

Nanobiotechnology: A new strategy to develop non-toxic antimicrobial textiles

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Consumers' attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of antimicrobial textiles, which in turn has stimulated intensive research and development. As a result, the number of biofunctional textiles with an antimicrobial activity has increased considerably over the last few years. In a near future, biomedical products will perhaps be the largest application of antimicrobial textiles since they find applications for prevention, as surgical lab coats, or therapy as wound dressings.

Several antimicrobial agents have been tested in textiles. They have powerful bactericidal activity as indicated by the MIC value. However, the majority has a reduced spectrum of microbial inhibition and may cause skin irritation, ecotoxicity and bacteria resistance. Moreover, the biocide can gradually lose activity during the use and launderings of the textile. In addition, wearing these textiles in a continuous manner can lead to sensitization and bacteria resistance. As a result, and to minimize such risks, there is a great demand for antimicrobial textiles based on non-toxic and ecofriendly agents.

Due to the relatively lower incidence of adverse reactions of natural products in comparison with synthetic pharmaceuticals, they can be exploited as an attractive ecofriendly alternative for textile applications.

Here, an innovative nanobiotechnological approach based on the application of natural defensive amino acids and peptides, as antimicrobial agents for textiles, is discussed. Moreover, peptide-based natural antimicrobial agents for the biofunctionalisation of textiles at nanoscale could not only find a sphere of influence in the wellness sector but the ambition is to use them as prophylaxis and therapy tools.

This paper reports a comprehensive short review on antimicrobial textiles as well as the present perspective and major challenges regarding the development of non-toxic and ecofriendly biomimetic solutions. As an example, a successful preliminary application is addressed.

Keywords defensive amino acids; antimicrobial peptides; antimicrobial textiles; wound-dressings

1. Antimicrobial textiles: a review

Textile industry continuously searches for new technologies in order to accomplish the consumers' demands. Especially in recent years, new developments allowed the production of functional and smart textiles which are capable of sensing changes in environmental conditions or body functions and responding to these changes. Likewise, consumers' attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of textile products finished with antimicrobial properties, which in turn has stimulated intensive research and development [1-3].

As a consequence, the number of biofunctional textiles with an antimicrobial activity has increased considerably over the last few years [2,4]. Application is nowadays extended to underwear, sportswear, home furnishing and protective clothing in areas with high risk of infection by pathogens (hospitals, schools and hotels) [4,5]; and because they are able to absorb substances from the skin and can release therapeutic compounds to the skin, they find applications for prevention, as surgical lab coats, or therapy, as wound dressings [1,6]. Thus, biomedical products will perhaps be the largest application of antimicrobial textiles [5,6].

1.1 Microorganisms and textiles

Textiles are an excellent substrate for bacterial growth and microbial proliferation under appropriate moisture, nutrients and temperature conditions [1-3]. In a clinical setting, they can be an important source of bacteria that may contaminate the patients and clinician personnel [1]. Bacteria and fungus, either pathogenic or not, are normally found on human skin, nasal cavities, and other areas, such as in the genital area.

Microbial shedding from our body contributes to microorganism spreading into a textile material either directly in clothes or on surrounding textiles. Recent studies strongly support that contamination of textiles in clinical settings may contribute to the dispersal of pathogens to the air which then settle down and infect the immediate and non-immediate environment. It is one of the most probably causes of hospital infections [1]. Typically, pathogenic microorganisms like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida albicans* have been found on textiles.

In addition, microorganism proliferation can cause malodours, stains and damage of mechanical properties of the component fibres that could cause a product to be less effective in its intended use. Additionally, may promote skin contamination, inflammation and in sensitive people, atopic dermatitis [7]. Fortunately, the use of antimicrobial textiles

may significantly reduce the risk of infections especially when they are used in close contact with the patients or in the immediate and non-immediate surroundings.

