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Technological Opportunities for the Implementation of Anti-Microbial Activities into Dry-Wet Shaped Cellulose Fibres

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Summary: Metal cations are valuable antimicrobial additives for controlling of bacteria growth on textile fibres. By means of multiple actions they are able to suppress the undesirable microbe action also during wound healing. Dry-wet shaping technology offers numerous opportunities for implementation of antimicrobial activities into textile structures (fibres, yarn, fabrics). The degree of load, insertion step as well as type of additive (Ag, Cu, Zn) presents a broad field of physical modification of dry-wet spun cellulose fibres. The investigation of antimicrobial activities of manufactured fibres and yarns exhibit bactericide or bacteriostatic effects against typical pathogenic germs. Ag contents higher than 30 ppm in fibres and 0.06 per cent in yarns as well as Cu contents higher than 100 ppm in fibres and about 0.5 per cent in yarns are fairly effective for a permanent antimicrobial effect. Fabrics based on the developed fibres and yarns could already been successfully commercialised by innovative small and medium sized enterprises (SME).

Keywords: additives; biocompatibility; cellulose; fibres; modification; polysaccharides

Introduction

Today, society is faced worldwide with about 13 million people suffering from chronic wounds such as ulcus cruris, decubitus or diabetic foot syndrome.[1] As people exhibit an increasing life expectancy because of improved living standards the numbers of patients with chronic wounds increase year by year.

Annual costs for chronic wound healing therapies are worldwide on the level of about 10 billion Euros per annum.[2]

Successful healing therapies may consume 6 month up to 6 years. One reason for that might be that the physiology of wound healing is not fully understood until today. An other reason is that wound healing will be blocked by a quantity of bacteria higher than 10^5 colony forming units (CFU).[3] In addition most of colonised wounds are affected in minimum by two different kinds of bacteria - movable plankton bacteria or sessile, bio-film forming bacteria. Both of the different bacteria types respond variably to antimicrobial concentrations. Whereas bacteria in a plankton form could be very easily affected by antimicrobial agents in concentration lower than 50 ppm.[4] Antimicrobial agents at higher concentrations (> 50 ppm) or even multiple doses will be necessary for the destabilisation of bio-film forming bacteria as shown in in-vitro examinations by Chaw et al.[5]

Important for the degree of antimicrobial activity is a sufficient interaction between antimicrobial agents and microorganisms inside the wound.[6] Effective antimicrobials might be metal cations, liberated from the pure metals or metal salts by means of liquids (wound exudate, humidity) or oxygen.[7]

The paper will concern with the most favourable mechanism for prevention as well as treatment of infected wounds - the inhibition or control of bacterial growth -,

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which can be reached easily by the action of submicron scaled silver particles, silver, copper or zinc ions or even metal oxide particles (Ag_2O, ZnO).

More pronounced, the paper will describe several opportunities for integration of antimicrobial activities inside a textile matrix. Beside the implementation of antimicrobial agents inside a fibre during fibres spinning, fibres finishing or textiles aftertreatment seem to be opportune for this purpose.

Methodology

Materials

The investigations had been carried out using a commercial spruce sulphite pulp with a DP of 465 (Domsjö AB). The alpha content was higher than 92 per cent. Before using in dissolution trials pulp sheets were purified and disintegrated by dispersing in water (1:20, w/w) using an Ultra-Turrax® shearing tool for 20 minutes. Thereafter cellulose pulp was filtered out of the excess water and was used without any further pretreatment.

Aqueous N-Methylmorpholine-N-Oxide (NMMO, delivered by BASF) was in technical grade and was used without any further purification.

Methods

Two different methods were used for the manufacturing of antimicrobial acting textile fibres.

Method 1[8]

15 weight per cent (regarding to the used amount of cellulose) of a powdered weakly cross-linked cation exchanger resin ($D_{99} \leq 10$ μm) based on a copolymerisate of acrylic acid and sodium acrylate was added to a cellulose solution containing 12 weight per cent dissolving pulp (DP = 465) dissolved in N-methylmorpholine-N-oxide monohydrate.

