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Antimicrobial Textiles – Evaluation of Their Effectiveness and Safety

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Abstract

The number of biofunctional textiles with an antimicrobial activity has increased considerably over the last few years. Whilst in the past it was predominantly technical textiles which had antimicrobial finishes, in particular to protect against fungi, nowadays textiles worn close to the body have been developed for a variety of different applications as far as medical and hygienic tasks. Together with the increase in new antimicrobial fibre technologies and possibilities in the hygienic and medical applications, the demand for proper test systems to evaluate the effectiveness as well as the safety of antimicrobial textiles rose. With the aid of agar diffusion and suspension tests, it is possible to record qualitative and quantitative data on the in vitro 'degree of effectiveness' of antimicrobial textiles. Test systems based on testing the biocompatibility of medical devices are suitable to evaluate the safety of antimicrobial textiles.

Applications and Objectives

The number of applications of textiles with antimicrobial activity has increased dramatically. A brief summary of the various fields of applications and associated products is shown by way of example in table 1.

From the data presented in table 1, one can deduce four main objectives behind the use of antimicrobial finishes [1]:

(a) to avoid the loss of performance properties as a result of microbial fibre degradation;
(b) to significantly limit the incidence of bacteria;
(c) to reduce the formation of odour as a result of the microbial degradation of perspiration;
(d) to avoid transfer and spread of pathogenic germs.

Table 1. Antimicrobial textiles and their fields of application

Medicine	Sport and leisure	Outdoor	Technology	Domestic
Support stockings	Shoes	Jackets	Wall hangings	Curtains
Antidecubitus mattress	Socks	Tents	Roof coverings	Coverings
Incontinence liners	T-shirts	Uniforms	Facade linings	Cloths
Encasings	Cycle wear	Personal protective	Air filters	Bath mats
Bedding filling	Team kit	Astro turf	Automotive	Sanitizers
Pillows	Jogging suits	Sunshades	Geotextiles	Underwear
Implants		Awnings		Carpets

Together with the increase in new antimicrobial fibre technologies and possibilities in the hygienic and medical applications [2], the demand for proper test systems to evaluate the effectiveness and safety of antimicrobial textiles rose [3].

Two Types of Activity

Before testing textiles with antimicrobial activities for their effectiveness, one has to take into account the technology and the bioactive substance which have been used to equip the textile. Three fundamental procedures have become established:

(a) the bioactive substance is applied directly to the spinning mass;
(b) the bioactive substance is applied to the fibre surface, e.g. with the aid of spacer molecules;
(c) the manufactured fabric is coated with a resinogenic finishing agent.

Depending on the type of technology and bioactive substance, textiles result with different antimicrobial active principles. Coarsely, these principles can be divided into two categories: materials with passive and materials with active effects [4].

Passive Antimicrobial Principles

Passive antimicrobial fibres do not contain any type of antimicrobial additives but have a quasi-antimicrobial effect, e.g. the microbial colonization of the textile is prevented by the surface structure of the fibre (microdomain-structured surfaces, lotus effect). In other words, the bacterial cells themselves are not affected – instead, the micro-organisms are prevented from adhering to the fibre surface (to prevent fibre degradation) or are attacked by non-leaching surface-active compounds. An example are so-called co-polyamines, antimicrobial polymers which have covalently attached polycationic compounds, that interact

with the bacterial cell wall [5]. More recently, anti-adhesive, 'intelligent' polymers have been developed which prevent the formation of a biofilm on implants and thus offer a preventive measure for infections associated with implants (so-called plastic infections) [6].

Active Antimicrobial Principles

The majority of textiles with antimicrobial activity are materials with active finishes which contain specific active antimicrobial substances acting upon micro-organisms; either on the cell membrane, during metabolism or within the core substance (genome). A number of metallic compounds are also used; various silver compounds are currently very popular. Besides this, quaternary ammonium compounds, biguanides, amines and glucoprotamine displaying polycationic, porous and absorbent properties are active substances. Natural products such as chitosan are also being used more frequently [7]. The effectiveness of textiles with active antimicrobial activity is based on the so-called diffusion principle, i.e. the bioactive substance diffuses out at a variable rate from the finish or from within the fibre by ion exchange, by the substitution of cations from perspiration.

