. coli* and *S. aureus* respectively; c-c') silver-treated samples tested after ten laundries on *E. coli* and *S. aureus* respectively.

Figure 9. Representative images of murine fibroblasts 3T3 stained at 24 (a, b, c), 48 (d, e, f) and 72 hours (g, h, i) for each experimental condition: cells 3T3 cultured with no extract at the different time points (a, d, g); cells 3T3 cultured with the extracts from untreated flax at the different time points (b, e, h); cells 3T3 cultured with the extracts of silver treated flax at the different time points (c, f, i).

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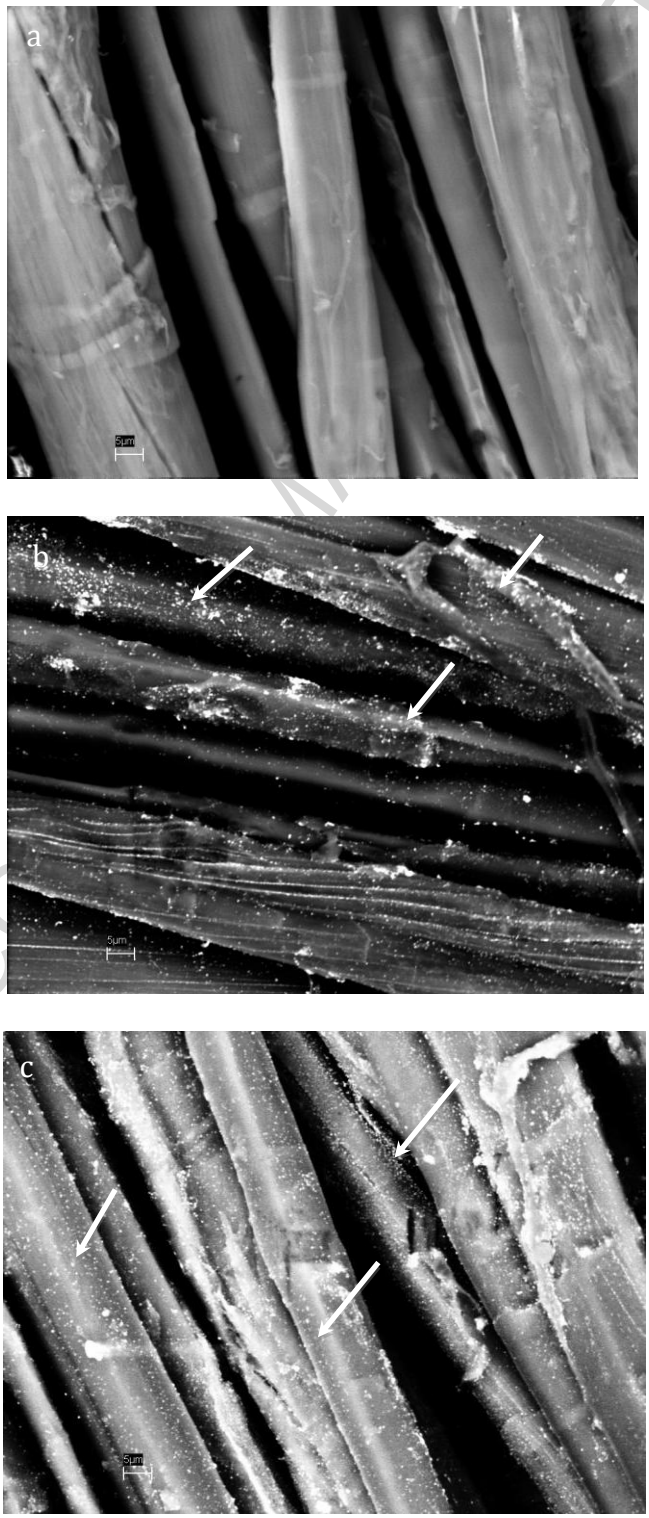