The pre-dope was homogenised in a keading machine at 90 °C and extruded through a spinning nozzle containing 480 holes with a single hole diameter of 80 μm. The filament bundle had been taken-up with a velocity of 30 metres per minute. The filaments were completely eluted from the solvent passing several washing bathes, cutted into fibres with a staple length of 38 mm and centrifuged. The resulted staple fibres were treated by 10 litres per kg of a 0.1 molar aqueous silver nitrate solution. Finally the treated fibres were centrifuged and completely eluted from the adherent silver nitrate and dried at 80 °C. Table 1 shows the fibres parameters as well as the silver content per kg of the fibres.

Method 2[9]

Powdered zinc compound (ZnO, $D_{99} \leq 15$ μm) was directly added to a cellulose solution containing 12 weight per cent dissolving pulp (DP = 465) dissolved in N-methylmorpholine-N-oxide monohydrate (NMMO). The pre-dope was homogenised in a keading machine at 90 °C and extruded through a spinning nozzle containing 480 holes with a single hole diameter of 80 μm. The filament bundle had been taken-up with a velocity of 30 metres per minute. The filaments were completely eluted from the solvent passing several washing bathes, cutted into fibres with a staple length of 38 mm, centrifuged and dried at 80 °C. Table 2 shows the fibres parameters.

Fibres produced according method 1 or method 2 were spun in combination with cotton to textile yarns with a yarn count of Nm 68/1 containing about 5 per cent (method 1) and 10 percent (method 2) of functional cellulose fibres. With these yarns hoses were knitted and examined on theirs bactericide effects.

Table 1.
Textile-physical characteristics of manufactured Lyocell staple fibres modified by IE resin adsorbed silver salts.

fineness	dtex	0.7
tenacity	cN/tex	22.5
elongation	%	14.8
loop tenacity	cN/tex	7.5
silver content	g/kg fibre	80.0

Table 2.
Textile-physical characteristics of manufactured Lyocell staple fibres modified by direct incorporation of Zn compounds

fineness	dtex	0.5
tenacity	cN/tex	31.2
elongation	%	14.2
loop tenacity	cN/tex	9.1
zinc content	g/kg fibre	160

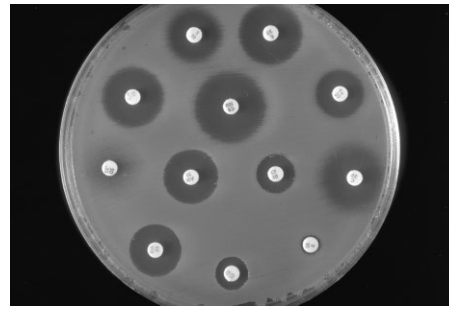

Figure 1.
Results of an Inhibition Areola Test using different kind of antimicrobials.[17]

Antimicrobial Evaluation

Basically microbiological studies for the assessment of an antimicrobial behaviour of topical acting materials include specific in-vitro assays for the determination of the qualitative and quantitative antimicrobial properties of the products made of them.

Log_{10}-Assay

The so-called *log_{10} -Assay* provides a quantitative estimation of the antimicrobial power of antimicrobial agents against defined test germs and defined concentration of bacteria over a fixed period. Primarily the *log_{10} -Assay* measures the rate of bacteria killing. The results are indicated as $\log_{10}$-reduction values according to equation (1) regarding to a non-active sample.

$$\Delta\log_{10} = \log_{10}(\text{initial bacteria quantity}) - \log_{10}(\text{final bacteria quantity}) \quad (1)$$

In the investigations carried out for this paper a modified $\log_{10}$-assay has been applied according to JIS L 1902:2002 ''Testing for antibacterial activity and efficacy on textile products''.[16] For the testing of antimicrobial activity *Klebsiella pneumoniae* and *Staphylococcus areus* were used as test germs at a concentration of about 6.6 x 10^5 CFU per millilitre. After incubation at 37 °C for 24 hours the numbers of remained bacteria are counted out manually.