1.2 Antimicrobial agents for textiles

Several major classes of synthetic antimicrobial agents are used in the textile industry and recent research is focused on natural compounds. They are in use in other industries, as food preservatives, disinfectants, etc. Antimicrobial agents should possess broad spectrum biocidal properties, be safe for use and highly effective against antibiotic resistant microorganisms including those that are commonly involved in hospital-acquired infections. In addition, they should not permit the development of resistant microorganisms to the active compound or cause skin sensitization [1-3].

Antimicrobial agents are natural or synthetic compounds that inhibit the growth (bacteriostatic or fungistatic) because they can be protein, lipid synthesis or enzyme inhibitors, all of which are essential for cell survival; or kill (biocidal) the microorganisms by damage in the cell wall [8]. Almost all antimicrobial synthetic agents in use on textiles are biocides.

1.2.1 Synthetic compounds

Several antimicrobial agents have been tested in textiles: Quaternary ammonium compounds, silver, polyhexamethylene biguanides (PHMB) and triclosan even in an industrial scale. They have powerful bactericidal activity, as indicated by the MIC value, and also different application methods, effectiveness on fibres depending on chemical composition, and side-effects, as reported in Table 1. However, the majority have a reduce spectrum of microbial inhibition and may cause skin irritation, ecotoxicity and bacteria resistance. Moreover, the biocide can gradually lose activity during the use and launderings of the textile. Thus, great amounts of these biocides are applied to the textiles to control the bacterial growth efficiently and to keep its durability. In addition, despite the fact that synthetic antimicrobial agents used in textiles can be effective against a wide range of microorganisms, wearing these textiles in a continuous manner can lead to sensitization and bacteria resistance [2,3].

Table 1 - Some synthetic biocides used for the treatment of various textile fibres (adapted from Gao and Cranston [2] and Kramer et al. [5]).

Biocide	Fibre	MIC	Toxicity	Fibre interaction and side-effects
Silver	Polyester Polyamide Wool Regenerated cellulose	0.05-0.1 mg/L Against <i>E.coli</i>	Little to nontoxic	Slow release; durable but Ag can be depleted.
QACs	Cotton Polyester Polyamide Wool	10-100 mg /L Against Gram-positive and Gram-negative bacteria	Moderate to highly toxic	Covalent bonding; very durable; possible bacterial Resistance.
PHMB (Vantocil)	Cotton Polyester Polyamide	0.5–10 ppm against Gram-positive and Gram-negative bacteria	Moderate acute aquatic toxicity	Large amount needed; potential bacterial resistance.
Triclosan	Polyester Polyamide Cellulose acetate Acrylic fibre	Less than 10 ppm against Gram-positive and Gram-negative bacteria	Breaks down into toxic dioxin.	Large amount needed; bacterial resistance.
N-Halamines	Cotton Polyester Polyamide Wool	Na	Moderate to highly toxic	Needs regeneration; odour from residual chlorine.

Na – Not available.

1.2.2 Natural compounds

To minimize the above mentioned risks associated with the application of antimicrobial agents, there is a great demand for antimicrobial textiles based on non-toxic and ecofriendly bioactive compounds [9]. Due to the relatively lower incidence of adverse reactions of natural products in comparison with synthetic pharmaceuticals, they can be exploited as an attractive ecofriendly alternative for textile applications [4]. Although there are many natural antimicrobial agents,

the study on their use in textiles is very limited and not well documented, except for the case of chitosan, natural dyes and natural plant extracts which has been widely reported elsewhere [9,10].

Natural bioactive compounds have been widely reported as antimicrobial agents for textiles in a finishing setting [9]. However, commercial applications were not reported yet, except for the case of chitosan. Typically, chitosan and plant extracts are the most explored. Yet, there are several major challenges regarding extraction, isolation of the bioactive compounds, application and durability. Nevertheless, due to their ecofriendly nature and non-toxic properties they are still promising candidates as antimicrobial agents for textiles.

Chitosan is a deacetylated derivate of chitin, non toxic, resistant to microorganisms, biodegradable and biocompatible. The antimicrobial activity of chitosan is influenced by several factors such as the type of chitosan, the degree of deacetylation, molecular weight and other physical and chemical factors such as pH, ionic strength and addition of non-aqueous solvents [9,11]. Chitosan can be considered an antimicrobial agent for textile finishing. However, its application in textile materials is effective against a wide range of microorganisms only at high concentrations, which causes a decrease of the air permeability on fabrics and turns the fabric very inflexible. Another disadvantage is the low durability after application [9].

