

New garment proposal for prevention of spreading Gram-negative bacteria resistant to carbapenem antibiotic class under hospital settings

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Abstract

Sensitive skin diseases, including atopic dermatitis, skin inflammation and bedsores, leave patients vulnerable under hospital setting. It is important for the development of a hospital gown with “soft hand” properties and at the same time as a protector against nosocomial infections. *Klebsiella pneumoniae* has developed resistance to antibiotics in the carbapenem antibiotic class, known as carbapenem-resistant *K. pneumoniae* (CRKP). CRKP is resistant to nearly all antibiotics and can kill up to 50% of infected patients.

This work consisted in the development of a washable recycled silk fibroin-based gown covalently linked with an amino acid L-Cysteine(L-Cys), focused on prevention of *K. pneumoniae* establishment, proliferation and spreading to community, for use under hospital settings. With the growing problem of resistance to antibiotics and few new therapies on the horizon, gowns adsorbed with L-Cys show to function as a barrier to the establishment and proliferation of microorganisms, providing user protection from infectious disease. This gown was knitted at a rectilinear needle loom with a Jersey knit

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structure. Then it was cross-linked with L-Cys, subjected to laundry, and subsequently characterized by energy-dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, contact angle, free energy of adhesion, scanning electron microscopy and transmission electron microscopy. Results presented a bactericidal effect against *K. pneumoniae* of 94.92% after three rinses and 88.88% after five washing cycles, with the few adhered bacteria with an altered and compromised morphology.

Keywords

L-Cysteine, antimicrobial-gown, nosocomial infections, aminolysis, *Klebsiella pneumoniae*

Introduction

The microbiota comprises all microorganisms usually found in healthy individuals. These microorganisms live on skin and mucosae of all persons after birth and are renewed until their death. Indigenous microbiota benefits host through early stimulation of immune system, prevention of colonization of pathogenic microorganisms, and synthesis of essential compounds (e.g. Vitamin K). On the other hand, various studies suggested that the development of potential pathogenic microorganisms upon alteration of local conditions, antimicrobial therapeutics, and immunosuppression may happen [1]. Healthcare-associated infections, such as pneumonia, bloodstream infections, wound or surgical site infections, and meningitis can provide conditions for *Klebsiella pneumoniae* establishment and infection development [2]. *Klebsiella pneumoniae* that belongs to Enterobacteriaceae family makes up comensal flora and causes opportunistic infections. It is a Gram-negative rod, non-motile, and it is characterized by a prominent polysaccharide capsule, which is responsible for its mucoid appearance and virulence [3]. Aside from colonization of gastro-intestinal tract, it colonizes skin and nasopharynx. One-third of individuals carry *K. pneumoniae* in stool; however, this number increases to 90–100% in hospitalized individuals and/or the ones taking antibiotics on a regular basis, as well as children [4]. These complications are likely to occur in immunocompromised patients under hospital settings, as elderly, malnourished, bedridden or paralyzed, and individuals who have a diminished pulmonary function [4]. The misuse of antibiotics has increased antibiotic resistance. Jim O'Neill stated that if nothing was done, 10 million annual deaths by infections caused by resistant bacteria would happen in the world, with an associated cost of 90 billion euros by 2050. One of contributing factors to emergence of resistance includes its widespread use in animals [5]. Hence, *K. pneumoniae* presents a risk of conversion to carbapenem-resistant *K. pneumoniae* (CRKP), especially in hospital settings. CRKP has become resistant to essentially all antibiotics, and can kill up to 50% of infected patients [6]. Moreover, when it becomes established, it proliferates and can spread rapidly throughout the community, through its fimbriae and polyssaccharide capsule to adhere and cause virulence [7]. These events add urgency to develop new strategies at the preventive level rather than at the curative level with antibiotics.