Inhibiting Areola Test (IAT)

A more qualitative estimation is given by the so-called IAT. It measures the antimicrobial activity as size of an inhibition zone around a bioactive sample over a period of 24 hours against a defined bacteria species (see Figure 1).

In those cases where a formation of an inhibition zone could be detected the sample is considered as antimicrobial active against the bacterial species tested. The IAT could be carried out in more stringent manner for the activity against one test germ by usage of a new slime mould after each 24 hours.

Nephelometric Analysis

Nephelometry is an optical testing procedure for a determination of the scattering intensity of finely dispersed, colloidal particles (Nephelometric Turbidity Unit – NTU) inside a liquid or gas.[18] While nephelometric analysis determines the changes of lateral scattered light, the turbidimetry measures the particle adsorption.

For the evaluation of antimicrobial materials nephelometric analysis is used as qualitative test method for the determination of that concentration limit of a bioactive component, which is needed for bacterial growth prevention.

In the investigations carried out for this paper a modified Lyocell fibre containing 50 g silver per kilogram fibre and 20 g copper per kilogram fibre, respectively. 1 gram of such a fibre had been extracted by 50 ml of sterile deionised water. The resulted solutions are used in 1:2 dilution series. For that purpose one part of

solutions are used as prepared and the others are contaminated by S. aureus and K. pneumoniae spores dispersion, respectively and incubated at 37 °C for 24 hours. Inoculated and pure samples are investigated every hour by means of a Nephelometer. Determined turbidity was plotted against time of incubation.

Results and Discussion

Mode of Impact

Application of metals ions as antibacterial agents is well known. Silver as the most famous antimicrobial agent exhibits an impressive history going back to ancient Greece and Roman Empire. Usage of silver containing materials against infects markedly decreased after invention of antibiotics at the beginning of the 40s of the last century. But the frequently emergence of multi resistance microbes like MRSA or VRE, an increasing number of nosocomial infections nowadays and its negligible allergenic potential as well as essential mobility led to reuse of silver for inhibition of bacterial growth and transfer.

Regarding to the degree of bacteria damage between three different mechanisms of antimicrobial action might be distinguished:

- inhibition of bacterial growth, which is named as bacteriostatic action
- the killing of bacteria, named as bactericide action, caused by antibiotics like penicillin or others and
- bacteriolytic, where the bacteria cell walls will be disintegrated.

The bacteriostatic action of metal cations, liberated only in very small, but well defined amounts from submicron metal powders, metal salts or metal oxides in functional textiles, is the most favoured strategy for a bacteria growth inhibition because of its low influence on bacterial skin flora.

Liberated metal cations are highly efficient vehicles, because of their multiple abilities for interaction with bacteria, proteins and physiological anions (see Figure 2).

So for instance, the cell wall structure might be changed by an interaction with metal cations, whereby vitally important exchange functions aren't any longer available.[10] Otherwise metabolic transportation through the cell membrane will be disrupted, caused by the formation of insoluble compositions between metal cations and membrane anions or by the interaction of metal cations with vitally important proteins or enzymes.

Finally, bacterial cell replication will be blocked by complexation of metal cations with DNA and destabilisation DNA double helix. Based on those multimodal activities silver, copper and zinc cations are highly efficient antimicrobials exhibiting a broad angle of impact with only a small risk for development of a bacterial resistance.[11–14]

In contrast on this, antibiotics normally exhibit only a single mode of action against bacteria, which could be relatively simple overcome and transferred from one bacteria population to another.