Sericin is a natural macromolecular protein derived from silkworm *Bombyx mori* which constitutes 25-30% of the silk protein. It is a biomolecule of great value since it has antibacterial properties, UV resistance, resists oxidation and has hydrating properties. It has several applications, such as moisturizing agent in shampoos and creams, and is also an important biomaterial for various applications including textiles. Although the application of sericin as an antibacterial agent for textiles has not been reported yet, it has been found evidence of such a potential application [9,12].

Neem (*Azadirachta indica*) is an evergreen tree of India, which belongs to the plant family *Meliaceae*. This is recognized as one of the most promising sources of compounds with antimicrobial and medicinal properties. The active ingredients of neem are found in all parts of tree. The extract of neem has been widely used in pesticide formulations that due to their pest repellent properties have the potential to inhibit the growth of Gram-positive and Gram-negative bacteria. At present, little has been reported of its use in textiles as an antimicrobial agent. Few studies concerning application of seed and bark extracts to cotton and cotton/polyester blends have been reported [9,13].

Aloe vera (*Aloe barbadensis*) belongs to the family *Liliaceae* and is known as "Lily of the Desert". Research has shown that Aloe leaf contains a large number and variety of nutrients and active compounds. Aloe vera also has antibacterial and antifungal properties that can be exploited in applications for medical textiles such as bandages, sutures, bioactive textiles, etc. [9]. Likewise to neem extracts, few studies have been reported and further investigation is needed.

Eucalyptus oil has amazing cleaning properties. It also has an effective effect against infections caused by bacteria, fungi and viruses. But application in textile substrates is still being explored [9].

Prickly chaff flower (*Achysanthus aspera*) is one of the herbs most commonly found in India. It presents antimicrobial activity against both Gram-negative and Gram-positive, however with a low activity [9]. It was tested in cotton fabrics but the results showed mild antibacterial activity against Gram-negative bacteria.

Clove oil (eugenol) is the main product of *Syzygium aromaticum*. The bioactivity of clove oil was explored as an agent for finishing of cotton fabrics [9] but still, further investigation has to be done.

Turmeric or curcumin, a fluorescent yellow pigment extracted from the rhizomes of several species, has been used as a dye for dyeing wool, silk and cotton. Because of its bioactive activity saffron also transmits antibacterial properties to textiles. The antimicrobial activity of plant extracts such as peppermint, primrose and perilla oil, has been also explored for applications in the textile industry [9].

In summary, despite some major challenges, natural antimicrobials are promising candidates as antimicrobial agents for textiles due to their ecofriendly nature and non-toxic properties.

1.3 Commercial antimicrobial fibres and finishes: a short review

Another common method for the incorporation of antimicrobial agents onto textiles is in the fabrication process of the fibre. In this method, the antimicrobial agents can be incorporated into the polymer prior to extrusion or blended into the fibres during their formation. In this way, several commercial antimicrobial fibres are now available in the market. Basically, the antimicrobial agents are the same synthetic agents previously described and the fibres assume different commercial names in a basis of the biocide used, type of fibre and manufacturer preferences.

Some commercial bioactive fibres with antimicrobial silver treatment and finishing products are: SeaCell® Active, a cellulose-base fibre; MicroFresh®, SoleFresh® and Guard-Yarn®, polyester or nylon yarns with AlphaSan®, a zirconium phosphate-based ceramic ion-exchange resin containing silver; Trevira Bioactive®: polyester fibre with silver incorporated prior to the extrusion process; SmartSilver®, wool fibres with silver added by typical exhausting dyeing methods and other finishing silver-based products like SmartSilver™, Silpure®, Sanitized®, AlphaSan® and Ultra-Fresh. Cotton fibres are also being commercialised under a pre-treatment with Reputex® (PHMB attached to cotton) and more recently, polyamide with PHMB is sold as Purista®. Moreover, polyamide and polyester fibres treated with Tinosan AM 100®, cellulose acetate yarns named Silfresh®, Microban® textile and Irgaguard® and Irgacare® products, all contain triclosan as antimicrobial agent.