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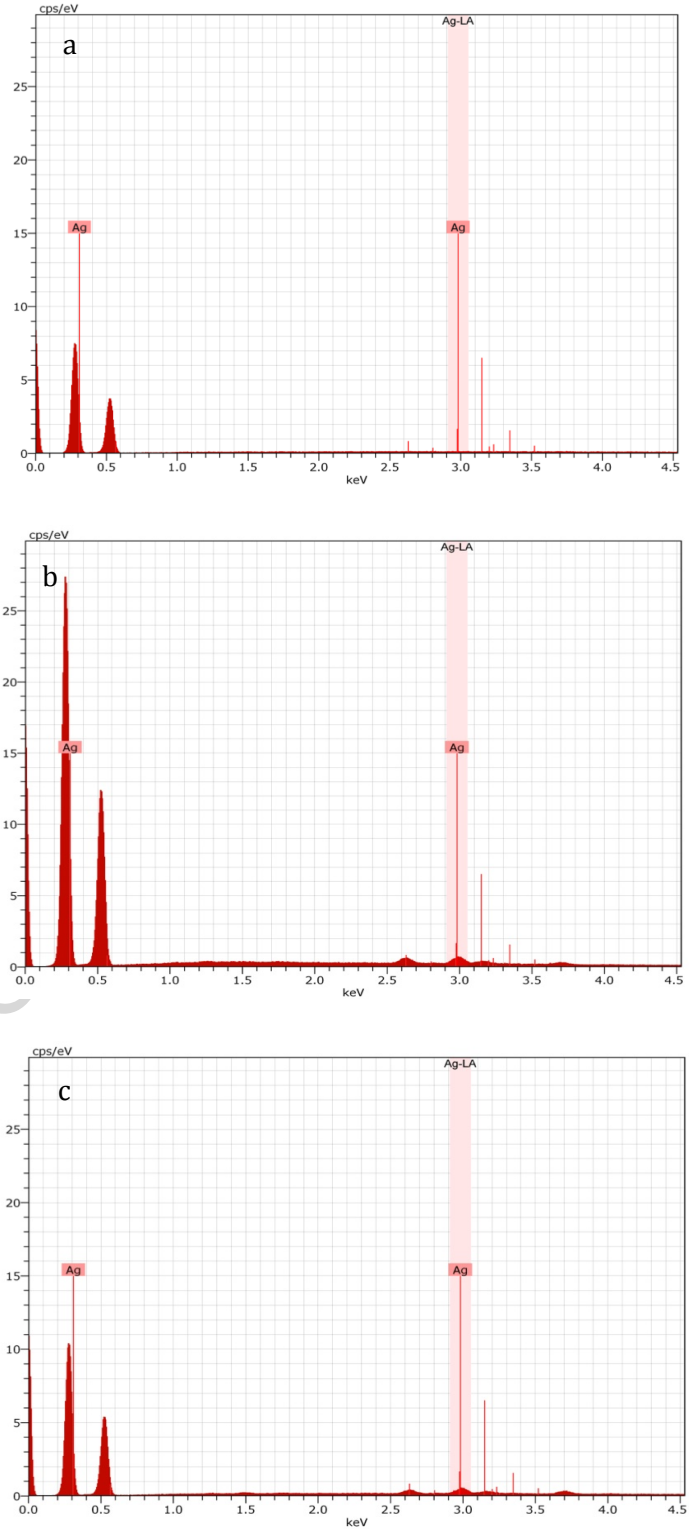

