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Antimicrobial functionalization of wool: assessment of the effect of Cecropin-B and [Ala5]-Tritrp7 antimicrobial peptides

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This investigation provides a new strategy to impart antimicrobial properties into wool-based materials using Cecropin-B and [Ala5]-Tritrp7 antimicrobial peptides (AMPs). The process was conducted using exhaustion method at 40 °C for 1–3 h. The presence of the AMPs in the modified-wool samples was confirmed by colorimetric assay of Bradford reagent and possible changes in the morphology of the fibers and damage to its surface were analyzed by scanning electron microscopy. Results showed that 1 h were long enough for the functionalization to occur effectively and that the morphology of the fibers was not influenced by the functionalization process. Furthermore, the antimicrobial activity of the AMPs applied on wool was assessment by JIS L 1902-2002 against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). The results showed that both AMPs have a high reduction in bacterial growth (Cecropin-B resulting in 71.67% reduction against *S. aureus* and 85.95% against *K. pneumoniae*. While [Ala5]-Tritrp7 resulting in 66.74% reduction against *S. aureus* and 88.65% against *K. pneumoniae*).

Keywords: wool-based materials; antimicrobial-textiles; antimicrobial-peptides; antimicrobial-agents

Introduction

Wool is a natural proteinaceous fiber that has been widely used as a high quality textile material, being lighter, warmer, softer, and smoother than other fibers. However, wool-based materials are a host for the generation and propagation of micro-organisms, resulting in fiber damages or even skin irritations. In order to impart antimicrobial properties to wool fabrics, as for example for geriatric and pediatric applications, in which skin tends to be more sensitive, several antimicrobial compounds have been studied and applied (Gouveia, Sá, & Henriques, 2012; Niu, Liu, Dai, Hou, & Xu, 2012; Tang et al., 2011). Synthetic organic agents such as quaternary ammonium compounds, biguanides, N-halamines, metals as silver, and naturally derived biopolymers such as chitosan, are among the most studied antimicrobial agents in recent years (Gao & Cranston, 2008; Gouveia et al., 2012; Windler, Height, & Nowack, 2013). However, these agents may cause side effects, problems of environmental pollution, resistance for the micro-organisms, low durability, and limited effectiveness for a broad spectrum of micro-organisms (Gao & Cranston, 2008; Gouveia et al., 2012; Windler et al., 2013).

Therefore, there is a pressing demand to new and effective antimicrobial compounds to develop the next generation of agents to control microbial infections.

The antimicrobial peptides (AMPs), important components of innate immunity of many vertebrate and invertebrate species, exhibit a broad spectrum of activity, high efficacy at very low concentrations and low propensity for developing resistance (Brogden, 2005; Nguyen, Haney, & Vogel, 2011).

The AMPs have diverse mechanistic modes of action. They can change the transmembrane electrochemical gradients necessary for microbial homeostasis, inhibit protein synthesis, induce membrane permeability and rupture, inhibit protein synthesis, or promote the synthesis of reactive oxygen species that cause cell death (Rahnamaeian et al., 2015). As such, due to their mechanism of action, AMPs act without high specificity towards a protein target, which reduces the likelihood of induced resistance (Wimley & Hristova, 2011). This is crucial to provide a permanent, nontoxic, and effective antimicrobial effect over wool-based materials used, in particular in pediatric and geriatric nursing unit services.

Therefore, in this study, we report a simple strategy to connect AMPs onto wool materials. Cecropin-B and [Ala5]-Tritrp7 were the peptides selected and used.

Cecropin-B is an inducible antibacterial peptide that is found in the hemolymph of the pupae of *H. cecropia*. Mature cecropin peptides lack cysteine residues, are 35–39 amino acids in length and assumes an amphipathic α-helice structure. Its antimicrobial activity is related to the ability to form amphipathic α-helices that facilitate its insertion into the microbial membranes. Thus, when Cecropin-B inserts in the microbial membranes cause

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changes in its permeability and consequently may occur rupture and release of cellular contents (Jacobi, Plourde, Charest, & Hamelin, 2000; Li, Xiang, Zhang, Huang, & Su, 2012).