More recently, natural resources have been used for the development of a composite fibre of chitosan and viscose with durable antimicrobial activity (Crabyon®). Like in finishing processes, fibre production sector is investigating natural compounds as a main source of antimicrobial agents.

1.4 Antimicrobial activity tests

There are several standard methods to assess antimicrobial activity on textile materials because there is not a unique test that is suitable for all the sorts of the antibacterial fibres and microorganisms. These standards are classified on the basis of the kind of the evaluation of microorganism inhibition: qualitative and quantitative. Qualitative methods include AATCC 147:2004, ISO 20645:2004, SN 195920:1992, and JIS L 1902:2002 – Halo method) for antibacterial assessment, and AATCC 30:2004, SN 195921:1992 and JIS Z 2911-1992 for antifungal assessment. Quantitative methods include AATCC 100:1999, JIS L 1902:2002– Absorption method and ISO 20743:2007 [14]. These are the most used standard methods, among others.

The qualitative methods are easy, fast and useful when a large number of samples have to be screened. The test specimen and an untreated control are placed into contact with nutrient agar plates containing the bacterial cells of Gram-positive and Gram negative bacteria [2]. These qualitative methods evaluate the bacterial activity by the halo formation (absence of bacteria growth around the edges of the test specimen). They also provide a formula to measure the inhibition zone width even though it cannot be considered as a quantitative indication of the antibacterial activity because the colonies are not counted. In addition, the halo size only provides some indication of antimicrobial activity against the tested strains.

In contrary, quantitative methods provide values of antimicrobial activity based on the reduction of microorganism population [2,14] e.g. based on the number of bacteria still living after incubation with the bioactive specimen. However, they are more time consuming than the qualitative methods and a greater amount of test specimens is required.

These methods are of utmost importance to assess antimicrobial properties of textiles but strongly depend on the mechanism of action of the antibacterial agent, with or without migration effect, and on the hydrophobic/ hydrophilic character of the bioactive material. Thus they should be selected according to these considerations.

2. Nanobiotechnology: Present perspective and major challenges

To overcome the risks, disadvantages and side effects associated with the use of synthetic antimicrobial agents along with the mild activity and durability associated with the above described natural compounds, new solutions are expected. In this way, an innovative strategy could be the utilisation of natural defensive amino acids and peptides that are found in every living organism as new biocides for material functionalisation.

2.1 Antimicrobial amino acids and peptides

Due to the widespread resistance of bacteria to the available drugs, naturally occurring antimicrobial peptides (AMPs) are considered promising candidates for future therapeutic use [5,15-19]. Virtually, all life forms express cationic AMPs as an important component of their innate immune defenses. AMPs isolated from bacteria, fungi, plants, invertebrates and vertebrates are very heterogeneous in length, sequence and structure, but most of them are small, cationic and amphipathic.

AMPs fall into 4 principal categories based on their size, conformational structure, or predominant amino acid structure [20]. These comprise group I: linear, α -helical peptides without cysteine (e.g., Cecropin, a family of 3-4 kDa linear amphipathic peptides); group II: cysteine-rich open-ended peptides containing single or several disulfide bridges (e.g., β -defensins a highly complex group of 4-kDa); group III: linear peptides rich in 1 or more amino acids (The smallest is represented by thanatin and brevinin); and group IV: peptides with sphere structures (e.g., bactenecin and ranalexin).

The cationic peptides represent the majority of antimicrobial peptides already registered, and there are more than a thousand peptides with antimicrobial activity characterized [21,22]. These peptides belong predominately to the three main classes above described: Group I, II and III.

Anionic peptides are a group of much smaller dimensions, only recently identified mainly in mammals. These peptides are small, hydrophilic and contain specific regions that confer a negative charge. Zinc can be used as a cofactor in anionic peptides to enable them to overcome the negative charge on the surface of microorganisms, thus obtaining a maximum bactericidal activity [20]. Subfamilies of these peptides can quote: neuropeptide derived molecules, aspartic acid-rich molecules, aromatic dipeptides and proteins derived from hemocyanin [19, 20].