The antimicrobial principles listed above brought their own problems, however: it was often difficult or impossible to detect antimicrobial activity using conventional test methods, although the active substances themselves are clearly recognized and described as antimicrobial. This is due to the fact that a range of test systems only react to highly diffusive leaching substances (active principle) and therefore often give false-negative results where non-diffusive or weakly diffusive compounds are present.

Evaluating the Effectiveness of Antimicrobial Textiles

To record the quantitative reduction of bacteria by antimicrobial textiles, test systems have become established which specifically record this process. It is far beyond the scope of this article to compare all of the most important recognized international test methods for antimicrobial textiles (table 2). Therefore, we focused on the pros and cons of two major test systems: the agar diffusion test and the suspension test (challenge test).

Agar Diffusion Test

The agar diffusion test has a long-standing tradition in microbiology (e.g. testing the antibiotic resistance of germs). A probe is placed directly onto the surface of a germ-containing agar plate [8]. The test can easily be run with various micro-organisms. In textile research, this method can be recommended as a quick and preliminary qualitative method to distinguish between active

Table 2. Overview of the most popular standards to test the performance of textiles, fibres, yarns and polymers for antimicrobial effectiveness

Designation	Title	Principle
SN 195920-1992	Textile fabrics: determination of the antibacterial activity	Agar diffusion test
SN 195921-1992	Textile fabrics: determination of the antimycotic activity	Agar diffusion test
EN 14119:2003-12	Textiles-evaluation of the action of microfungi	Agar diffusion test
ASTM E 2149-01	Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions	Challenge test
JIS Z 2801	Antimicrobial products – test for antimicrobial activity and efficacy	Challenge test
JIS L 1902-2002	Testing for antibacterial activity and efficacy on textile products	Challenge test

(i.e. clear zone of growth inhibition around the probe) and passive antimicrobial principles (no zone of inhibition). The diameter of the zone of inhibition may give an indication of the dimension of the antimicrobial activity or the release rate of the diffusive compound. Thus, it is not more than a semi-quantitative analysis. Nevertheless, the agar diffusion test is also favourable when testing textiles with passive antifungal activity (fig. 1), since fungi do not grow or are impaired in their growth on the specific probes. For textile materials other than fabrics, only very poor or imprecise conclusions can be drawn using this method.

Suspension Tests

With the aid of suspension tests such as the JIS 1902-2002 it is possible to record the maximum achievable in vitro 'degree of effectiveness' of the finished textiles as growth inhibitors of test bacteria [9]. This standard was not designed as a wear test for textiles, but it is an excellent method to assess the degree of the antimicrobial activity of textiles. Suspension tests can also be used to differentiate between a specific or general antimicrobial activity, and they can be modified to check the activity of textiles with the passive principle. When calculating and evaluating the antimicrobial activity, it is advantageous that the composition of the control and sample materials is identical, as in the test for specific antibacterial activity. The only difference must be the antibacterial component of the sample. This is the only way to guarantee that the test results reflect the specific antibacterial activity of the treatment over the test period.

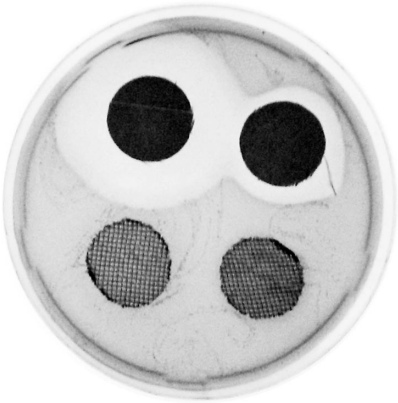

Fig. 1. Agar diffusion test with antimicrobial textiles. The two upper samples on the plate show a clear zone of inhibition (bacteria have been destroyed around the samples). This can be taken as an active antimicrobial principle. In contrast to this, the two lower fabrics do not show any signs of an active zone, although these probes are also treated with an antimicrobial finish (non-diffusive, passive antimicrobial principle).