On the other hand, [Ala5]-Tritrp7 is a synthetic peptide that results of replacing of the first Pro at position 5 in tritrpticin by Ala (Tritrp7). The substitution of Pro-5 to Ala in Tritrp7 was characterized by having a greater ability to form amphipathic α-helices, promoting an effective cell leaching which may be the cause of the higher reduction rates (growth inhibition) for this peptide (Schibli, Nguyen, Kernaghan, Rekdal, & Vogel, 2006).

The option for each one should lie on the type of application because they have distinct features. Cecropin-B is effective for pathogen clearance and can affect host cell to promote wound repair with a known antiviral and antifungal activity, while [Ala5]-Tritrp7 is a member of the cathelicidin family of AMPs with effective antibacterial activity (Jacobi et al., 2000; Li et al., 2012; Schibli et al., 2006).

Therefore the aim of this investigation was to assess the ability of Cecropin-B and [Ala5]-Tritrp7 binding onto wool fibers through ionic interaction. A promising strategy to impart antimicrobial properties into natural fibers, namely wool-based materials.

Experimental

Textile material

The textile substrate used in this work was a 100% wool fabric. The wool was washed in an alkaline bath pH 8.5 (2.00 g/L Na_2CO_3) for 30 min at 60 °C with the nonionic detergent Nekanil 907 (0.3 g/L), which has a good wetting effect. After prewashing, the wool was rinsed with distilled water and placed to dry at room temperature. The washing process is recommended to remove any residue of chemicals commonly used in the finishing of wool, so that false positives can be avoided in relation to antimicrobial activity. Furthermore, washing also eliminates any fat that may still be present in the fiber itself and thereby improve its hydrophilicity/wettability.

Peptides

Sequences of the peptides used are shown in Table 1. The lots containing lyophilized powder of Cecropin-B (AnaSpec) and [Ala5]-Tritrp7 (AnaSpec) were stored at −20 °C. When the peptide solution was prepared the content present in the lots (1 mg) was dissolved in 1 mL of sterile water and a stock solution of peptide of 10 μg/mL was prepared.

Determination of minimal inhibitory concentration

The minimal inhibitory concentration (MIC) values of Cecropin-B and [Ala5]-Tritrp7 for bacterial strains *Staphylococcus aureus* (ATCC 6538) and *K. pneumoniae* (ATCC 4352) were determined using the broth microdilution test in 96-well plates according to standard M07-A6 of the CLSI.

In this procedure, the inoculums were prepared in sterile water from fresh overnight liquid culture and bacterial turbidity was adjusted to 0.5 McFarland (1–2 × 10^5 CFU/mL) according to standard.

The MIC values of AMPs was determined by successive volumetric dilutions in a ratio of 1:2 in Mueller-Hinton Broth with a solution of AMP of initial concentration 10 μg/mL.

The 96-well plates were then inoculated with total of 50 μL of the bacterial inoculum suspension and 50 μL of the AMPs dilutions. After incubated at 35 ± 2 °C, antibacterial activity was assessed and the MIC was determined.

Wool functionalization process

The process of functionalization was conducted using exhaustion method at 40 °C for 1–3 h with constant stirring of 15 rpm. The wool samples were immersed in a solution of AMP (10 μg/mL) in sterile water (3 mL). The concentration tested was above the MIC value for each strain and AMP, in order to ensure the antimicrobial effect.

Afterwards, all samples were washed in a 1 g/L of a solution of AATCC 1993 Standard Reference Detergent WOB, in five washing cycles performed at 40 °C during 60 min, a method adapted from the international

Table 1. Amino acid sequences of Cecropin-B and [Ala5]-Tritrp7.

AMPs	Site of production	Amino acid sequence
Cecropin-B	Silk moth (*Hyalaphora cecropia*) – imune haemolymph	KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL-NH_2
[Ala5]-Tritrp7	Synthetic	VRRFAWWWPFLRR-NH2

standard EN ISO 105-C06:2010, followed by drying at 37 °C for approximately 4 h. The soaping procedure was performed over the samples treated with the AMPs prior to further investigation and assessment of antibacterial activity in order to give evidence of a durable and stable functionalization effect.

Assessment of the effectiveness of functionalization

The presence of the peptide at the AMPs-functionalized samples was determined using the colorimetric assay of Bradford reagent. The colorimetric method of Bradford reagent allows the determination of protein content in solution and consists in forming a stable complex between the dye Coomassie Brilliant Blue G, and the proteins in solution (Bradford, 1976).

