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Evaluation of Antimicrobial Textiles for Atopic Dermatitis

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SUMMARY

Background: Functional textiles have been proposed as safe adjunct treatment for atopic dermatitis (AD). Some data has been published regarding their antimicrobial properties and their clinical efficacy.

Objective: This study examined the physical and functional properties of 11 commercially available functional textiles, including their antimicrobial activity in vitro, as a function of multiple laundering cycles.

Methods: All materials were weighed and examined under scanning electron microscopy (SEM) before and after laundering for fiber morphology and silver coating. Bioburden of newly purchased textiles was assessed by measuring bacterial colony forming units (CFU). Deliverable antimicrobial efficacy was evaluated in vitro for each specimen, before and after 30, 70, 100, 150 and 200 laundering cycles.

Results: Textile weight showed high variability. Damaged silver-coating of variable degree was observed under SEM in most materials after laundering. Products made of silk showed smoother and tighter fiber morphology compared to cotton. The bacterial load of unwashed material ranged from <1 CFU to 35 CFU per 50x50 mm specimen. Most silver-containing products lost their antimicrobial activity rapidly after laundering. Silk and cotton retrieved products had no deliverable antimicrobial effect even in their original state.

Conclusion: Elastic, light weight textiles with smooth fibers are comfortable for daily use. Functional textiles rapidly losing their deliverable antimicrobial activity in vitro are not advisable for AD patients. Recommendations for functional textiles should be based on a

combination of in vitro analysis of products in their original state and after laundering, together with real life data obtained from controlled clinical trials.

INTRODUCTION

Staphylococcus aureus (S. aureus) plays a major role in the pathophysiology of atopic dermatitis (AD), and its density on skin is correlated with disease severity. *S. aureus* produces super-antigenic toxins leading to a pro-inflammatory cascade of events, [1] and they may get internalized in keratinocytes or produce destructive enzymes, leading to necrotic cell death. [2]

According to recent AD treatment guidelines, emollients and topical anti-inflammatory drugs are the mainstay of therapy. Antibiotics may be useful to treat impetiginization, but their long term use is not recommended. [3-6] Topical antiseptics may reduce the bacterial load of AD skin, and are in most cases preferred over topical antibiotics because they have a lower risk for contact allergy and development of resistance. For this reason, triclosane, polyhexanide, chlorhexidine or silver particles may be compounded into emollients for application on AD skin. Functional textiles with an antimicrobial activity could also play an important role in AD management, when they diminish *S. aureus* colonization. [7]

The most commonly used fibers for garments are either synthetic, such as polyamide or polyester, or natural fibers, such as silk, angora, cotton, modal and Lyocell. The addition of antimicrobial properties to these fabrics may be achieved by coating the material with functional substances such as silver particles, or 3-trimethylsilylpropyl-dimethyloctadecyl ammonium chloride (AEGIS) on silk.

Some materials with antimicrobial components have deliverable antimicrobial activity, whereas other combinations may only result in a textile which is protected from bacterial colonization itself, but does not possess deliverable antimicrobial activity. A deliverable

antimicrobial activity, however, is important for the therapeutic usefulness of a "functional" textile, because the ultimate goal of reducing *S. aureus* on the skin is not the same as carrying a garment with low bacterial load.

As garments need to be changed, cleaned and washed on a regular basis for hygienic reasons, a clinically useful, durable, functional coating is required when products are laundered over hundred times. On the other hand, garments should be made of light and stretchy elastic fabrics to ensure optimal wearing comfort.

Since many different products are offered as therapeutically useful "functional textiles", we sought to compare the physical properties, durability of coating, bacterial load of newly purchased products, as well as deliverable antimicrobial activity of different textiles with a potential usefulness in AD patients.

MATERIALS AND METHODS

Fabric data

This study investigated 13 textile products present in German market. One AEGIS-coated silk textile, 10 silver-coated textiles and two untreated cotton textiles as control were compared (see Table 1).

Weight determination

The product weight was determined according to the industrial norm standard DIN EN 12127:1997-12 "Textiles - Fabrics - Determination of mass per unit area using small items": The weight was measured on a rectangular cut, 50x50 mm ± 2 mm test specimen. The results are given in grams per square meter.