Gowns are a type of underwear worn by inpatients and should be changed once a day. The microbial contamination and probability of infection with CRKP increase as the day goes on, which implies microbial movement or transfer from gown to rashes or blisters developed in skin-sensitive patients. A gown can absorb an amount of sweat and microorganisms from human body and environmental contaminants in hospital settings, which is the ideal growth environment for microbial growth and *K. pneumoniae* transformation to CRKP.

Silk fibroin (SF) is a non-toxic hydrophilic natural textile, tissue compatible, with high tensile strength, being a product already approved under Food and Drug Administration (FDA) for clinical applications. In order to develop a silk recycled waste-based gown, four dimensions were considered: (i) material, where consumers have preferences for innovative raw materials; (ii) ecological, stimulating the rapprochement between cultures and nature as well as the criterious usage of resources; (iii) economic, with the use of recycled silk waste of cocoons as a low-cost process; (iv) antimicrobial, preventing *K. pneumoniae* establishment and colonization [8,9]. As antibiotics are ineffective and even counterproductive in fighting drug-resistant *K. pneumoniae*, L-Cys amino acid proves promising, since no microbial resistance has been reported.

In this research, L-Cysteine (L-Cys) was used to covalently modify silk fibroin surface through aminolysis. L-Cys is a common α -amino acid coming from a variety of food sources, and its thiol group at the end of variable side group is nucleophilic and provides a wide spectrum of bioactivity [10]. L-Cys activity is based on metabolic disturbance and membrane depolarization [11]. L-Cys targets the bacterial membrane, decreases its enzymatic activity, and compromises bacterial metabolism.

The present research reports on the study of recycled SF gown that embodies antimicrobial properties. More specifically, its covalent linking with L-Cys shows laundry-resistance and prevents the establishment, spreading, and conversion of *K. pneumoniae* to CRKP under hospital settings.

Experimental

Materials

Bacteria cultivation: *Klebsiella pneumoniae* (ATCC 4352) strain was cultivated on Nutrient Agar (NA) and was then grown on Nutrient Broth (NB) (Panreac).

Silk fibroin (SF) samples preparation: SF recycled waste was obtained from silkworm *Bombyx morii* (Brazil), Mn 30000, knitted at a rectilinear needle loom with seven needles per inch, with a Jersey knit structure and a linear density of 21.68 number metric (Nm). Its sericin was removed using a soxhlet apparatus with dichloromethane, as a standard procedure in our lab (Figure 1).

Covalent modification on SF samples surface was performed via aminolysis with 1% (w/V) L-Cysteine (L-Cys) (Sigma-Aldrich), for 3 h, under pH 9.5, at 90°C (Figure 2).

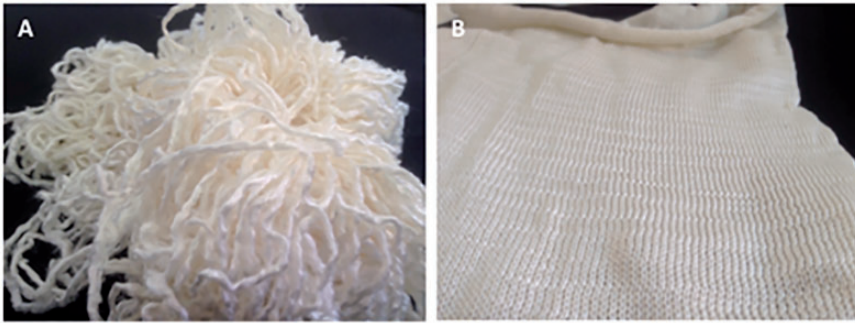


Figure 1. Silk recycled waste products (a) as raw materials for the development of Silk knitted structure (b).