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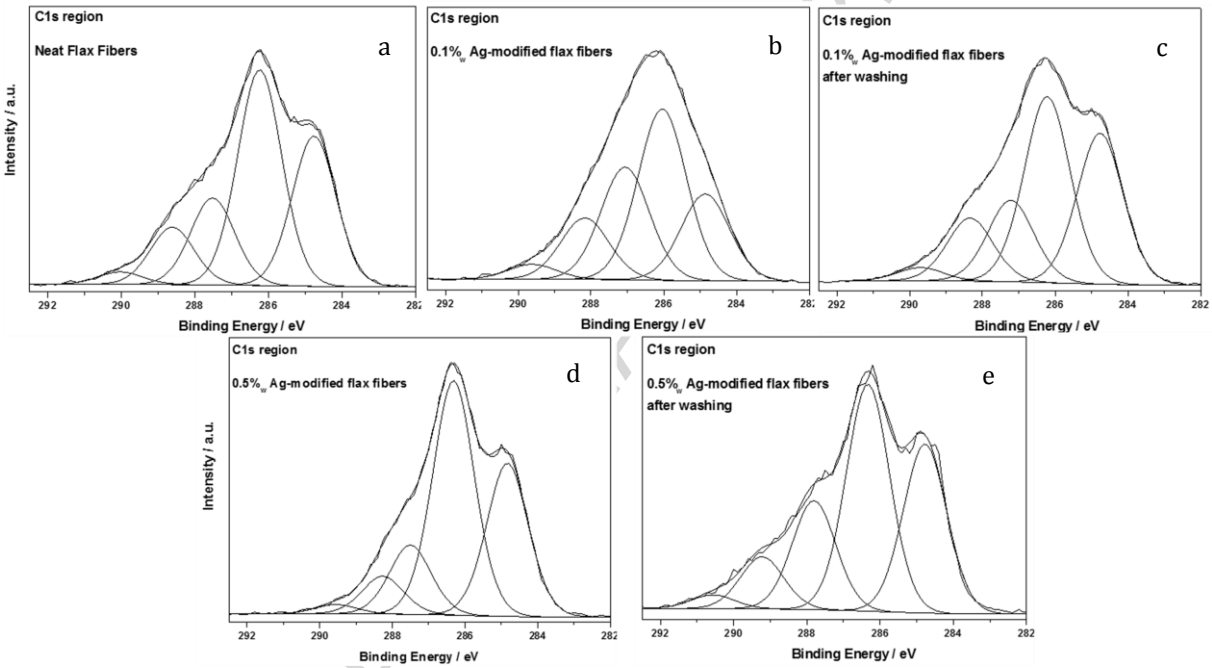

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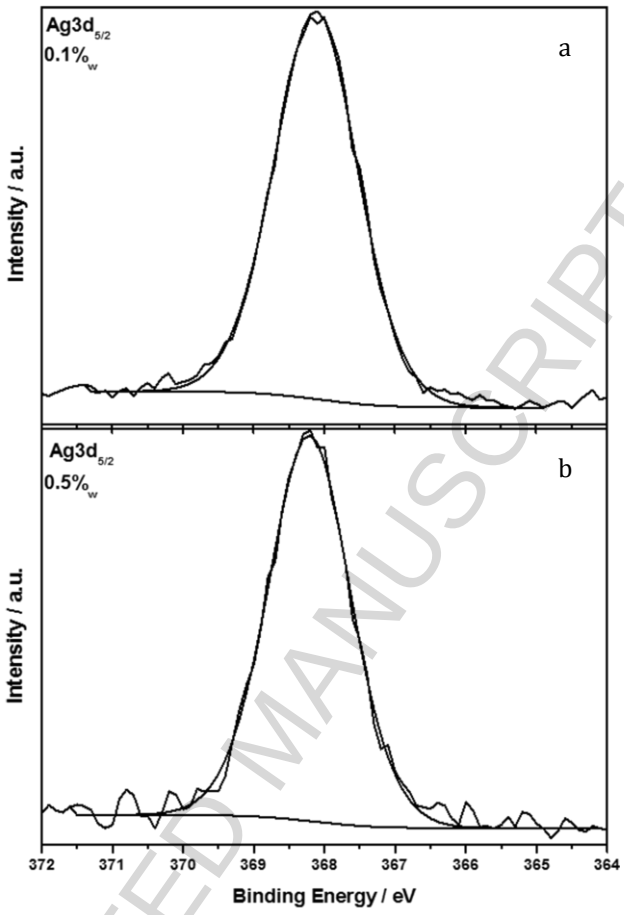