Laundering procedure

Standardized washing was performed at an independent institution (Ipi Institute for Product Market Research, 70190 Stuttgart, Germany) to ensure objectivity of the procedure: As a simulation of wearing usage, all textile samples were laundered repeatedly in accordance to the standard DIN EN ISO 6330 "Textiles - Domestic washing and drying procedures for textile testing" in a front load washing machine, Siemens WM14E3A0 (Type WLM 40) using the "Easy care 40 °C" (German: "Pflegeleicht") program. Each fabric was washed with ¼ cloth ballast soil (SBL2004) per 2 Kg washing load and ECE-2 phosphate-free washing detergent at a dosage of 20 g per laundering cycle (ISO105 C 08) according to the local water hardness of 9-13 °dH. In-between washings, specimens were left for 24 hours on a laundry rack to air-dry and stored overnight. For each specimen, the same washing machine was used.

Electron microscopic examination

All products were examined under 600x, 1200x and 5000x magnification using a HITACHI TM 3000 scanning electron microscope to obtain visual analysis results of fiber morphology and integrity of the silver coating. This visual analysis was performed in original unused stage of products, as well as after 30, 70, 100 and 150 laundering cycles.

Bacterial load determination on unwashed textile products (Bioburden Test)

All microbiological tests were performed at an independent institution (Eurofins BioPharma Product Testing Munich GmbH, 82152 Planegg, Germany) to ensure objectivity of the procedure: Fabric samples were cut out, wearing gloves to avoid contamination. All samples were immediately packed light- and air-tight in lockable plastic bags and aluminum foil, and sent directly to the microbiological testing laboratory (Eurofins, Planegg) for the examinations described below. The bacterial count on the textile's surface in its original state

was determined according to the international standard EN ISO 11737-1, 2009 "Sterilization of medical devices - Microbiological methods - Part 1: Determination of a population of microorganisms on products". Briefly, a 50 ± 2 mm square piece of fabric was treated with a buffer solution in a stomacher, and the microbial count of the rising fluid was determined using a membrane filtration method. Unused buffer solution served as a negative control. The filter was transferred onto a TSA agar plate and incubated first at 30-35 °C and afterwards at 20-25 °C. This sequential incubation easily allowed the differentiation between bacteria and yeasts based on morphologic criteria. The colony forming units (CFU) per filter were counted, and the total microbial count per test item was calculated.

Deliverable antimicrobial activity determination of products in original state and after launderings

The deliverable antimicrobial activity against *S. aureus* was determined before and after 30, 70, 100, 150 and 200 laundering cycles, according to international standard ISO 22196 (JIS 2801) "Measurement of antibacterial activity on plastics and other non-porous surfaces". Briefly, a 50 x 50 ± 2 mm square piece of fabric was cut out and inoculated with 1 to 4 $x10^5$ CFU of *S. aureus*. The microbial count was determined using the TSA-plate count method in duplicate for a reference item without incubation and test item after 24 hours of incubation. The antimicrobial efficacy was calculated as: $R = [\log (B/C)]$; with R being the value of antimicrobial activity, B being the average number of viable bacterial cells on the reference sample after incubation for 24 hours, and C the average number of viable bacterial cells on the test sample after incubation for 24 hours. Samples with an R-value ≥ 2 were considered having an efficient antimicrobial activity.

RESULTS

The functional textiles investigated differed largely in physical properties.

Weight

The weight varied between 80 g/m^2 for the silk product and 210 g/m^2 for 2 products – one with composition of 5% silver coated yarn mixture together with Modal/Polyamide/Lycra and the other one with 25% silver coated yarn together with ecological cotton mixture (see Table 1).

Bioburden

Two products containing 100% cotton, which were examined for control purposes, presented sharp and largely interspaced fibers under SEM (Figs 1a, 1b), had a high bioburden result in their original state (Fig. 2) and no deliverable antimicrobial activity before and after laundering (Fig. 3). The AEGIS-coated silk fabric showed long, smooth fibers with minimal interspace (Figs 1c, 1d) corresponding to the known, favorable wearing comfort, and a low bioburden result of <1 CFU / specimen in its original state (Fig. 2). New purchased silver-coated textiles had a variable bacterial burden, with results ranging from <1 CFU / sample up to 33 CFU / sample (Fig. 2). This was confirmed by SEM results, since only few unwashed textiles had an intact silver-coating (Images not shown).