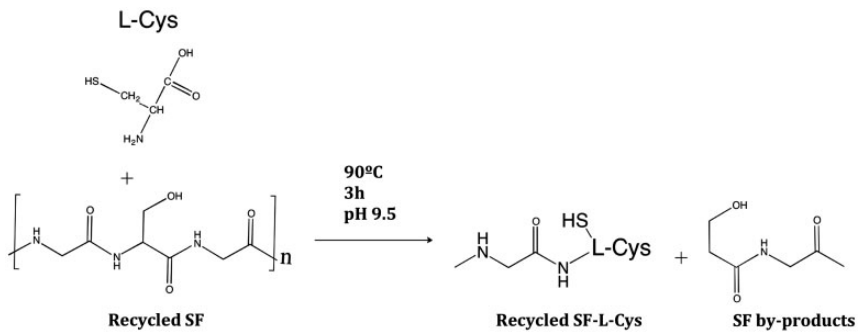


Figure 2. Schematic representation of the mechanism of covalent bonding of L-Cys on recycled SF (SF-L-Cys). Chemical structures were designed in CS ChemDraw Ultra.

Five washing cycles were done according to standard EN ISO 105-C06:2010, in a 4 g/L solution of AATCC 1993 Standard Reference Detergent WOB for 30 min each, at 40°C , rinsed, and dried afterwards at 30°C until used.

Methods

Minimal inhibitory concentration: The susceptibility of *K. pneumoniae* to L-Cys was performed with the microdilution method following M07-A6 guidelines applied to *K. pneumoniae* (ATCC 4352) – Standard JIS L 1902:2002. Briefly, a serial dilution of L-Cys was carried out in Mueller-Hinton Broth (MHB) (Sigma-Aldrich) to yield concentrations from 0.3 mg/mL to 20 mg/mL. Overnight liquid *K. pneumoniae* culture was adjusted to 0.5 McFarland (1×10^8 CFU/mL for *K. pneumoniae*) with sterile H_2O , from which 500 μL was added to 4500 μL MHB – bacterial work suspension. 50 μL of work suspension and L-Cys dilutions were added to wells in a 96-multi-well plate. Samples were incubated for 24 h at 37°C . A smear of

Table 1. Energy-dispersive X-ray spectroscopy.

Condition	C (Elements [C(atom)])	N (Elements [C(atom)])	O (Elements [C(atom)])	S (Elements [C(atom)])
A	46.48	21.69	31.83	0.00
B	47.33	21.31	31.29	0.07
C	47.89	21.12	30.92	0.07

Note: Control SF (A); SF-L-Cys after 0 washing cycles (B); SF-L-Cys after five washing cycles (C).

microorganisms in the bottom of wells demonstrate microbial killing and constitute the minimal inhibitory concentration (MIC). Triplicates were used.

Energy-dispersive X-ray spectroscopy: The sulphur of adsorbed L-Cys was quantified using an energy-dispersive X-ray spectroscopy (EDS) attachment of a HITACHI S 2700 scanning electron microscope, with a high voltage of 20 kV (Table 1).

Fourier Transform Infrared Spectroscopy: The chemical composition of native SF and SF functionalized with 1% L-Cys after five washing cycles was analyzed by Fourier transform infrared spectroscopy (FTIR) (Thermo-Nicolet is10). Each sample was scanned 64 times, with a spatial frequency resolution of 4 cm^{-1} .

Contact angle: Contact angles were measured with an OCAH-200 set-up device (Dataphysics) outfitted with a high-speed video system with CCD video camera with a resolution of 752×484 .

Free energy of adhesion: The surface tension of SF samples was determined (equation (1)) by the sessile drop contact angle method at RT, for three different types of components: the apolar diiodomethane and both polar water and ethylene glycol, whose surface tension components have already been determined.

$$(1 + \cos\theta)\gamma_{\text{SF}}^{\text{TOT}} = 2\left(\sqrt{\gamma_{\text{S}}^{\text{LW}}\gamma_{\text{SF}}^{\text{LW}}} + \sqrt{\gamma_{\text{S}}^{+}\gamma_{\text{SF}}^{-}} + \sqrt{\gamma_{\text{S}}^{-}\gamma_{\text{SF}}^{+}}\right) \quad (1)$$

where θ is the contact angle, and $\gamma^{\text{TOT}} = \gamma^{\text{LW}} + \gamma^{\text{AB}}$. The parameter γ^{LW} corresponds to the Lifshitz-van der Waals component of the surface free energy, and γ^{+} and γ^{-} the electron acceptor and electron donor parameters, respectively, of the Lewis acid–base component γ^{AB} , being $\gamma^{\text{AB}} = 2\sqrt{\gamma^{+}\gamma^{-}}$ [12].