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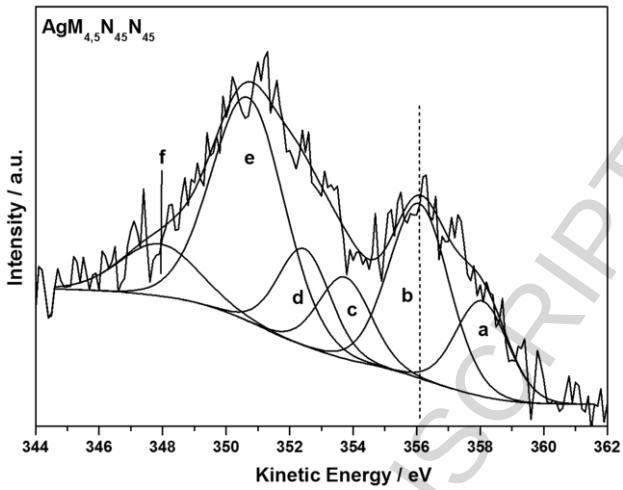

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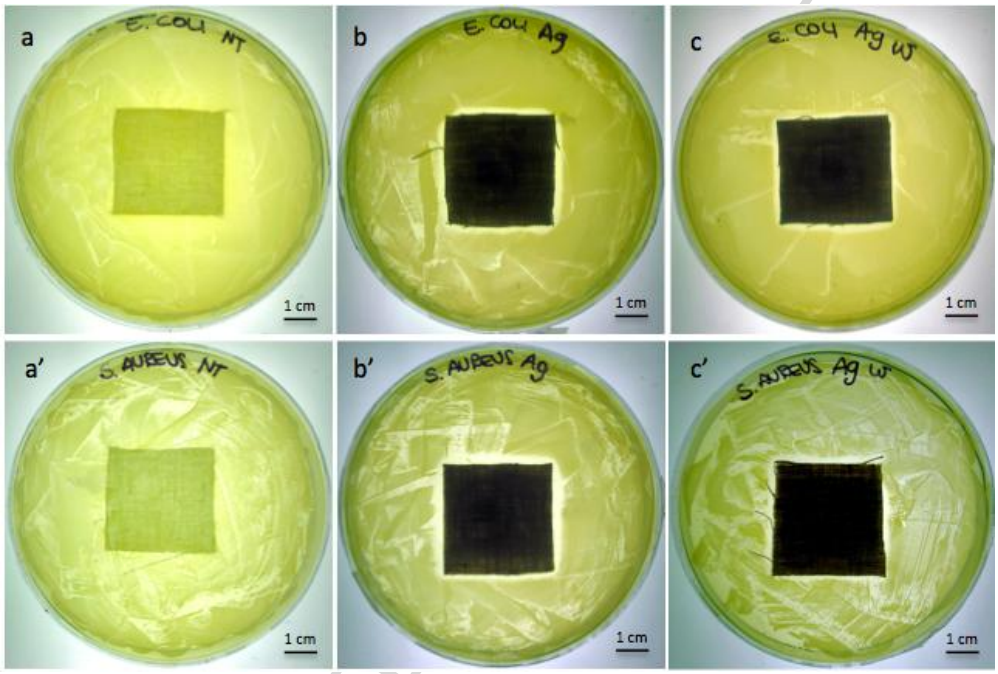