These cationic and anionic peptides exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria, yeasts, fungi and enveloped viruses [16,17,19]. Consequently, considerable efforts have been expended to exploit the therapeutic potential of AMPs, especially regarding the pharmaceutical industry [18-22]. Moreover, because of the membrane-disturbing mode of action of most AMPs, there is a reduced likelihood of the acquisition of resistance by bacteria [20].

2.1.1 The basis of peptide activity, specificity and mode of action

The relationships between peptide structure and antimicrobial activity have been widely described [15-22], but little is known about the molecular basis of the marked differences in peptide activity and specificity [22].

According to Brogden [22] the susceptibility of a single microorganism to a panel of antimicrobial peptides depends on the size, the sequence, the degree of structuring, the charge, the overall hydrophobicity, the amphipathicity and the respective widths of the hydrophobic and hydrophilic faces of the helix. These characteristics of peptides are all considered important and their alteration, even if of one single parameter, can result in significant changes in the others. As a consequent, alteration of peptides antimicrobial activity can also occur.

Likewise, the composition of the microbial surface and cytoplasmic membrane is equally important as indicated by the differences in the susceptibility of several different microorganisms to a single peptide [22].

In summary, structural differences of peptides, differences among bacterial surfaces and cytoplasmic membranes are just a few of the variables that determine the extent of antimicrobial activity and specificity. Therefore, designing antimicrobial peptides of more efficient and with a broad-spectrum require targets other than just membranes [22].

Different modes of action can be proposed for AMPs: destabilisation of the cell membrane of the organism, inhibition of protein synthesis, arrest of DNA synthesis, DNA strand breakage and production of hydrogen peroxide that can trigger autolysis (bacteria) or apoptosis (eukaryotic organisms) of the cell [16].

One of the general mechanisms of action proposed for cationic AMPs involves the following steps: electrostatic bond between the outer surface of the negatively charged microorganism and an antimicrobial peptide positively charged forming the helix structure and insertion of the peptide in the membrane and, finally, aggregation of several helices to form a pore. For this process to occur micromolar concentrations of AMPs are required and four or more peptides are needed to form pores in order to obtain actual cell death [21,22]. This mechanism or other additional modes of action such as induction of hydrolases that degrade cell membranes, disturbance of membrane functions, or damage of key intracellular structures, allow that AMPs are effective against some species of bacteria that create resistance to most common antibiotics [16,21,22].

2.1.2 The potential for material functionalisation

Our earlier studies revealed the success of biofunctionalisation of wool and polyamide fibres with L-Cysteine (L-Cys) (patent pending). A durable antimicrobial effect over *Staphylococcus aureus* and *Klebsiella pneumoniae* was obtained without cytotoxicity. Apparently, the effect results from the interaction of free sulphhydryl groups of L-Cys with the sulphhydryl groups from the proteins and enzymes that are essential to the survival of microorganisms, as discussed by Kyung and Lee in studies with tiosulfates from garlic [23].

In addition, due to the widespread resistance of bacteria to the available drugs, naturally occurring antimicrobial peptides (AMPs) are considered promising candidates for future therapeutic use. Furthermore, the peptides should have a broad spectrum against Gram-positive bacteria, Gram-negative bacteria and fungi.

Few studies have exploited the immobilization of AMPs in several films through a covalent attachment and by oppositely charged polypeptide to make 10-bilayer polyelectrolyte films by electrostatic layer-by-layer self-assembly (LbL), with success. In both cases, several advantages are related including long-term stability and lower toxicity of the AMPs when compared to the incorporation into release-based systems [24,25]. Bagheri et al. concluded that among the various methods of immobilization of AMPs, covalent attachment of two highly active α -helical peptides (that rendered the different substrates (resins) antimicrobial properties) offers several advantages, including long-term stability and lower toxicity of the AMPs compared to incorporation into release-based systems [24]. In another work, Zhong et al. used oppositely charged polypeptide to make 10-bilayer polyelectrolyte multilayer films by electrostatic layer-by-layer self-assembly (LbL), with success. Cysteine was included in the peptides to promote disulfide bond formation and film stability [25].