The interaction between *K. pneumoniae* and SF was predicted in terms of total interaction energy, and it is represented in the equation 2 by KP and SF, respectively.

$$\Delta G_{\text{Adhesion}}^{\text{TOT}} = \left(\sqrt{\gamma_{\text{KP}}^{\text{LW}}} - \sqrt{\gamma_{\text{SF}}^{\text{LW}}}\right)^2 - \left(\sqrt{\gamma_{\text{KP}}^{\text{LW}}} - \sqrt{\gamma_{\text{W}}^{\text{LW}}}\right)^2$$

$$\begin{aligned}
 & - \left(\sqrt{\gamma_{SF}^{LW}} - \sqrt{\gamma_W^{LW}} \right)^{KP} + 2 \left[\sqrt{\gamma_W^+} \left(\sqrt{\gamma_{KP}^-} + \sqrt{\gamma_{SF}^-} - \sqrt{\gamma_W^-} \right) \right. \\
 & \left. + \sqrt{\gamma_W^-} \left(\sqrt{\gamma_{KP}^+} + \sqrt{\gamma_{SF}^+} - \sqrt{\gamma_W^+} \right) - \sqrt{\gamma_{KP}^-} \gamma_{SF}^+ - \sqrt{\gamma_{KP}^+} \gamma_{SF}^- \right] \quad (2)
 \end{aligned}$$

If $\Delta G_{Adhesion}^{TOT} < 0$, *K. pneumoniae* adhesion was expected favorable. On the contrary, adhesion would be unfavorable if $\Delta G_{Adhesion}^{TOT} > 0$ [12].

Antimicrobial activity: The antimicrobial properties of SF samples modified with L-Cys were tested according to JIS L 1902 Standard test method for assessing antimicrobial activity of incorporated antimicrobial agent(s) in textile materials. The Gram-negative strain *K. pneumoniae* (ATCC 4352) was chosen because its resistance to recommended antibiotic treatments has been raised over time, most recently becoming resistant to carbapenem antibiotic class, known as carbapenem-resistant *K. pneumoniae* (CRKP).

Briefly, a *K. pneumoniae* suspension of $1-5 \times 10^6$ bacteria/mL was inoculated into SF samples, and added with NaCl plus surfactant. After 0 and 24 h of incubation, samples were vortexed for 20 s to release any adsorbed *K. pneumoniae*. The antimicrobial activity was calculated by a quantitative method in order to determine the percentage of bacterial reduced growth rate (% of reduction) at 0 and 24 h

$$\text{Reduction} = (C - A)/C \times 100 \quad (3)$$

where C represents the average number of Colony Forming Units (CFU/mL) of non-modified SF samples, and A represents the average number of CFU of modified SF.

In order to elucidate whether SF-L-Cys presents a bacteriostatic or bactericidal effect, equations (4) and (5) were resolved [13]

$$\text{Bacteriostatic activity} = Mb - Mc \quad (4)$$

$$\text{Bactericidal activity} = Ma - Mc \quad (5)$$

where Ma = log₁₀ of the average of three replicas at T0h controls, Mb = log₁₀ of the average of three replicas at T24h controls, and Mc = log₁₀ of the average of three replicas at T24h SF-L-Cys.

Scanning electron microscopy: Adsorbed *K. pneumoniae* were reticulated overnight with 1.5% glutaraldehyde under 4°C. Samples were then serially dehydrated with an increasing ethanol-water gradient (50–99% v/v) for 10 min each, and subjected to critical point drying (K850, EMITECH). Then, they were sputtered with a thin layer of gold and examined by scanning electron microscopy (SEM) (Hitachi S2700).