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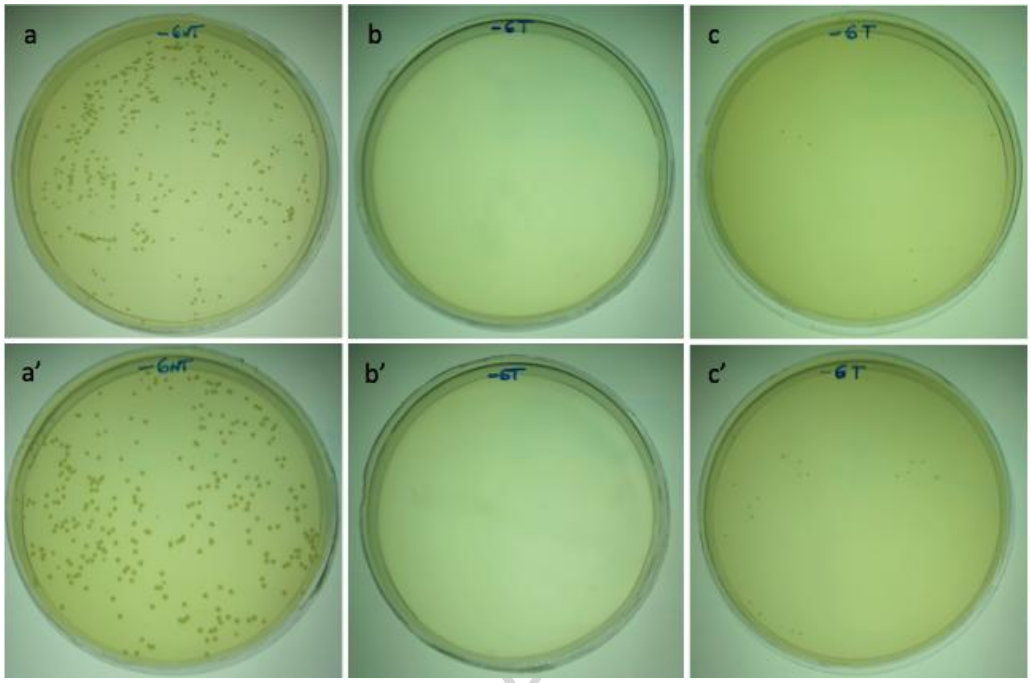

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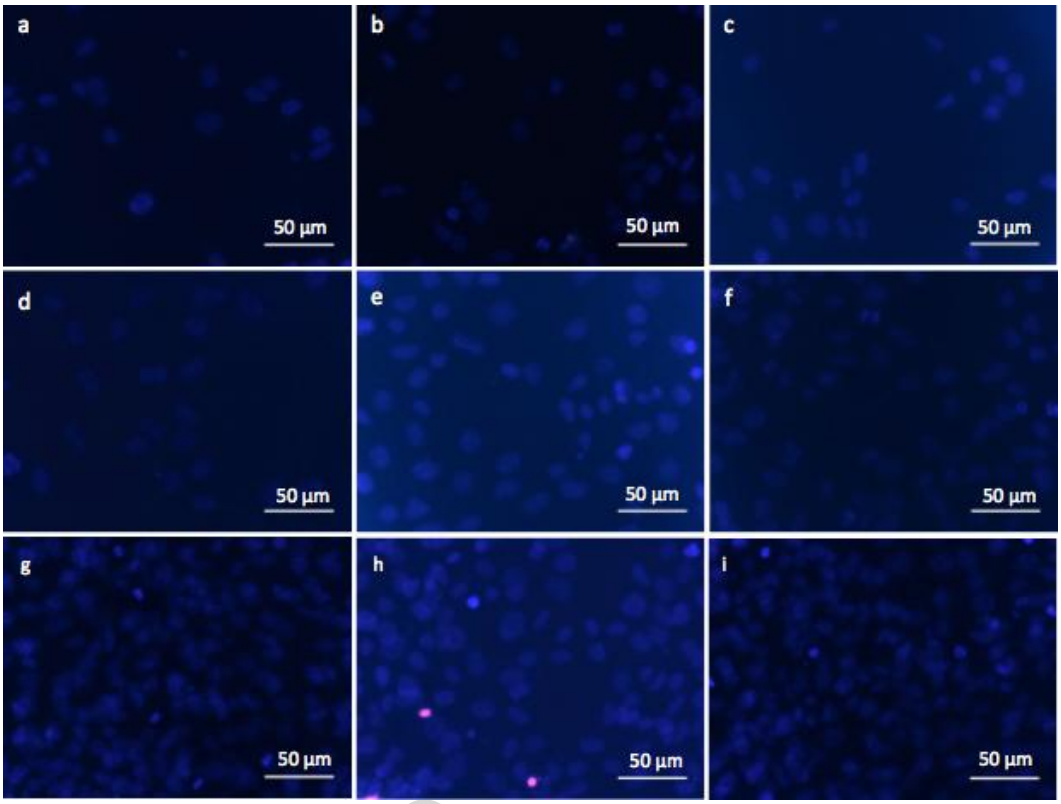