Deliverable antimicrobial activity and sustainability of silver coating

Cotton and AEGIS-coated silk textiles, which showed no deliverable antibacterial activity in unused stage, were not laundered and not repeatedly examined under SEM. Among silver-coated textiles, the silver-coating showed highly variable sustainability with standard care. One durable product showed a homogenous silver coating before washing (Figs 1e, 1f),

which remained intact after 150 laundering cycles (Figs 1g, 1h). Some silver-coated products were highly damaged and presented peeling of silver coating after 150 (Figs 1i, 1j) or even 70 laundering cycles (Figs 1k, 1l).

Heterogeneous results concerning deliverable antimicrobial activity of products in their original unused state were also noted. The AEGIS-coated silk fabric had no deliverable antimicrobial activity even before laundering (Fig. 3).

The deliverable antimicrobial activity of the silver-coated textiles, expressed by the R-values, varied largely from the beginning, and declined rapidly after laundering the textiles up to 200 cycles. Two textiles had lost their deliverable antimicrobial efficacy ($R<2$) just after 30 cycles, 1 lost it after 70 cycles, 3 after 100 cycles, 2 had a highly destructed, peeled-off coating after 150 cycles, and only 1 retained its full efficacy even after 200 cycles (Fig. 3).

DISCUSSION

In our study, the functional textiles varied in terms of weight, bioburden, stability of coating and deliverable antimicrobial activity over time. Many silver-coated fabrics were heavy in weight, and some had a high bacterial burden in their original state. The AEGIS-coated silk product showed a low surface bacterial count, but did not possess any deliverable antimicrobial activity in original state. Laundered products with silver content showed degradation in the coating's intactness under microscopy, and their initially sufficient antimicrobial activity declined rapidly.

The weight of the products with silver content differed largely between 120 and 210 g/m^2, and was higher than the lightest textile in our study - the silk textile with 80 g/m^2. Functional textiles should be comfortable to wear to be accepted by the patients. In our atopic dermatitis special clinics, the vast majority of patients favor lighter, elastic fabrics over heavier ones. Knitted fabrics are more flexible than woven ones, and therefore usually preferred by

patients. Light-weight knit materials with thin yarns are valued as functional textiles, but the lower weight comes on the cost of less stability and durability. Hence, choosing the best material with silver content retaining its functionality is a complex decision.

The high bioburden and low deliverable antimicrobial activity of several products with silver coated yarn content in their original state was an unexpected result. Silver is a well-established broad spectrum antiseptic agent. [8-10] Therefore, the high bioburden of many unused silver products is arguing against a relevant antimicrobial activity of the fabric. In other words, it seems that the silver contained in some of the products is not functional.

On the other hand, a previous study showed that the amount of bacteria present on the unwashed silver containing fabric "Juzo Skin Protect Silver" was lower than on placebo fabrics. [11] This might correspond to a possible destruction of the silver coating due to aggressive knitting, depend on the technology and machinery used, or result from the suboptimal release of Ag+ antibiotic ions from certain types of fibers. Latest customized and milder knitting techniques might lead to the production of more stable functional textiles. A higher silver concentration in the fabric or in a Lyocell fiber/silver ions combination seems to have a higher efficiency, since the Padycare and Schiesser products had apparently a lower bioburden than Binamed or Medima antisept products. However, the bioburden of new garments is clearly a less relevant parameter for clinical usefulness of a functional textile for *S. aureus* reduction in AD patients than the deliverable antimicrobial activity of the fabric.

The AEGIS-coated silk fabric in our study showed the lowest bioburden when purchased (<1 CFU/specimen). However, there was no deliverable antimicrobial activity detectable even before washing. AEGIS can efficiently protect silk garments against microbial invasions in vitro. [12]

The clinical signs and symptoms of AD patients may improve somewhat better than with untreated silk and significantly better than with cotton, [13, 14] reaching an effect size in the range of a corticosteroid in short term use. [15] These promising results are in contradiction to the CLOTHES trial, which is a recently published, large-scale, controlled clinical trial conducted in the UK under real life conditions: The results of the CLOTHES trial showed zero benefit from the use of AEGIS-coated silk as an adjunct treatment for AD. [16, 17] The unexpected findings from the CLOTHES trial may be explained by our results, which indicate that the antimicrobial effect of AEGIS is limited to the textile itself and is not being delivered to the patient's skin.