The performed test was a micro-Bradford assay, which differs from the standard test in the concentration range used to prepare standard solutions (1–10 µg of total protein in 1 mL). The concentration of peptide in the solution was determined by a calibration curve using standard protein such as bovine serum albumin. For analysis, to each tube containing 1-mL incubation solution before and after functionalization was added 1-mL Bradford dye. After agitating and incubating for 5 min at room temperature, the absorbance was read on a spectrophotometer at 595 nm. The exhaustion rate (% E) was estimated through the difference of the amount of AMP in the solution before and after the functionalization.

The absorption rate of the AMP was also measured directly in textile samples through color strength (K/S). Briefly, the process of staining in AMPs-functionalized samples and control samples (without AMP) consisted in immersing the samples in reagent solution Coomassie Brilliant Blue G-250 at room temperature and under constant stirring. Afterward, the samples were washed with distilled water to remove unbound dye out of the fibers. The color strength measurement of samples was performed by spectrophotometer readings in the spectrophotometer Spectraflash SF300 from Datacolor at 595 nm. The device measures the reflectance (R) of the sample at all wavelengths of the visible range and the reflectance can be related with the concentration trough the Kubelka–Munk Equation (1):

$$\frac{K}{S} = \frac{(1-R)^2}{2R}, \tag{1}$$

where R is the reflectance and K and S are the absorption and diffusion coefficient of the medium, respectively, because $K/S = \alpha C$, being C the concentration of dye in the fiber, and α a constant.

Evaluation of the quality of functionalized material

X-ray diffraction

In order to verify if the functionalization caused alterations in the structural characteristics of the wool fibers an analysis by X-ray diffraction (XRD) was carried out. In this work, the X-ray diffractometer used was the Rigaku model Rigaku DMAXIII/C with radiation Kα of one ampoule of copper ($\lambda = 1.5406$ Å) operating at 40 kV and 30 mA. The diffractograms were recorded at values of 2θ between 0 and 50 °C. The crystal structure of wool fibers were evaluated by comparing the diffractograms of wool functionalized with diffractograms of their respective controls.

Scanning electron microscopy

The functionalized samples with AMPs were analyzed by scanning electron microscopy (SEM) to evaluate possible change s and determine the morphology of the fibers, before and after functionalization. SEM images were obtained on a HITACHI S2700 scanning electron microscope, operated at 20 kV.

Evaluation of antimicrobial activity

Suspension test

The evaluation of the antimicrobial effect was carried out according to the Japanese Industrial Standard JIS L 1902-2002. This protocol specifies the test method for assessing the antimicrobial activity and effectiveness of functionalized textile samples.

In this work, the suspension test (quantitative) was the selected and performed method, and was applied to two bacterial strains: Gram-positive *S. aureus* (ATCC 6538) and Gram-negative *K. pneumoniae* (ATCC 11296).

Briefly, Falcon tubes containing the control samples (without AMP) and AMP-functionalized samples were inoculated with a standardized bacterial suspension of 1–5×10^5 CFU/mL, prepared from fresh overnight liquid cultures. Afterward, half of the samples were incubated for 18–24 h at 37 °C ($T_{24\ h}$) and the other half was immediately processed and analyzed ($T_{0\ h}$). The tubes referring to $T_{0\ h}$ were, immediately after inoculation, shaken in vortex after adding 20 mL of 0.85% (w/v) sodium chloride solution with 0.20% (v/v) Tween-80. Afterward, the tubes were shaken on vortex and several dilutions were prepared in sterile 0.85% (w/v) sodium chloride solution and plated in Nutrient Agar. The plates were incubated at 37 °C for 18–24 h. Following incubation, the number of colonies present on the plates was determined and it was calculated the percentages of bacterial reduction. The above procedure for the tubes

$T_{0\ h}$ was repeated to $T_{24\ h}$ tubes, after incubation for 18–24 h.