In general, cationic AMPs with best antimicrobial activity are molecules that have the charged and hydrophilic portions separated from the hydrophobic areas. This means that either amphipathic or cationic double-wing structures with a hydrophobic core separating two charged segments are preferred [23]. In accordance to this, Cathelicidin peptides range in length from 12 to 80 residues, and may have α -helical, β -sheet or other types of tertiary structures [22,24]. In contrast, defensins are more uniform in their appearance. They are small cysteine-rich AMPs that mainly form β -sheet structures stabilized by three or (rarely) four conserved intramolecular cystine disulphide bridges [21,22,24].

Hence, AMPs which can both be attached to polymers or form films are expected to bind several polymer-based textiles by exhaustion or LbL assembly. Likewise, our previous results with L-Cys give a guarantee of success for these new antimicrobial textiles because peptides can be attached to textiles in a similar way which is further described below.

Therefore, to obtain effective antimicrobial textiles the peptides should be selected attending the above considerations in order to give a starting point for the biofunctionalisation, since no references can be used from literature, regarding the functionalisation of textiles. In this way, linear and non-linear structures, opposite charges for LbL self-assembly methods, small and moderate molecular weight to be applied by well-known exhaustion processes,

and peptides with/without cysteine, for possible crosslinking are, in author's opinion, promising candidates. However much has to be studied before starting any attempt to apply these peptides since, unfortunately, they can be rather expensive. In addition, this new approach should be considered for special applications as for example new textile-based antimicrobial wound-dressings and other bioactive medical textiles, due to economic reasons.

Therefore, L-Cys and AMPs used as natural antimicrobial agents for textiles could not only find a sphere of influence in the wellness sector but the ambition is to use them as prophylaxis and therapy tools.

Consequently, biofunctionalisation of textile-based materials with defensive amino acids and peptides at nanoscale is an innovative approach and may open new avenues for the design of medical and healthcare textiles. To author's knowledge, this is a new strategy and approach aiming to mimetize nature through nanobiotechnological tools to give "protective skin" to textiles.

3. Nanobiotechnology: An application study on wool fibres

This investigation reports a short resume of a new biotechnological process (patent, final registration pending) that uses L-Cysteine (L-Cys) which provides a permanent, non-toxic and effective antimicrobial effect over wool-based materials.

The antimicrobial activity of the bioactive wool was assessed by the international standard JIS 1902:2002 against *Staphylococcus aureus* and *Klebsiella pneumoniae*., and the confirmation of L-Cys immobilization on wool substrates was assessed by the Ellman's reagent (5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB method).

3.1 Nanobiofunctionalisation of wool

Fresh solutions of L-Cys (Sigma-Aldrich) were prepared in a concentration range of 0.5% to 12% (owf; over the weight of the fabric) in 25 mM acetate buffer solution pH 4.8. Functionalisation of the wool material was performed by an exhaustion method which promotes the reaction of L-Cys with the wool using a liquor ratio of 50/1 (L/kg). In this way, the wool fabrics were incubated for 50 minutes at 60 °C. These conditions were selected in order to preserve both the quality of wool and to ensure the reduced form of L-Cys aiming to endorse an increase in the number of free sulfhydryl groups on wool in order to give the desired antimicrobial properties. In the end, several rinses were made followed by a soaping procedure at 40°C (in accordance with the recommendations of the Standard NP EN ISO 105-C06). The soaping procedure was performed over the samples treated with L-Cys prior to the assessment of antibacterial activity in order to give evidence of a durable biofunctionalisation effect.

3.2 Assessment of antibacterial activity and cytotoxicity

The evaluation of the antibacterial activity of the functionalised samples was carried out according to the Japanese Industrial Standard JIS L 1902:2002, widely used for the antibacterial assessment of textiles. In addition to qualitative method, quantitative tests were also used to determine more accurately the percentage of microbial growth inhibition.