Though AEGIS-coated silk textiles did not show deliverable antimicrobial activity in our study and did not show clinical benefit under real life conditions in the CLOTHES trial, the good wearing comfort and resistance of the garment against bacterial colonization still makes it a recommendable garment option compared to cotton fabric and especially to wool. [4]

Silver textiles act by releasing silver ions onto the patient's skin. [8-10] For reasons of hygiene, household machine washing is crucial to keep the clothing clean of bacteria. [11] Therefore, clinically useful silver textiles must be resistant to household washing.

In our study, some silver containing fabrics with an initially good deliverable antimicrobial activity lost their activity very fast after 30, 50 or 100 laundering cycles. This was accompanied by physical damage of the silver coating under SEM magnification.

Grafting of silver nanoparticles to previously oxidized cotton textiles leads to highly sustainable antibacterial activity after 50 home laundering cycles, with a maintained 96% bacterial reduction. [18] Another study showed that household washing affects the Ag^+ concentration only minimally, and thus would not interfere with the deliverable antimicrobial activity of commercially available functionalized silver textiles. [19]

Silver containing fabrics were previously shown to decrease *S. aureus* on AD skin, and to improve the course of AD, [20] especially compared to conventional cotton clothing, [7, 13, 20, 21] Intact silver garments would reduce the need of topical corticosteroids and improve the patient's signs and symptoms of AD. [7, 21, 22] A significant decrease of transepidermal water loss and increase of stratum corneum hydration have also been noted. [20, 23] Consequently, a causal relationship between the silver coating loss after washing and the decrease of deliverable antibacterial activity of the fabric sample may be assumed.

All previous clinical trials investigating silver-coated fabrics were conducted for 7 to 28 days of textile use intervention only, which obviously seems too short to observe a clinically important decrease of deliverable antimicrobial efficacy and clinical efficiency (SCORAD, *S. aureus* reduction, amount of Prednicarbate cream used…) of the textiles due to laundering. Yet, they are proof that silver in textiles could have an important role as an adjunct AD therapy. For most of the tested products, the sustainability of the metal coating needs improvement.

An ideal functional textile should be worn 24 hours per day during the treatment period, should ideally be soft, comfortable, light-weight, easy to wash and dry and should not absorb moisture. [24] If silver is properly grafted to the fibers of the textile, conventional cleaning methods should not affect silver's concentration or its antimicrobial function. [18] A resistant

and prolonged antimicrobial activity is quite substantial in order for these fabrics to contribute to treatment outcomes.

In practice, silk garments might represent the most comfortable garment option to wear for our AD patients. However, the clinically most useful adjuvant treatment for AD might be the Padycare, Binamed or Platatex products due to their durable deliverable antimicrobial activity unaffected by laundering. Products with a rapidly damaged silver coating seem less advisable.

This was an experimental study, performed on textile specimens in vitro. The different steps of the experiment were strictly standardized according to well established, previously published protocols. All experiments are thus exactly reproducible and had clear, predefined outcome parameters.

The bacterial load of unused products was a non-*S.aureus*-specific measurement. We consider this parameter clinically less relevant than the deliverable antimicrobial activity of the textile to the patient's skin. The latter was tested using exclusively *S. aureus* colonies.

The collection of clinical patient data was not an aim of our study, which could be considered a limitation. Additionally, our study was sponsored by a manufacturer of functional textiles, but this would neither influence the conduction of our study nor the objectivity of the analysis.

In conclusion, our new data add to the existing knowledge on functional textiles. Silver coated textiles seem to be the most promising adjunct therapy, even if only some of them offer sustained deliverable antimicrobial activity for our patients' skin. Most currently marketed textiles are not likely to be recommended as "functional textiles" for AD patients. In order to formulate strong recommendations in new AD treatment guidelines, the choice of

fiber, light-weight, elasticity and durability of the silver coating should be well considered and verified under clinical settings.

