

Percutaneous penetration of silver from a silver containing garment in healthy volunteers and patients with atopic dermatitis



Olivier A. Pluut^a, Carlotta Bianco^b, Ivone Jakasa^c, Maaïke J. Visser^a, Petra Krystek^d, Francesca Larese-Filon^e, Thomas Rustemeyer^f, Sanja Kezic^{a,*}

^a Coronel Institute of Occupational Health, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ, Amsterdam, The Netherlands

^b Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via Giorgeri 1, 34127 Trieste, Italy

^c Laboratory for Analytical Chemistry, Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, Zagreb, Croatia

^d Philips Innovation Services, High Tech Campus 11, 5656 AE, Eindhoven, The Netherlands

^e UnitaClinicoOperativa di Medicina del Lavoro, Dipartimento di Scienze di MedicinaPubblica, UniversitadegliStudi di Trieste,Via della Pietà, 19-24120 Trieste, Italy

^f Department of Dermatology, VU University Medical Centre, Amsterdam 1081 HV, The Netherlands

HIGHLIGHTS

- Silver is increasingly used in functionalized products for consumers and medicinal products.
- Dermal absorption of silver after wearing a silver containing garment did not exceed the current reference dose.
- Dermal absorption of silver in healthy volunteers and patients with atopic dermatitis did not significantly differ from each other.
- Dermal exposure to silver containing textile did not lead to increased levels of IL-1 cytokines in the stratum corneum.

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ABSTRACT

Human data on dermal absorption of silver under “in use” scenario are scarce which hampers health risk assessment. The main objective of the present study was to determine percutaneous penetration of silver after dermal exposure to silver containing garment in healthy individuals and atopic dermatitis (AD) patients. Next to assess pro-inflammatory effect of silver in the skin. Healthy subjects ($n = 15$) and patients with AD ($n = 15$) wore a sleeve containing 3.6% (w/w) silver on their lower arms for 8 h during 5 consecutive days. The percutaneous penetration parameters were deduced from the silver concentration-depth profiles in the stratum corneum (SC) collected by adhesive tapes. Furthermore, silver was measured in urine samples collected before and after exposure. Inflammatory response was assessed by measuring IL-1 α and IL-1RA in the exposed and non-exposed skin sites. Dermal flux of silver in healthy subjects and AD patients was respectively 0.23 and 0.20 ng/cm²/h. The urine silver concentrations showed no increase after exposure. Furthermore, exposure to silver did not lead to the changes in the profiles of IL-1 α and IL-1RA. Dermal absorption of silver under “real life scenario” was lower than the current reference dose. Furthermore, dermal exposure did not lead to altered expression of inflammatory IL-1 cytokines in the skin.

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1. Introduction

Antimicrobial properties of silver have been recognized since ancient times. Silver has mainly been used for medical purposes *e. g.* in wound care, medicating clothing for health care personnel and in garments designed to combat superinfections in patients with atopic dermatitis (AD). The treatment of wounds and burns with silver evolved from topical application of the silver nitrate solution

* Corresponding author at: Coronel Institute of Occupational Health, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. Tel.: +31 20 5665321; fax: +31 20 6977161.

E-mail addresses: carlotta.bianco@phd.units.it (C. Bianco), ijakasa@pbf.hr (I. Jakasa), petra.krystek@philips.com (P. Krystek), T.Rustemeyer@vumc.nl (T. Rustemeyer), s.kezic@amc.uva.nl (S. Kezic).

followed by silver sulfadiazine cream and more recently by medical garments and dressings containing silver salts or metallic silver (including nanosilver) (Moimen et al., 2011). Nowadays, silver is also increasingly used in functionalized products for consumers like in outdoor- and sport clothing, and personal hygiene products aiming at prevention of malodour. The most used form of silver in textiles are silver salts (79%) followed by metallic silver and silver ion exchangers (respectively 13% and 8%) (Windler et al., 2013). As silver containing textiles are in direct contact over large body surface areas for prolonged periods this has raised concerns over possible adverse health effects, also realizing that these products are worn by individuals who might be increasingly susceptible, e.g. children and individuals with skin diseases. For substances in textiles no registration is required in many EU countries, since textiles in the EU are treated as articles (von Götz et al., 2013). Substances in articles also do not need to be listed on a product label or be evaluated according to their potential for consumer exposure (von Götz et al., 2013). Dermal exposure to silver might potentially lead either to local skin effects or systemic adverse effects after its absorption in the body (Samberg et al., 2010). The likelihood of such effects is dependent on the amount of silver which penetrates into and across the skin. The *in vivo* data on percutaneous penetration of silver from clothing and garments under “in use scenario” are scarce. Furthermore, little is known on dermal absorption of silver in the individuals with impaired skin barrier. Reduced skin barrier can be intrinsic, like in patients with AD, or acquired, e.g. due to skin irritation caused by sweating or exposure to skin irritating chemicals, UV light or mechanical friction. In addition to the skin barrier condition, actual exposure can be influenced by various factors that can influence migration of silver from fabrics like skin pH and temperature, physical stress acting on the fabrics (wear-and-tear), type of silver and the way it is incorporated in the textile (von Götz et al., 2013).