Mode of Implementation into Textiles

Common antimicrobial textiles should possess specific product requirements, like for instance[10]:

- a lasting antimicrobial effect
- a constant release profile of its active agents
- an effective adsorption of wound exudates in wound exudation phase
- the ability to establish a wet wound climate
- safety barrier properties against bacterial germs, that means it should adhere or adsorb bacteria from inside the wound and should protect against a bacterial contamination from outside the wound and
- finally, it should be non traumatic removable.

Based on these requirements, three basic techniques for implementation of

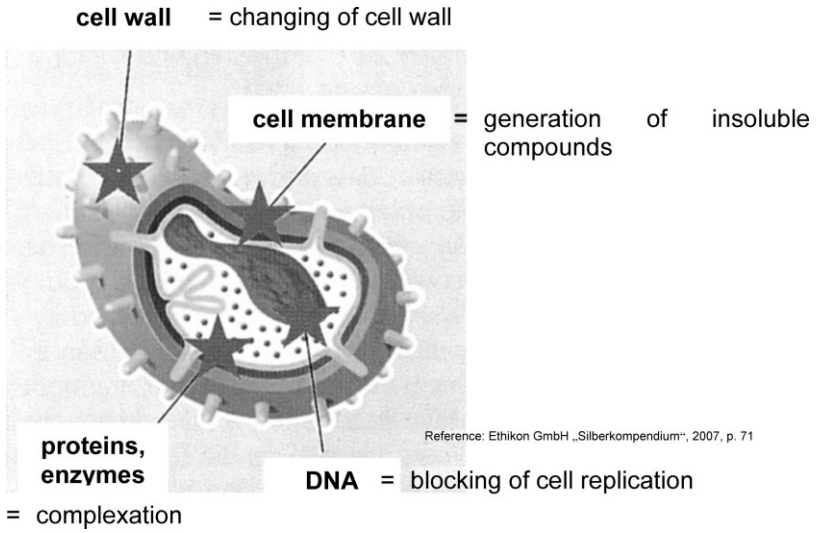

Figure 2.
Pathways of antimicrobial action of metal (cation)s.[15]

antimicrobial activities on or into a textile fabric seem to be possible in principle from technological standpoint of view.

First, it would be possible to incorporate such functionality into a textile fibres matrix by direct implementation of submicron scaled particles of antimicrobials like suitable metals, metal salts or metal oxides, by incorporation of powdered micron sized IE resin particles coupled with subsequent charging of metal cations and by incorporation of encapsulated antimicrobials or heat and chemical insensitive antibiotics.

Second, antimicrobial agents (particles, capsules) might be coated onto the surface of fibres or yarns by means of suitable crosslinkers while running a fibre finishing process.

Finally antimicrobials might also be covered onto the surface of knitted or weaved fabrics by means of impregnation, coating or lamination of antimicrobials dispersions or capsules into the textile structure.

Thanks to its relatively low changes in textile properties the incorporation of antimicrobial substances into a man-made cellulose fibre matrix is the most favourable strategy.

In the case of regenerated cellulose fibres the direct dissolution process as well as the dry-wet-shaping offers a huge field of optional process parameter for manufacturing of functional fibres. Besides the changing of fibres morphology especially the physical and/or chemical functionalisation provide suitable opportunities (see Figure 3).

Running a physical modification procedure of textile cellulose fibres, a lot of several options would be opened up.

The different additives, in the form of submicron metals, metal salts adsorbed on IE resins or metal oxide, will be the carrier of shape functionality and might be incorporated heterogeneously into the spinning dope before or after cellulose dissolution step, in low, moderate or high concentration. Regarding to the used amount of cellulose up to 200 weight per cent of functional additives could be introduced in Lyocell type fibres.

Alongside, the broad field of chemical modifications, which will be carried out comfortably in homogeneous phase, open-up a second area of prospects for changing the fibres functionalisation.

The spinning scheme for the manufacturing of physical modified bioactive fibres is shown in Figure 4.