Antimicrobial activity expressed in percentage reduction was calculated by the following Equation (2), comparing the control population size, C, with the population size on the functionalized samples, A (Tang et al., 2011).

$$\text{Percentage reduction} = \frac{C - A}{C} \times 100, \quad (2)$$

where C corresponds to the average value of Colony Forming Units (CFU – µg/mL) of control samples (without AMP), and A to the average value of CFU of AMP-functionalized samples.

In order to evaluate the efficacy of the test, the growth value was calculated according to equation 3:

$$\text{Growth value} = M_B - M_A, \quad (3)$$

where M_B matches to the average of common logarithm of number of living bacteria in control samples after 18–24 h incubation, and M_A to the average of common logarithm of number of living bacteria in control samples immediately after inoculation of inoculum.

Furthermore, were calculated the bacteriostatic and bactericidal activity values according to the Equations (4) and (5), respectively.

$$\text{Bacteriostatic activity value} = M_B - M_C; \quad (4)$$

$$\text{Bactericidal activity value} = M_A - M_C, \quad (5)$$

where M_C is the average of common logarithm of number of living bacteria in AMP-functionalized samples after 18–24 h incubation.

Results and discussion

Determination of minimum inhibitory concentration (MIC)

The determination of MIC was used to estimate the concentration of peptide to be applied in the antimicrobial functionalization of wool textile samples. The MIC values obtained were reported in Table 2 and analyzed in comparison with values already reported in the literature. In previous studies, Cecropin-B showed to have preferential activity against Gram-negative bacteria, with MIC values between 0.5 and 16.00 µg/ml (Hu et al., 2013). Thus, comparing the value obtained for *K. pneumoniae*, 4.06 µg/mL, with the range reported by Hu et al. (2013), it can be concluded that this value is in agreement with previously described in literature. However, preferential activity of Cecropin-B against Gram-negative bacteria is not visible because was obtained a low MIC value against *S. aureus*, 1.44 µg/mL.

For the [Ala5]-Tritrp7 peptide, literature shows MIC values of 4.00 µg/mL for the *S. aureus* and 20.00 µg/mL for the *Escherichia coli* (Schibli et al., 2006).

The result obtained in this research for the *S. aureus* (MIC value of 4.58 µg/mL) proved to be very similar to that of MIC value previously found by Schibli et al. (2006) for *S. aureus* (MIC value of 4.00 µg/mL). However, the MIC value obtained to *K. pneumoniae* (5.00 µg/mL) was lower than that obtained by Schibli et al. (2006) for *E. coli* (20.00 µg/mL).

Indeed, is visible that Cecropin-B and [Ala5]-Tritrp7 exhibit MIC values very low, which is a major benefit in comparison with other antimicrobial agents for textiles. Furthermore, when the AMPs are used there is a reduced probability of acquisition of resistance to the bacteria.

The MIC values show a similar behavior against Gram-negative and Gram-positive bacteria.

Effectiveness of functionalization wool

The wool fibers have functional groups which make the surface chemically reactive. The main functional groups which occur naturally in wool structure are amino (–NH_2), carboxyl (–COOH), and hydroxyl (OH–) groups (Ibrahim, Abdalla, El-Zairy, & Khalil, 2013). Therefore, the binding reaction occurred by electrostatic interactions between the –NH_2 groups and –COOH groups of the peptides and wool. This is more important since the selected peptides [Ala5]-Tritrp7 and Cecropin-B both have a terminal amino group which facilitates the binding though the terminal carboxylic sites of wool fibers.

Thus, the presence of the AMPs on wool was detected by the Bradford assay reagent in solution by calculating the Exhaustion rate (%E) and on the wool itself by the color strength values (K/S). This

Table 2. MIC values of AMPs against *Staphylococcus aureus* and *Klebsiella pneumoniae.*

MIC values (µg/ml)	*Staphylococcus aureus* (ATCC 6538)	*Klebsiella pneumoniae* (ATCC 11296)
Cecropin-B	1.44	4.06
[Ala5]-Tritrp7	4.58	5.00

determination is possible, because the Coomassie Brilliant Blue G has specific affinity for the $-NH_2$ groups in solution and for the wool itself (Bradford, 1976).

The absorption of AMPs on the surface of the wool samples measured by %*E* and *K*/*S*, showed that the AMPs are present even after five washing cycles. From Table 3, it is obvious that both %*E* and *K*/*S* values increase for the functionalized samples with AMPs, compared with the non-functionalized wool sample.