The magnifications used were 2000× and 7500× with accelerating voltage of 20 kV.

Transmission electron microscopy: The morphology of adsorbed *K. pneumoniae* was observed by transmission electron microscopy (TEM). They were stained with 2% uranyl acetate for 5 min, and mounted on a mesh with thin bar copper grids covered with formvar. Each sample was examined at a magnification of 30,000 × in a HITACHI HT7700 TEM with accelerating voltage of 80 KV.

Statistical analysis: SPSS Statistics software, version 21.0, was used for statistical analysis with comparisons between results (*t*-test). $p < 0.05$ was considered significant.

Results and discussion

The reduced functional capacity of immunocompromised patients and complex crosstalk between Enterobacteria make the phenomenon of multidrug resistance even worse [14,15] which can open the door to establish infection and bacterial proliferation resulting in patients fighting a losing battle.

This research paper describes the development and testing of a silk-based gown made from cocoon of silkworm *Bombyx morii* recycled materials, by focusing on prevention of disease, with the compound (L-Cys) that does not trigger microbial resistance, rather than treatment with antibiotics, against an *in vitro* infection caused by *K. pneumoniae*.

Bacteria cultivation

In order to perform the simulation of adhesion assays (1×10^8 *K. pneumoniae*/mL), *K. pneumoniae* was grown in its optimal condition with vigorous shaking until reaching exponential growth. During this stage, the bacterial metabolic machinery is entirely adapted to the medium (Nutrient Broth), in which each bacterial cell divides into two cells.

Susceptibility testing

MIC results of L-Cys against *K. pneumoniae* were 10 mg/mL. These results suggested that L-Cys in aqueous solutions would require 10 mg/mL to kill *K. pneumoniae*.

Silk fibroin (SF) samples preparation

Antimicrobial textiles show that besides protecting user, they can also prevent the formation of malodors released from bacterial growth. The developed silk fibroin (SF) gown was covalently modified by aminolysis, with the amino acid L-Cys. SF structural amides were hydrolyzed to carboxylic acid under alkaline environment (pH 9.5) and heat (90°C) through “saponification”, in which the amino groups of L-Cys were then cross-linked. Amino acid L-Cys, with antimicrobial properties,

was chemically bound onto textile fibers, which improved the orientation of the bound molecule (SF-NH₂-L-Cys-SH), decreased the quantity required (1% owf (over the weight of the fabric, in a m/V solution of 1 g fiber/50 mL; hence, 0.2 mg/mL)), and improved laundry resistance, and its mechanism of action only interfered with microorganisms when they contacted each other [16].

Energy-Dispersive X-ray spectroscopy

L-Cys covalent reaction onto SF fibers can be indirectly detected by the presence of sulphur, which did not decrease after five washing cycles. Furthermore, SF does not have sulphur in its native chemistry. After functionalization and three rinses, the amount of L-Cys was 0.07 wt.%. In addition, after five washing cycles, the amount of L-Cys was maintained at 0.07 wt.%. Controls confirmed no presence of sulphur (Table 1). EDS showed that L-Cys was grafted to SF fibers, as well as its maintenance after five washing cycles, which demonstrated the effective mechanism of functionalization.

Fourier transform infrared spectroscopy

The infrared spectra of unmodified SF is shown in Figure 3(a). Peaks at 3300 cm⁻¹ and 3100 cm⁻¹ are associated with amides B and A. At 1650 cm⁻¹ vibrations are due to C = O stretching vibrations of amide I, and the peak at 1520 cm⁻¹ is related to N-H in-plane bending together with the out-of-phase C-N stretching of amide II.