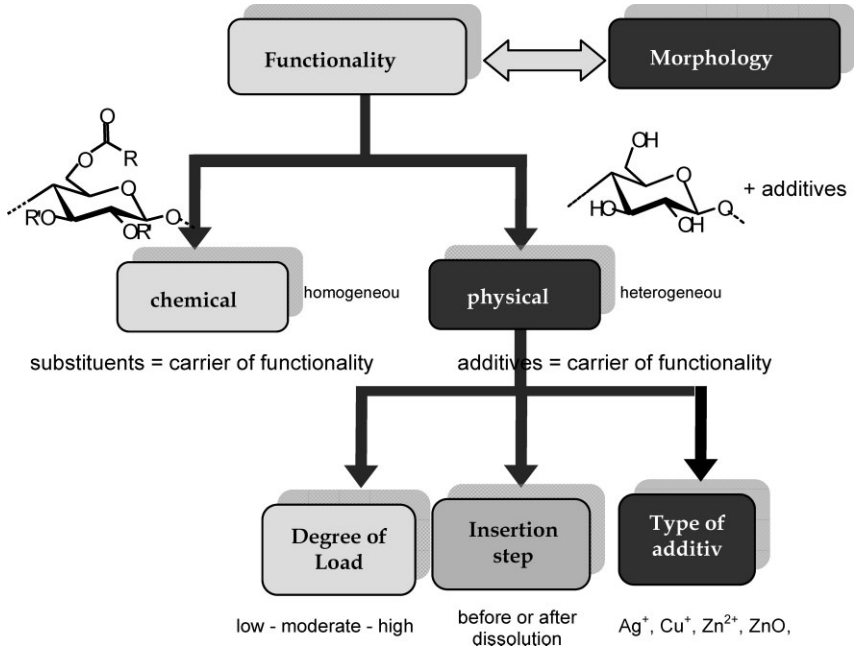

Figure 3.
Technological opportunities for fibres functionalization.

The spinning scheme demonstrates the two different options for the incorporation of antimicrobials into the fibre matrix. In that case, where functional additives are compatible with heat and chemical sensitive solvent used, additives could be implemented already while dope preparation step. For all those additives, which are affecting the solvent or cellulose decomposition (especially Ag^{+} and Cu^{+}), they have to be incorporated into the cellulose fibre matrix by means of an aftertreatment step using IE resins as adsorbing particles inside the fibrous shapes. By both pathways antimicrobial active cellulose fibres become available, containing the different additives

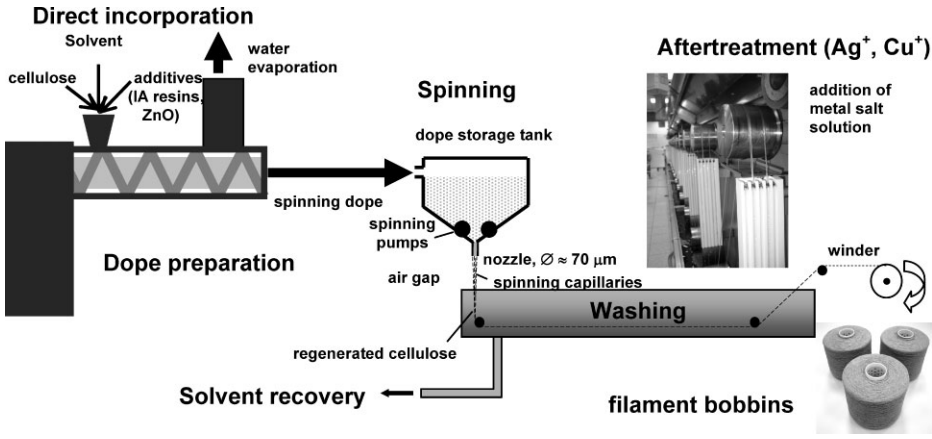

Figure 4.
Spinning scheme for manufacturing of antimicrobial cellulose fibres.

in a needed concentration for a permanent activity over the whole lifecycle of functional textile fabrics.