Also, an increase on functionalization time showed a gradually decreases in these values. Furthermore, functionalized samples for 1 h showed that there was a rapid absorption of the AMPs on the surface of the wool samples ($\%E_{(\text{Cecropin-B 1 h})} = 75.68$; $K/S_{(\text{Cecropin-B 1 h})} = 5.18$ and $\%E_{(\text{[Ala5]-Tritrp7 1 h})} = 65.11$; $K/S_{(\text{[Ala5]-Tritrp7 1 h})} = 7.06$). However, after 3 h functionalization, a slight decrease in these values was observed ($\%E_{(\text{Cecropin-B 3 h})} = 47.92$; $K/S_{(\text{Cecropin-B 3 h})} = 5.16$ and $\%E_{(\text{[Ala5]-Tritrp7 3 h})} = 43.66$; $K/S_{(\text{[Ala5]-Tritrp7 3 h})} = 5.06$). This decrease may be due to the degradation of the wool peptides with incubation time, which occurs in the incubation solution during functionalization. Results showed in Table 3 seems to indicate that after 1 h of functionalization, the wool samples absorbed "all the AMP" of the solution and the test method using Comassie blue was able to detect peptides of wool fibers dropped into the solution due to functionalization. It is well known that even immersing wool into cold water causes some protein loss. Accordingly, results also showed a slight decrease in the %*E* and *K*/*S* values after further increase of incubation time up to 3 h. The explanation of this may be that since no further AMP absorption was verified (note that it was such a low amount in the solution!) a higher protein content of wool natural peptides was observed.

Consequently, the *K*/*S* values were consistent with the %*E*. This concordance allowed us to conclude that 1 h was sufficient time for the AMPs to be effectively adsorbed onto wool samples.

Table 3. Results of the assay of Bradford reagent.

	%*E*	*K*/*S*
Wool samples functionalized with Cecropin-B		
1 h	75.68	5.18
2 h	55.80	5.07
3 h	47.92	5.16
Wool samples functionalized with [Ala5]-Tritrp7		
1 h	65.11	7.06
2 h	47.76	6.48
3 h	43.66	5.06
Non-functionalized wool/control	–	3.24

In comparison with previous studies by Monier, El-Sokkary, and Sarhan (2010) and by Wang et al. (2009), the obtained results are in accordance with the existing ones. In prior studies by Monier et al. (2010), was developed a method to immobilize lipase from *Candida rugosa* on modified natural wool fibers and the presence of the enzymatic proteins on fibers was qualitatively characterized using this staining test. While the investigation by Wang et al. (2009) the staining test was used for determination of the lysozymes immobilized onto wool fibers.

Finally, it is possible to conclude by the %*E* and *K*/*S* values that the diffusing capacity of the Cecropin-B and [Ala5]-Tritrp7 was similar for both AMPs. The Cecropin-B peptide has in its composition a higher arginine content which leads to increase of amino groups. However, the Cecropin-B (molecular mass of 3834.7) in terms of size is greater than the synthetic peptide [Ala5]-Tritrp7 (molecular mass of 1875.3) (Kulagina, Shaffer, Ligler, & Taitt, 2007; Schibli et al., 2006). Thus, despite [Ala5]-Tritrp7 peptide to have tryptophan residues in its own constitution, also showed very positive results. Consequently, since both the AMPS have a terminal amino group, size is the factor that most influences the absorption and diffusion of AMPs in the wool, which is visible by the %*E* and *K*/*S* values.

Evaluation of the quality of functionalized material: XRD wool

Chemically, wool can be regarded as a composite material consisting of mainly keratinous protein polymers. Keratin in wool is a mixture of chemically linked amino acids with strong hydrogen and covalent bonds (Horrocks & Anand, 2000), i.e. wool has a composite structure consisting of fibrils with α-keratin structure embedded into an intermacrofibrillar matrix (Pakdel, Daoud, & Wang, 2013). The keratin macromolecule in wool fiber presents two different crystal structures, which represent two different chain conformations. The conformation of α-helices (α-keratin) is characteristic for wool fiber in its natural state, this is when the fiber is not stretched along its axis. But if wool is stretched, there is a gradual and reversible transformation the α-keratin for β-keratin. β-keratin presents an unstable conformation and when the stretching force disappears the conformation of keratin reverts to α-helices (Wojciechowska, Włochowicz, & Wesełucha-Birczyńska, 1999). Furthermore, the α-helix is stabilized by intramolecular hydrogen bonds. While, in β-keratin structure, the peptide chains are largely stretched to form what is called a pleated sheet structure and each sheet has hydrogen bonds between the peptide

groups of opposing chains, i.e, intermolecular bonds (Cao, 2000, 2002; Feughelman & Danilatos, 1980).