Common Procedure: Test for Specific Antibacterial Activity

It is favourable to start with a log culture of bacteria (e.g. *Staphylococcus aureus* 6538 ATCC, *Klebsiella pneumoniae* DSM 789) diluted with sodium chloride to a definite suspension with 10^5 CFU (colony-forming units). In this dilution stage, the inoculum is deprived of virtually all reserve nutrients, in order to minimize factors influencing growth and directly record the specific effect of the antibacterial treatment applied to the textile fibres. It is also of advantage to conduct the suspension test with a sample material that has been given an antibacterial treatment and a reference material. The latter is a fabric of the same structure and chemical/physical properties and composition, but without the antibacterial treatment. Both materials are inoculated and incubated for 18 h at 36°C. The average value is taken from parallel tests (e.g. triple control). After the incubation, both materials are washed out with physiological salt solution with detergent supplement, and the number of separated bacteria is recorded in terms of CFU, e.g. using a spiral plater procedure. The bacterial growth over 18 h is calculated for the reference material and the sample material (CFU sample), respectively.

Calculation and Assessment

The reduction value in the growth of micro-organisms R is calculated, which expresses the difference in bacterial growth between the sample material and the reference material RM over 18 h. It is expressed in log stages as the bacterial growth-inhibiting, antibacterial activity:

Table 3. Assessment of the antimicrobial activity

Antibacterial activity	Specific antibacterial activity
Slight	0 to <1*
Significant	≥1 to <3
Strong	≥3

*Because of the instability of the bacterial growth, the biological variation (laboratory standard ± 0.5 log stages) should be taken into account in the assesment criteria, especially in the lower range/where the effect is slight. When bacteria are used, the term 'antimicrobial' can be specified to 'antibacterial'.

$$\text{specific antibacterial activity} = \log_{10}(\text{CFU}_{\text{RM/18 h}}) - \log_{10}(\text{CFU}_{\text{sample/18 h}}) = \text{R}$$
$$\text{specific antibacterial activity} = \text{BG}_{\text{RM}} - \text{BG}_{\text{sample}}$$

where BG = bacterial growth.

The $\log_{10}$ values for the specific antibacterial activity can be rated according to the assessment described in table 3.

When nutrients are limited during the incubation phase, depending on the fibre type changes may be observed in the bacterial population: on substrates/fibre types containing no nutrients, stagnation occurs, while natural fibres which contain nutrients (wool, silk) may promote growth. Occasionally a slight reduction in the bacterial culture may even be detected. However, by directly comparing a reference material with the treated sample, it is always possible to record the direct effect of the additional antibacterial treatment, because external factors (e.g. supply of nutrients) can largely be excluded and, because of the characteristics of the sample and reference materials, it can be assumed that any potential growth pattern will be the same.

Controls

To check the capacity for growth of the test bacteria, an internal growth control test should always be carried out using a defined test material. In experiments, values between ±0.5 log stages can been achieved. To check the recovery rate, the 0-hour value should also be calculated. The bacterial suspension is also plated at 0 and 18 h and the CFU are counted.

Common Procedure: Test for General Antibacterial Activity

In the absence of a reference material with the same chemical/physical properties, the internal growth control material can be used as a reference value. In this case, the assessment should be specified to the term 'general antibacterial activity of textile' in order to distinguish clearly from the term 'specific

antibacterial activity'. If there is no reference material, the sample and reference cannot be compared and evaluated purely with regard to the antibacterial treatment, i.e. directly. Additional treatment of the fibres in finishing processes, dyeing, washing etc. can affect the growth of bacteria and so contribute to the general antibacterial activity.

By comparing the 18-hour value for the internal growth control material with the 18-hour value for the sample, the total activity of the treated textile can be expressed using the following formulas:

$$\text{general antibacterial activity} = \log_{10}(CFU_{IGM/18\,h}) - \log_{10}(CFU_{sample/18\,h})$$
$$\text{general antibacterial activity} = BG_{IGM} - BG_{sample}$$

where BG = bacterial growth and IGM = internal growth control material.