Measurement of Antimicrobial Effectiveness

In a first approach antimicrobial effectiveness should be evaluated by means of quantification of action of metal compounds like different kind of metal cations (Ag^+, Cu^+, Zn^{2+}) adsorbed on incorporated ion exchange resins and incorporated metal oxide (ZnO), respectively. The needed amount of bioactive metal cations for a secured antimicrobial action should be determined in a second test. Furthermore the effect of textile processing using sufficient amounts of bioactive agent had to be demonstrated.

For that purpose JIS 1902:2002 for quantitative evaluation and nephelometric analysis for qualitative evaluation had been used. From the test results should be expected practical experiences for the antimicrobial permanence of structures while textile application as for work clothes, underwear or even wound dressing.

In Table 3 the type and content of the bioactive agents of antimicrobial textile structures used in these examinations are listed. As reference specimen common polyester staple fibres fleece, a Tencel® staple fibres fleece and a Lyocell staple fibres fleece containing 15% of IE resin were used, respectively.

For examinations of biofunctionality samples had been inoculated at the beginning with culture medium containing about 6.6 x 10^5 CFU per millilitre. *Klebsiella pneumoniae* as a member of gram-negative bacteria and *Staphylococcus areus* as a member of gram-positive bacteria were selected. as test germs. Both are well known for their resistance across to antibiotics and are often the source of nosocomial infections inside hospitals.[19]

The obtained results of biofunctionality testing are summarised in Figure 5 (test germ: Staphylococcus areus) and 6 (test germ: Klebsiella pneumoniae). All of the samples modified by metal cations or metal oxide exhibit a strong antimicrobial activity. Depending on the type of additive the activity decreases in the order Ag^+ (LC-S) > Cu^+ (LC-K) C > Zn^{2+} (LC-ZIA).

Because of this well known fact, the amount of incorporated additive had to been increased from silver to zinc. It couldn't detect any difference between antimicrobial effect neither of Zn^{2+} adsorbed at incorporated IE resin (LC-ZIA) nor of Zn^{2+} liberated from incorporated zinc oxide (LC-Z).

As one may recognise, already commercial non-modified Tencel® fibres manufactured by Lenzing AG surprisingly exhibit a strong bacteriocidic activity against Klebsiella pneunomiae, but only a weak bacteriostatic activity against Staphylococcus areus. The reason for that anomalous bioactivity might be the usage of specific

Table 3.
Contents of antimicrobials of physically modified Lyocell staple fibres fleeces.

Sample name	Additive type	Content [weight %]
Polyester	–	–
LC/T	–	–
LC/IA	IE resin	15[2]/–
LC/S	IE resin/silver cation	15[2]/0.03[3]
LC/K	IE resin/copper cation	15[2]/0.1[3]
LC/IAZ	IE resin/zinc cation	15[2]/2[3]
LC/Z	zinc compound	2[2]
LC/GZ[1]	zinc compound	0.02[3]

[1]weaved fabric manufactured of 88% modal staple fibres, 10% zinc compound modified Lyocell fibres and 2% spandex fibres (elasthane)
[2]regarding to cellulose content used
[3]g/kg fibre