Thus, analysis by XRD in this work was thought to detect possible changes in the structural characteristics of the wool which are reflected in the fiber crystallinity changes.

The diffractograms of the samples are shown in Figure 1. The graph for non-functionalized wool display the typical XRD pattern with a prominent 2θ peak at 20.2° and a minor peak at about 9.2°, which were attributed to the β-sheet and α-helix structure of peptide chains in wool. This result is according to what is sustained by other authors (Fan & Yu, 2012).

Comparing the intensity of the peaks $2\theta = 9.2°$ and 20.2° of the diffractogram of the non-functionalized wool sample with the diffractograms of treated wool, it appears that the intensity of the peak at $2\theta = 9.2°$ increases in the case of wool immersed in distilled water, a test sample provided to check and compare with the simple water effect, and in case of functionalized wool samples during 1 h with Cecropin-B and [Ala5]-Tritrp7. This increase is more evident than the changes in the intensity peak at $2\theta = 20.2°$, as shown in Table 4, i.e, the intensities of the peaks at $2\theta = 9.2°$ of 587 obtained for the non-functionalized wool is lower than the one obtained for the wool treated with distilled water, 1127, and an even greater increase in the intensity was recorded for the functionalized wool samples, being 1265 for wool treated with Cecropin-B and 1324 for wool treated with [Ala5]-Tritrp7.

Table 4. The intensities of the peaks at $2\theta = 9.2°$ and 20.2° of non-functionalized wool, wool in distilled water, and functionalized wool with AMPs.

	Intensity	
Samples	$2\theta = 9.2°$	$2\theta = 20.2°$
Non-functionalized wool	587	1235
Wool in distilled water_1 h	1127	1069
Functionalized wool with Cecropin-B	1265	1353
Functionalized wool with [Ala5]-Tritrp7	1324	1181

On the other hand, the peak intensity at $2\theta = 20.2°$ decreases for the water-treated wool: 1069 and for functionalized wool with [Ala5]-Tritrp7: 1181, compared with the value obtained for the non-functionalized wool: 1235; being slightly higher for wool functionalized with Cecropin-B: 1353.

Thus, it can be seen that in what concerns to the sample treated with distilled water, a slight increase in