SF covalent structural modification before and after five washing cycles was also assessed by FTIR. IR spectra confirmed L-Cys bonded on SF surface, as shown by

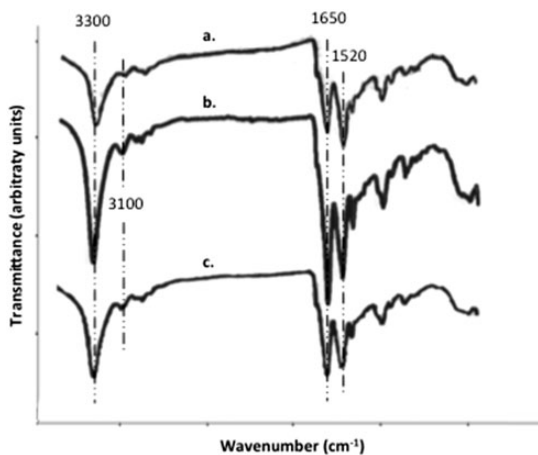


Figure 3. FTIR. Control SF (a). SF-L-Cys after 0 washing cycles (b). SF-L-Cys after five washing cycles (c).

the increase of peaks associated with L-Cys immobilization on a surface, around 3300, 3100 and 1650 cm^{-1} , as shown in Figure 3(b) and (c). Amide I is related to vibrations of the peptide bonds. However, the peak at 1520 cm^{-1} that is related to amide II diminishes, which shows that SF protein secondary structure was modified/unfolded based on the extent of L-Cys cross-linking to SF. Bound L-Cys on SF before (Figure 3(b)) five washing cycles was higher than after laundry (Figure 3(c)) because the peak at 1650 cm^{-1} , due to vibrations of bound L-Cys, was higher than the peak at 1520 cm^{-1} , which is related to the modification of SF [17].

EDS and Fourier transform infrared spectroscopy (FTIR) both showed that L-Cys was successfully grafted to SF fibers. Results also demonstrated the maintenance of cross-linked L-Cys after five washing cycles, which confirms the efficient mechanism of functionalization showed by EDS.

Contact angle

The wettability of SF (silk fibroin) and SF covalently modified samples (SF-L-Cys) at pH 9.5 was measured by Contact Angle Measuring System between their surface and water drop by the sessile drop method, at 10 s. The native/control SF fiber surface showed to be at the interface hydrophobicity–hydrophilicity, $111.83^\circ \pm 21.10^\circ$. On the other hand, after five washing cycles, SF-L-Cys became hydrophilic, $71.30^\circ \pm 4.41^\circ$.

This gown showed the capacity to transport moisture away from the skin, which improves the comfort in patients with sensitive skin [18].

Free energy of adhesion

A thermodynamic methodology of the free energy [12] was used to predict the *K. pneumoniae* binding potential to gown, according to van Oss [19,20]. SF altered from the hydrophobic–hydrophilic interface to hydrophilic character after its covalent treatment. Results showed that these modifications changed SF-L-Cys into a more favorable garment to bind to *K. pneumoniae*, therefore a method of killing bacteria. When *K. pneumoniae* was exposed to SF-L-Cys ($\Delta G_{\text{Adhesion}}^{\text{TOT}} = -7.37$) and SF-L-Cys after five washing cycles ($\Delta G_{\text{Adhesion}}^{\text{TOT}} = -3.96$), the $\Delta G_{\text{Adhesion}}^{\text{TOT}}$ decreased when compared to control SF ($\Delta G_{\text{Adhesion}}^{\text{TOT}} = 8.91$), meaning that the adhesion trend of SF-L-Cys to *K. pneumoniae* increased with the introduced modification. SF with an isoelectric point (pI) of 4.2 is negatively charged at neutral physiologic pH's [21]. SF fibers took for granted their affinity with water molecules in an environment which exposes the –OH and –SH groups to environment. This is important to enhance hydrophilic properties of gown and its self-cleaning. The negatively charged membrane potential of *K. pneumoniae* makes it repelled by native SF. In spite of the ubiquity of microorganisms, the ubiquity of microorganisms, even on hydrophobic surfaces, a gown whose fibers demonstrate an ability to quickly bind and kill microorganisms would seriously increase their odds of success as adjuvant in the prevention of establishment of *K. pneumoniae*.