Evaluating the Safety of Antimicrobial Textiles

More and more antibacterial textiles aim in particular at clients from hygiene-sensitive sectors such as the health sector and the foodstuffs industry. The fact that the clothing worn by doctors and nursing staff, as well as that worn by workers in food-processing companies, can play a critical role in the transfer of dangerous pathogens is indisputable [10, 11]. This is particularly true if the effectiveness has been proven beyond all doubt, i.e. on the basis of practical tests carried out by a neutral body. On the other hand, the term 'antibacterial' inevitably raises questions amongst wearers as to the skin compatibility of antimicrobial textiles worn next to the skin. Compelling legal reasons such as § 30 of the Ordinance on Foodstuffs and Articles for Domestic Use [12] require the use of innovative textiles to ensure there are no risks to health. The user of the textile products can therefore expect them to be safe in use and not to pose a health risk. If, for instance, an employer demands that specific workwear is worn or actually provides such workwear, he must ensure that this is safe as part of his duty of care towards his employee. For this reason, validated test methods have been developed to objectively assess the biological safety of textiles with antimicrobial activity on a scientific basis. The risk analysis is central to this test, in other words whether and in what way it is possible to assess the biological effects of these types of textile finishes on humans, what benefits the materials offer the wearer and whether these benefits are achieved without posing additional risks for the wearer.

Biological Safety Tests on Skin Test Systems

The basis for the biological safety tests for textiles is the EN ISO 10993 [13] for the biological evaluation of medical devices. This stipulates, depending

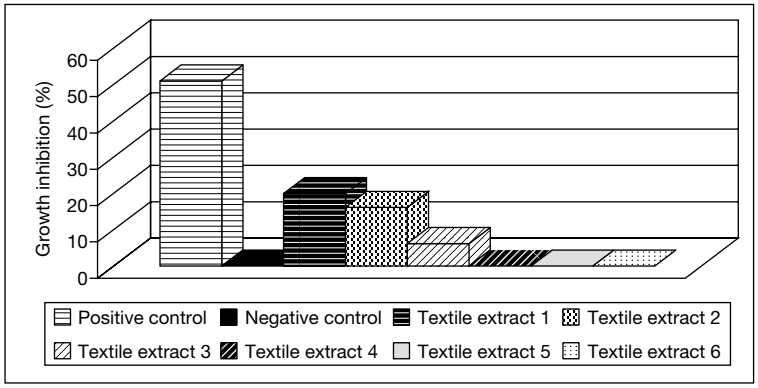

Fig. 2. Textile test for biocompatibility: dilutions of a textile extract (1–6) do not lead to a significant reduction in cell growth of fibroblasts. Only the positive control results in a tremendous and significant reduction in cell proliferation (>30%), indicating severe cytotoxicity.

on the type and duration of body contact, what risk analyses need to be carried out and which test methods need to be employed for this. The cytotoxicity (tissue compatibility) and sensitization and irritation potential are tested [14].

Cytotoxicity

When considering the tissue compatibility of textiles with antimicrobial finishes, it is particularly important to look at whether potentially cell-toxic substances (i.e. bioactive leaching substances) could be released from the material during normal wear. In the cytotoxicity test in accordance with EN ISO 10993, an extract from the textile is prepared using an artificial perspiration solution. The effects of this on the L 929 fibroblasts and HaCaT keratinocytes from the human epidermis provide information on potentially cell-toxic components. Figure 2 shows the results of a test for cytotoxicity, carried out on a antimicrobial active textile which was finished with silver and is used to treat cases of atopic dermatitis [15, 16].

Irritation

A classic test to determine the irritation potential of a substance is the Draize test, in which the substance to be tested is dripped onto the conjunctiva of a laboratory animal's eye in order to identify any potential irritants. The scientifically recognized hen's egg test on the chorio-allantoic membrane is an alternative to the animal test [17]. This has been validated among others by the European Centre for the Validation of Alternative Methods. It is possible to

determine the irritation potential of substances which could be released from the textile material just as accurately by observing the blood vessels of the treated egg as using the animal test. In addition to the cytotoxicity test, the hen's egg test on the chorio-allantoic membrane therefore offers decisive additional security regarding the use of antimicrobial textiles. In conclusion, new biological test systems make it possible to scientifically determine the interactions between textiles and the skin accurately and to recognize and evaluate potential benefits and risks. The methods can be used as safety tests for textiles with antimicrobial activity.

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