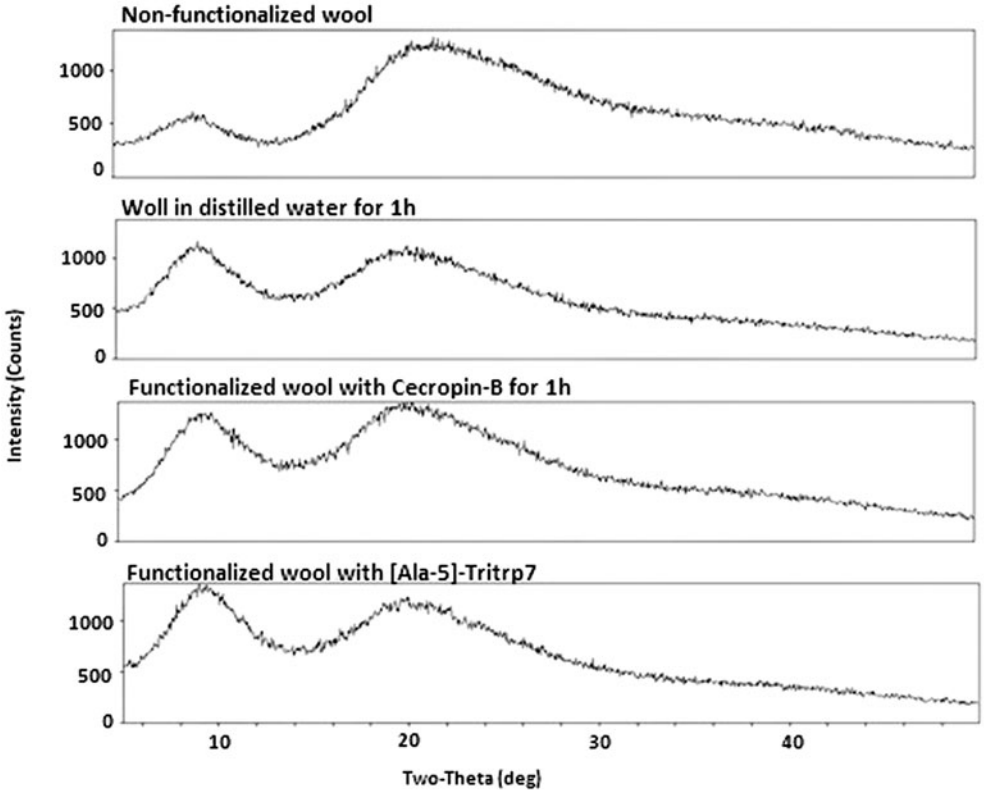

Figure 1. X-ray diffraction spectra of wool samples functionalized during 1 h.

peak intensity at $2\theta = 9.2°$ was obtained. This effect is due to reorganization of macromolecular chains, caused by the swelling effect of fiber because of water absorption. Regarding the functionalized samples with AMPs, there is a slight increase, but the intensity of the peak at $2\theta = 9.2°$ is more notorious, which results in a higher crystalline fiber, when the peptide is connected. In contrast, in relation with the $2\theta = 20.2°$ peak it can be seen that the intensity decreases for the wool treated with distilled water. This decrease causes a reduction in the disorientated β configuration and is a result of the effect that water causes in organization of the wool (Fan & Yu, 2012). The same effect is visible for the functionalized wool with [Ala5]-Tritrp7. This result is due to the fact of [Ala5]-Tritrp7 shows a low molecular mass (1875.3) (Schibli et al., 2006) that interferes less in the organization of wool. Rather, Cecropin-B, being larger (molecular mass of 3834.7) (Kulagina et al., 2007), led to an increase in the disorientated β configuration, visible by a slight increase in the intensity. This increase may be due to the hydrogen bonding break in the wool protein structure (Monier, Ayad, & Sarhan, 2010).

These results are in agreement with the obtained by Eslahi, Dadashian, and Nejad (2013) in production of nanoparticles for enzymatic hydrolysis of wool fiber. Therefore, in spite of the slight modifications in the diffractograms, the applied procedure did not cause significant changes in the macromolecular composition of wool.

Scanning electron microscope analysis of wool (SEM)

The morphology of the wool surface samples, before and after the functionalization, shows that there are no significant differences or damage. This means that there was no degradation or significant changes on wool fibers, Figure 2.

Similar results were obtained in a study by Silva, Prabaharan, Gübitz, and Cavaco-Paulo (2005) in which the wool fiber was treated with subtilisin and subtilisin-PEG. In this study also there were no significant changes on wool surface after having been pre-treated with a nonionic surfactant and bleached.

Evaluation os antibacterial activity: Suspension test

The antibacterial effect of wool functionalized with AMPs for 1 h was tested according to the Japanese Industrial Standard JIS L Standard 1902:2002. The test effectiveness was assessed through growth values and determined by comparing bacterial growth between the control/non-functionalized wool immediately after inoculation of inoculum and the control/non-functionalized wool after 24 h incubation. When control samples were compared showed a growth of bacterial population from 2.43E + 05 to 5.33E + 08 for *S. aureus* and from 8.00E + 05 to 3.15E + 08 for *K. pneumoniae* (Table 5). These results validate and confirm the test effectiveness.

The assessment of the antimicrobial effect of the AMPs showed that Cecropin-B and [Ala5]-Tritrp7 exhibited very good results for growth inhibition of *S. aureus* and *K. pneumoniae* (Table 5). Also, all AMPs-functionalized samples presented bacteriostatic effect, as shown in Table 6.