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TABLES

	Product name	Manufacturer	Silver content	Weight (g/m^2)
COTTON	Curaderm	Lohmann & Rauscher GmbH & Co. KG	No	160
	Dermifant	Allergika GmbH	No	160
SILK	DermaSilk	Dr. Beckmann GmbH	No	80
SILVER	Padycare	Texamed GmbH	Yes	130
	SkinProtect	Julius Zorn GmbH	Yes	120
	Binamed	Binamed Moll GmbH	Yes	210
	Platatex	AAT Alber Antriebstechnik GmbH	Yes	185
	Pulmanova Bioactive	MediTech Grosshandel fuer medizintechnische Produkte GmbH	Yes	165
	Silver-Skin	SilverSkin	Yes	210
	Best4Body	Trigema Inh. W. Grupp. e. K.	Yes	150
	Schiesser	Schiesser AG	Yes	145
	Medima Antisept	Medima Vertriebs GmbH	Yes	195
	Sansita	Frank sportswear	Yes	120

Table 1. Characteristics of the tested antimicrobial textiles. For each product, the following is listed: product type, brand name, manufacturer, presence or absence of silver and weight in g/m^2.

Figure legends

Figure 1. Electron microscopy images. Curaderm was examined before washing using a scanning electron microscope (SEM) under (a) 600x and (b) 1200x magnification. Its fibers were curly and woven, with numerous and large inter-fiber spaces. In original condition, DermaSilk's examination under (c) 600x and (d) 1200x magnification using SEM showed smooth, straight fiber morphology, with decreased inter-fiber space. Padycare was examined using SEM, before and after washing, under (e) 1200x and (f) 5000x magnification. Its silver coating was seen to be homogenous and uniform, covering the fibers of the textiles entirely. After 150 washing cycles, under (g) 1200x and (h) 5000x magnification, the silver coating was still homogenous, almost unchanged. Binamed's silver coating presented with substantial damage after 150 washing cycles, which was detected at (i) 1200x and (j) 5000x magnification with the SEM. Fissures and sloughed off patches were seen. Platatex presented with more severe damage after 70 washing cycles, also seen at (k) 1200x and (l) 5000x magnification using the SEM. The surface of the textile material appeared to have lost most of its silver coating.

Legend:
(a) Unwashed Curaderm (600x)
(b) Unwashed Curaderm (1200x)
(c) Unwashed DermaSilk (600x)
(d) Unwashed DermaSilk (1200x)
(e) Unwashed Padycare (1200x)
(f) Unwashed Padycare (5000x)
(g) Padycare after 150 washing cycles (1200x)
(h) Padycare after 150 washing cycles (5000x)

(i) Binamed after 150 washing cycles (1200x)

(j) Binamed after 150 washing cycles (5000x)

(k) Platatex after 70 washing cycles (1200x)

(l) Platatex after 70 washing cycles (5000x)

Figure 2. Bioburden of textiles in original state. The bacterial count on the textiles was determined before washing according to the International Standard ISO 11737-1 (2009). This graph shows the heterogeneous results regarding the bacterial density in the textiles before washing. The products Padycare and DermaSilk showed <1 CFU/specimen and for the rest of the products, the results ranged between 3 CFU/specimen to 35 CFU/specimen. Best4Body, SkinProtect and Curaderm showed a relatively high bacterial count above 30 CFU/specimen.

Figure 3. Antimicrobial activity as a function of number of washing cycles. The products' antimicrobial activity was measured before and after 30, 70, 100, 150 and 200 washing cycles. The bacterial count was determined on three untreated and three treated textile specimens after 24 hours incubation, and on three untreated specimens directly after inoculation. The antimicrobial efficacy was calculated as: R [log (B/C)]. R is the value of antimicrobial activity; B is the average of the number of viable cells of bacteria on the test pieces of the reference material after incubation for 24 hours. C is the average of the number of viable cells of bacteria on the test pieces of the test item after incubation for 24 hours. The experiment's protocol was performed according to the International Standard ISO 22196 (JIS 2801). Test items with microbial test result after several laundries with an R-value ≥ 2 have good antimicrobial activity and bacteria were efficiently eliminated. DermaSilk, Curaderm, Silver-Skin and Dermifant had no detectable antimicrobial activity before washing. The rest

of the products lost their functionality progressively, after 30, 70, 100, 150 washing cycles. Padycare had persistent antimicrobial activity even after 200 washing cycles.