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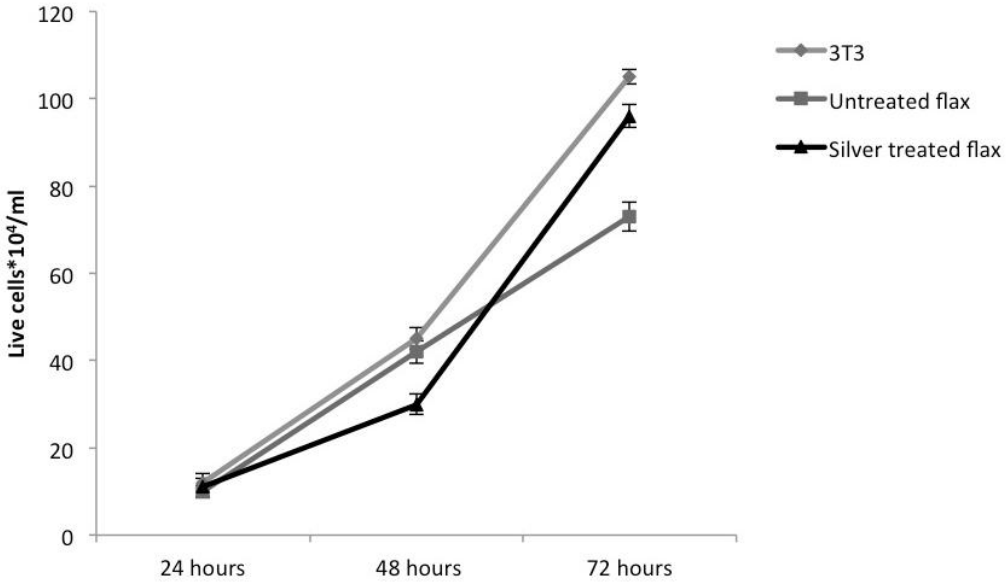

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Table 1. Surface chemical composition of flax fibres, expressed as atomic percentages (At%). Data acquired in triplicate.

Element (At%)	Neat Flax	0.1% ^{w} Ag-treated Flax	0.1% ^{w} Ag-treated Flax (after washing)	0.5% ^{w} Ag-treated Flax	0.5% ^{w} Ag-treated Flax (after washing)
C	69.0 ± 0.9	68.0 ± 2.0	69.2 ± 2.0	67.0 ± 2.0	66.7 ± 0.8
O	28.7 ± 0.9	28.5 ± 1.0	28.3 ± 1.0	30.0 ± 1.0	30.2 ± 0.5
N	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.3 ± 0.3	1.3 ± 0.2
Si	1.0 ± 0.2	1.4 ± 0.4	1.2 ± 0.3	0.9 ± 0.2	1.3 ± 0.5
Ag	-	0.5 ± 0.2	0.4 ± 0.2	0.8 ± 0.1	0.5 ± 0.1
Ca	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Na	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Cl	≤ 0.5	0.6 ± 0.1	≤ 0.5	≤ 0.5	≤ 0.5

Table 2. Quantification of carbon moieties in neat and Ag-treated flax fibres.

Sample	(C-C)% 284.8 ± 0.2 eV	(C-O/C-N)% 286.3 ± 0.2 eV	(C=O)% 287.3 ± 0.4 eV	(O-C-O)% 288.3 ± 0.5 eV	(O-C=O)% 289.7 ± 0.6 eV
Neat flax	21 ± 1	26 ± 5	13 ± 2	8 ± 1	2.0 ± 0.7
Ag-treated Flax	17 ± 3	28 ± 3	14 ± 3	7 ± 2	1.6 ± 0.8
Ag-treated Flax (after washing)	20 ± 1	26 ± 2	12 ± 1	8 ± 2	1.8 ± 0.6

HIGHLIGHTS

1. Silver nanophases are increasingly used as effective antibacterial agent for biomedical applications.
2. Silver coatings were deposited on textiles through the *in situ* photo-reduction of a silver solution.
3. Flax fabrics were characterized from a biological and surface chemical point of view
4. Scaling up of the process was confirmed