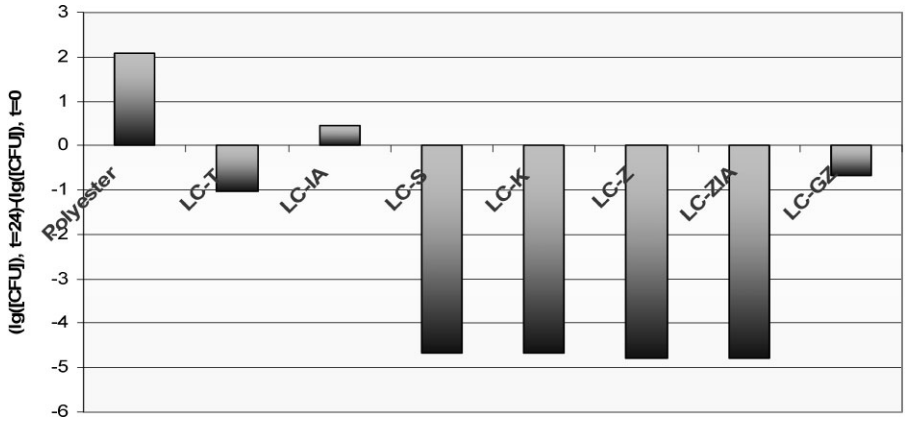

Figure 5.
Bioactivity of different textile structures against Staphylococcus areus ([CFU] at $t = 0$: $(6.6 \pm 1.8)10^5\ ml^{-1}$).

preparation liquids while fibres finishing. The also examined polyester and IE resin modified Lyocell textiles show a slight bacterial growth under the incubation conditions used. Investigation of the antimicrobial permanence and a determination of the threshold value for a growth control had been carried out by means of a nephelometric analysis.

For that purpose extracts from IE-fibres containing adsorbed silver and copper cations, respectively, were examined for their ability to inhibit a bacterial growth inside a cultural medium (see Figure 7).

As it is deducible from the JIS 1902:2002 test, K. pneumoniae exhibits a stronger bacterial growth under the same incubation conditions than S. aureus (compared on polyester bar in Figures 5 and 6). Consequently, K. pneumoniae might be less affected by liberated metal cations independent of the type of cation as S. aureus. The picture arises from the results of the nephelometric analysis is supporting that conclusion partially, only. So, a 1:32 dilution of the silver fibre extract namely allows controlling the K. pneumoniae growth up to 14 hours in contrast to S. aureus, where

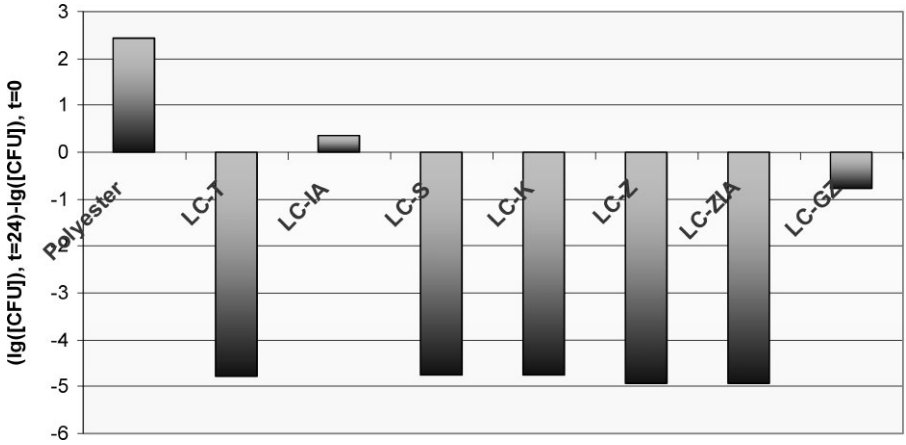

Figure 6.
Bioactivity of different textile structures against Klebsiella pneunomiae ([CFU] at $t = 0$: $(6.6 \pm 1.8)10^5\ ml^{-1}$).