Antimicrobial assays - quantitative method (JIS L 1902 2002)

Antimicrobial assays were conducted under recommended conditions for evaluating antimicrobial finishes on textiles [13]. The SF-L-Cys gown showed significant reduction of microorganisms when compared to controls ($p < 0.05$), as shown in Figure 4. This image illustrates how *K. pneumoniae* load diminished 94.92% after three rinses ($p = 0.0001$) and 88.88% after five washing cycles, none of the previous two significantly different from each other ($p = 0.2705$). Although L-Cys was functionalized using a very low quantity of 1% owf (0.2 mg/mL), most bacteria were eliminated, even after five washing cycles. Furthermore, bacteriostatic and bactericidal activity values were calculated, which showed to be -0.855 and 0.34 , respectively. According to JIS L 1902 [13], bacteriostatic and bactericidal activity values for *K. pneumoniae* (ATCC4352) cannot be inferior to 2.0 and 0 [13], respectively. Likewise, our results support the theory that SF-L-Cys has a bactericidal activity. Furthermore, *K. pneumoniae* was not able to develop resistance to L-Cys, which has the capacity to disrupt enzymatic activity as well as bacterial metabolism [11]. This microbicidity of SF-L-Cys may cause bacterial lysis, after its hydrolytic action against peptidoglycan. L-Cys potentiated its $-SH$ exposition against the external environment (bacteria) when cross-linked by their amines to SF fibers' surface. Otherwise, L-Cys in solution would require 10 mg/mL to kill *K. pneumoniae*, as reported by MIC results. In accordance with latter results, qualitative experiments – SEM and TEM – showed low bacteria adhesion and their morphology compromised, when comparing to controls.

Scanning electron microscopy

Fiber topography and adhered *K. pneumoniae* were observed for controls and SF-L-Cys after five washing cycles. As illustrated in Figure 5, and contrasting to

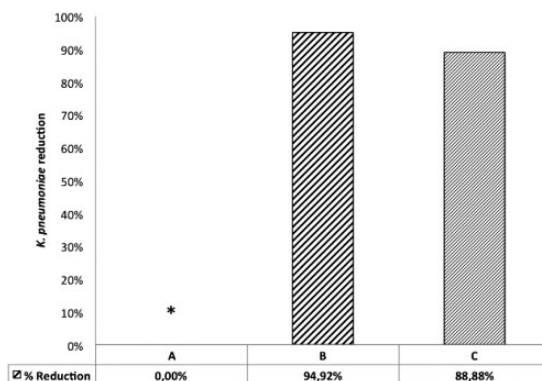


Figure 4. Percentage of *K. pneumoniae* reduction at 24 h ($n = 6$) – Control SF (a), SF-L-Cys after 0 washing cycles (b), SF-L-Cys after five washing cycles (c). *Statistically significant ($p < 0.05$).

controls, SF-L-Cys fiber surface became homogeneous and clean, as a consequence of a fall in the number of adhered *K. pneumoniae* after 24 h of incubation. Also, the presence of cross-linked SF fibers with L-Cys may prevent microbial contamination of the gown.

Transmission electron microscopy

The morphology of *K. pneumoniae* was also observed after five washing cycles under TEM. Results showed membrane-compromised *K. pneumoniae* when adsorbed to SF-L-Cys (Figure 6).

People experiencing sensitive skin, including atopic dermatitis, skin inflammation, bedsores, and related diseases, can benefit from the “softer hand” properties unique to this silk-based gown. Furthermore, it addresses fluid retention and self-cleaning due to L-Cys properties (hydrophilicity) [22], as well as bactericidal activity against *K. pneumoniae* after five laundering [23–25].

Hence, this hospital gown was constructed so that the comfort of sensitive skin sufferers and healing of the patients’ skin were enhanced beyond the requirements that hospitals meet. Furthermore, it may help prevent rashes and other skin irritations, due to a unique combination of silk and L-Cys properties.