In order to evaluate the release of toxic substances from a textile product with skin contact, a perspiration extract of the test material at high L-Cys concentration (6% owf) under which total inhibition of bacterial growth was achieved, was produced according to E DIN EN ISO 10993-5.

3.3 Results and discussion

The increase in colour intensity on the fabrics (DTNB method), measured on a spectrophotometer at 412 nm, clearly point out the presence of a greater number of free sulfhydryl groups in the biofunctionalised wool for all L-Cys concentrations tested, indicating that the sulfhydryl groups are on their free form once immobilized on the wool material. In addition, the exhaustion rates were more or less the same for all the concentrations used highlighting a good diffusion process of L-Cys whichever the concentration in the range used (data not shown).

The results of the quantitative antimicrobial tests show evidence of 40% inhibition for L-Cys concentrations of 1% and a total inhibition (more than 99%) above 6%. In addition, the MIC₅₀ is obtained in samples with 5% of L-Cys, approximately.

The antimicrobial effect that L-Cys provides to the wool is in accordance with the results found out for the antimicrobial activity of tiossilfinates claiming that the bioactive effect results from the interaction of sulphur groups present in tiossilfinates with the sulfhydryl groups of the proteins and enzymes that are essential to the survival of microorganisms, leading to growth inhibition and death [23]. Moreover, it become visible that L-Cys doesn't possess migration properties once immobilized onto the wool fibres, as Figure 1 shows. It also appear that these tiolated molecules have a high structural diversity and a broad spectrum of activity that includes Gram-positive and Gram-negative bacteria, fungi, and in particular cases, some viruses with the added advantage of not displaying cytotoxicity to mammalian cells, as described elsewhere [26,27].

The perspiration extract of the test material (biofunctionalised wool with 6 % of L-Cys owf) showed a growth inhibition of 25 % (<30%) of the connective tissue cells L 929. In accordance to this, it can be concluded that no

cytotoxic substances are released during the assigned use of these test materials, which can lead to irritations with skin contact.

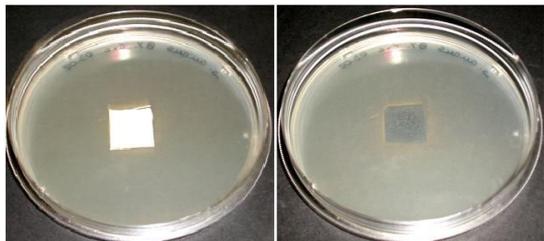


Fig. 1 Antimicrobial activity of the test specimens (6% L-Cys owf) assessed against *S. aureus* by the qualitative method. Left image shows the absence of a halo while right side image show evidence of inhibition under the test specimen.

4. Conclusions

There is a measureless resource of natural antimicrobial peptides which can be exploited for imparting antimicrobial properties to textile substrates. The main advantage of antimicrobial peptides is that they are small molecules that can be impregnated or covalently bound to textiles in a very effective and homogeneous deposition together with a low toxicity. Furthermore, surface immobilization seems to provide a powerful strategy to get information on the mode of peptide action.

The report on the case study on wool describes a novel method to give antibacterial property to wool fibres using a non-toxic and biodegradable agent - L-Cysteine, a natural compound never studied before as a potential bioactive agent for textiles which can grant antibacterial properties without cytotoxicity. The major advantages of this method in comparison with others that are commonly used are the non-toxicity both to the potential users and to the environment, the high bioavailability once immobilized on wool, and the durability. In addition, because L-Cysteine is part of several living organisms it is not expected to cause bacterial resistance. This is totally new and open promising perspectives for the functionalisation of polymeric materials with AMPs which can have an effective antimicrobial activity against a broad spectrum of microorganism.

Therefore, Nanobiotechnology e.g. nanoscale biofunctionalisation with biomolecules like AMPs can be a new and very promising strategy to develop non-toxic antimicrobial textiles that will be able to open new avenues for the biomimetic design of polymeric materials for special applications in the medical field.

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