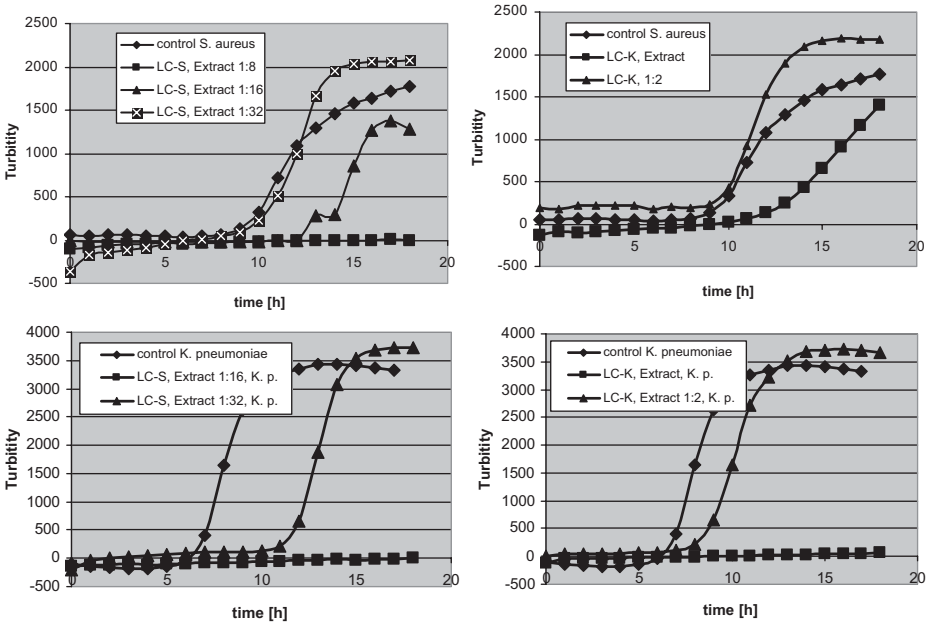

Figure 7.
Results of the nephelometric analysis of antimicrobial textile fibres (LC-S = extracts from fibre modified by silver cations; LC-K = extracts from fibre modified by copper cations).

bacterial growth could be controlled up to 12 hours, only.

In contrast to this, the 1:16 dilution seems to be more active in case of K. pneumoniae than in case of S. aureus. Finally, only in case of S. aureus a 1:8 dilution of silver fibre extract is sufficient for bacteria growth controlling.

Nephelometric analysis of fibre extracts manufactured from copper charged fibres confirms a lower antimicrobial activity compared with the silver fibre extract. Because of low copper concentration adsorbed at the initial fibres only the pure extract and the 1:2 dilution allow controlling of bacterial growth. While the pure extract is able to suppress the growth of K. pneumoniae over the whole testing time a clear, unaffected growth of S. aureus could be detected at the end of the testing period. Consequently, the 1:2 dilution allows to control the bacteria growth up to about 10 hours, only.

Derived from nephelometric analyses, Ag contents higher than 30 ppm in fibres as well as Cu contents higher than 100 ppm in fibres are fairly effective for a permanent antimicrobial effect.

Conclusion

Derived from the investigations of biofunctionality of physically functionalised cellulose fibres manufactured by a modified Lyocell technology it could be stated, that textile fibres containing silver at a concentration of equal or higher than 30 ppm and (0.06% in a blended textile yarn) or copper containing fibres at a copper concentration higher than 100 ppm (about 0.5% in a blended textile yarn) exhibit sufficient bacteriostatic activity over the whole textile lifecycle. There is no difference between biofunctionality of fibres contain antimicrobial component adsorbed at IE resins incorporated inside the fibre matrix and fibres containing direct incorporated zinc oxide, respectively. The already known differences regarding the bioactivity of varied agents had been tested could be confirmed in the order $Ag^{+} > Cu^{+} > Zn^{2+}$. For that reason, amount of bio-functional

additives had to been increased in fibres modification from silver to zinc.

Meanwhile biocompatibility (absence of cytotoxicity, skin irritation as well as environmental safety) had been successful demonstrated. The technology for bioactive fibres manufacturing had been scaled up into a semi-technical scale and fibres had been commercialised by smartfiber Corp. Rudolstadt under brand name *smartcel™ bioactive* and very recent *smartcel™ sensitive*. Both fibres types have also been included into the list of bioactive substances accepted by Eco-Tex standard. A couple of finished textile goods, like for instance Blue Wish® tissues or Blue Magic Ball®, are already available on the market.

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