According to published work, fabrics treated with antimicrobial compounds, namely anthranic acid, copper, silver, triclosan, polyhexamethylen biguanid (PHMB), quaternary ammonium compounds, lavender oil and most nanoparticles, have demonstrated leaching over laundering and/or cytotoxicity for skin [26–30].

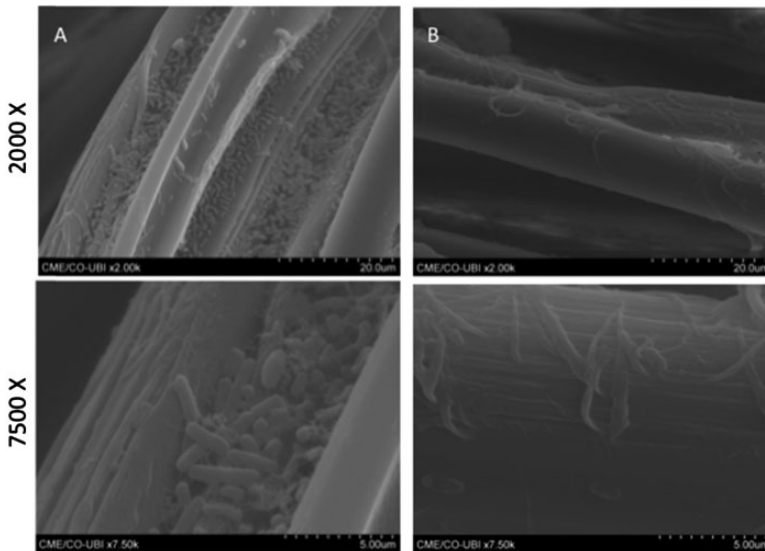


Figure 5. SEM images of control SF (a) and SF-L-Cys after five washing cycles (b).

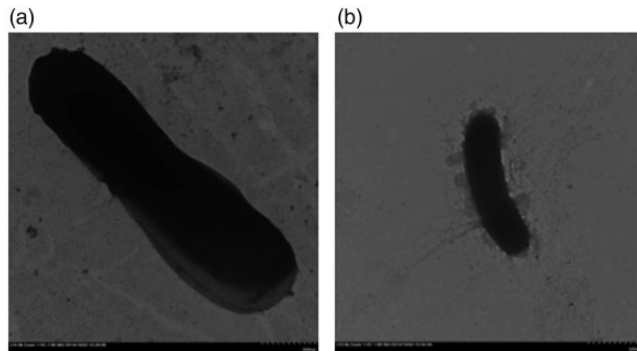


Figure 6. TEM images of *K. pneumoniae* that was in contact with control SF (a) and SF-L-Cys after five washing cycles (b).

Conclusions

Hospital gowns are a type of underwear worn by inpatients which should be changed once a day. The microbial contamination and probability of infection of patients affected by the problem of sensitive skin, with carbapenem-resistant *K. pneumoniae* (CRKP), increase as the day goes on. CRKP has become resistant to essentially all antibiotics, and can kill up to 50% of infected patients. Moreover, when it becomes established, it proliferates and can spread rapidly throughout the community, through its fimbriae and polysaccharide capsule to adhere and cause virulence. These events add urgency to develop new strategies to prevent antibiotic resistance from spreading.

This research paper describes the development and testing of a silk-based gown made from cocoon of silkworm *Bombyx morii* recycled materials, by focusing on prevention of disease, with the compound (L-Cys) that does not trigger microbial resistance, rather than treatment with antibiotics, against an *in vitro* infection caused by *K. pneumoniae*. There was a reduction of 94.92% after three rinses and 88.88% after five washing cycles, concomitantly with an altered morphology of adherent bacteria.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors would like to acknowledge the Fundação para a Ciência e Tecnologia (FCT) for the funding for the PhD grant SFRH/BD/91444/2012 and Programa Operacional Capital Humano (POCH) and European Union for the co-funding.

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