Wool samples functionalized with Cecropin-B showed 71.67% reduction against *S. aureus* and 85.95% against *K. pneumoniae*, while wool samples functionalized with [Ala5]-Tritrp7 showed 66.74% reduction against *S. aureus* and 88.65% against *K. pneumoniae*. Therefore, it can be observed that low concentrations of AMP tested (10.00 µg/mL) are enough to promote a great antimicrobial inhibition rate (>50%) in both bacterial strains after 24 h.

Results also showed that all samples functionalized with AMPs at low concentration (10.00 µg/mL) exhibited bacteriostatic activity and no bactericidal activity against *S. aureus* and *Klebsiella pneumonia*, as showed in Table 6.

In comparison with other studies about wool, in which new multifunctional properties were achieved by

Figure 2. SEM images of non-functionalized wool and AMP-functionalized wool samples.

Table 5. Percentages of microbial reduction against *S. aureus* and *K. pneumoniae.*

		Staphylococcus aureus			*Klebsiella pneumoniae*	
Samples		CFU/ml	Growth reduction (%)		CFU/ml	Growth reduction (%)
Control/non-functionalized wool	0 h	2.43E + 05	–	0 h	8.00E + 05	–
	24 h	5.33E + 08	–	24 h	3.15E + 08	–
Cecropin-B_1 h		4.09E + 08	71.67%		4.43E + 07	85.95%
[Ala5]-Tritrp7_1 h		3.67E + 08	66.74%		3.58E + 07	88.65%

Table 6. Antibacterial activity values (bacteriostatic and bactericidal activity values).

	Staphylococcus aureus		*Klebsiella pneumoniae*	
Sample	$M_B - M_C$	$M_A - M_C$	$M_B - M_C$	$M_A - M_C$
Cecropin-B	0.12	−3.23	0.85	−1.74
[Ala5]-Tritrp7	0.16	−3.18	0.94	−1.65

the combination of silica and silver nanoparticles on its surface, verified a reduction percentage in growth of *E. coli* (Gram-negative) of 66.00 and 71.00%, for wool with silver and for wool with silica and silver, respectively (Tang et al., 2012). These percentages, compared with the obtained in this study to *K. pneumoniae* (85.95% to Cecropin-B and 88.65% for [Ala5]-Trirtp7) are relatively lower. This result allows to conclude that the incorporation of the Cecropin-B and [Ala5]-Tritrp7 influence positively the antimicrobial activity of the wool.

The reduction growth of *S. aureus* in this work was compared with the results obtained in a study by Wang et al. (2009) where lysozymes were immobilized in the wool. The values obtained in present research, 71.67% for Cecropin-B and 66.74% for [Ala5]-Tritrp7 showed to be relatively lower than the obtained by Wang et al. (2009) (80.95%). Thus, the antimicrobial effect that the Cecropin-B and [Ala5]-Tritrp7 cause in the wool fiber was successfully achieved.

It is also important to highlight that there was no color change in the wool fabric after the incorporation of the AMPs, therefore the present study overcome the problem of yellowing on textiles that occur when other antimicrobial agents are used.

Conclusion

In the present study, the selected peptides showed to be promising candidates for the functionalization of textile materials. The selection of peptides, Cecropin-B and [Ala5]-Tritrp7 was carefully done, paying particular attention to the molecular size and the properties thereof, taking into account their application to proteinaceous fibers. According to the results for the antimicrobial activity it was verified that Cecropin-B and [Ala5]-Tritrp7 exhibited high inhibition percentages against *S. aureus* and *Klebsiella pneumonia* (Cecropin-B 71.67% and [Ala5]-Tritrp7 66.74% against *S. aureus*; Cecropin-B 85.95% and [Ala5]-Tritrp7 88.65% against *Klebsiella pneumonia*). Furthermore, the macromolecular composition and morphology of the fibers was not influenced by the functionalization process, as it was seen by XRD spectra and SEM images.

The present work proposes the implementation of a new antimicrobial process and it is intended to add effective, durable and nontoxic bioactive properties to the wool fibers, which can be used in textiles and other polymeric proteinaceous materials for biomedical applications.

The efficacy of AMPs at low concentrations and the low risk of inducing bacterial resistance makes them suitable for therapeutic applications. AMPs may become the drugs of choice for emerging bacterial infections in the future.

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