In fabrics, silver can be present as metallic silver in a natural crystalline or nano state which ionizes in the presence of water or

body fluids. The antimicrobial action of silver or silver compounds is believed to be proportional to the bioactive silver ion (Ag^+) released.

In the present study we investigated the percutaneous absorption of silver in healthy individuals and patients with AD under “real life” scenario. AD patients have been selected from two reasons. Firstly, AD patients, in particular children, are frequent users of silver containing garments due to often occurring bacterial superinfections. Secondly, one of the main features of AD is reduced skin barrier, thus they represent a model for damaged skin barrier (Kezic et al., 2014). In addition to percutaneous penetration we investigated for possible proinflammatory effects associated with dermal exposure to silver and measured the levels of the inflammatory mediators, interleukin (IL) cytokines, IL-1 and IL-1RA in the skin after exposure to silver.

2. Materials and methods

2.1. Investigational material

For this study the Argentum company (Geneva, IL 60134, USA) provided two sleeves marked with “L” (left arm) and “R” (right arm) in 5 different sizes to avoid that the sleeves are not too tight or too loose. One sleeve contained silver, the other one was a placebo, i.e. silver free. The participants got 2 pairs of sleeves. The silver containing garment, registered as medical device class I powered by Silverlon[®] technology, consisted of 79% Micromodal fibre, 13% Polyamide Ag and 7% Lycra. The surface of the fibers are covered by a layer of 0.2–0.4 μm of pure metallic silver using an electroless chemical plating method. The content of silver in the textile was 3.6% (w/w). A fiber coated with pure silver is knitted in such a way that silver yarn lies exclusively on the inside of the textiles. The SEM/EDX (Scanning Electron Microscopy/Energy-dispersive X-ray spectroscopy) images of the fibers containing silver (Spectrum 2) and silver free fibers (Spectrum 1) are shown in Fig 1. The area of

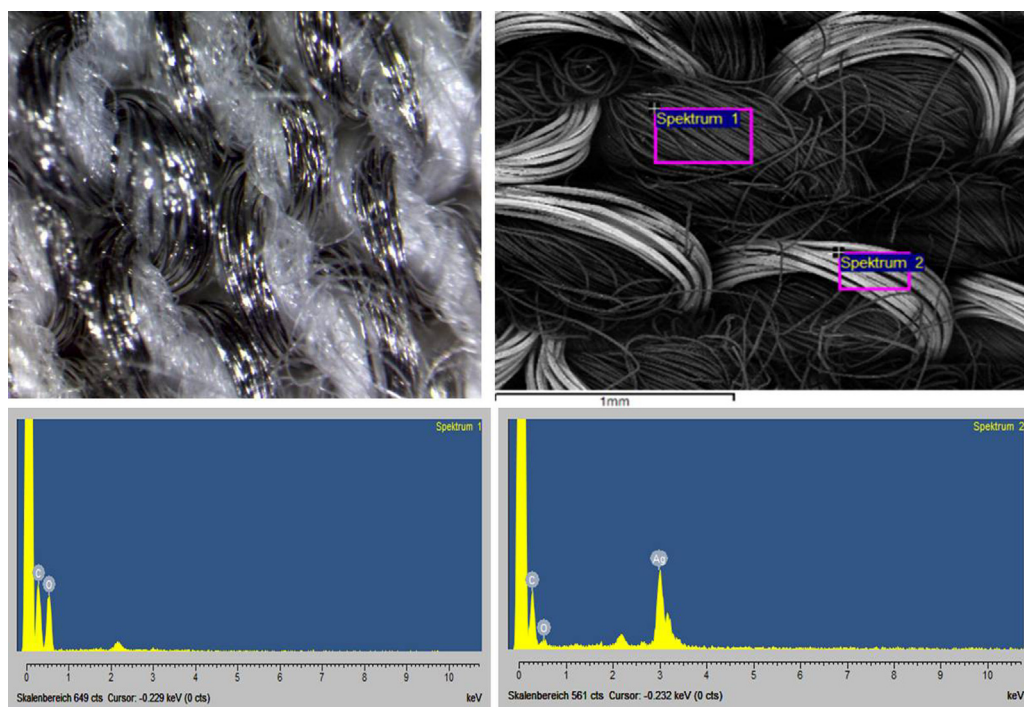


Fig. 1. Scanning Electron Microscopy images of the Silverlon[®] sleeve (Upper level) and Energy-dispersive X-ray spectra of the silver free fiber (Spektrum 1) and silver coated fiber (Spektrum 2) (Lower panel).

the sleeve was on average 600 cm². The sleeves were worn for 8 h during the night on 5 consecutive days. The sleeves were elastic ensuring good contact with the skin during wearing. The exposed skin sites, sampling schedule and investigated parameters in the stratum corneum (SC) are shown in Fig. 2.

2.2. Study population

30 Caucasian subjects (fifteen healthy subjects and fifteen subjects with AD; fifteen males and fifteen females), with a mean age of respectively 41.9 and 26.5 years participated in the study. The Medical Ethics Committee of the Academic Medical Center, University of Amsterdam, approved the experimental protocol (METC 2013_031). The study was conducted according to the Declaration of Helsinki Principles and informed consent was obtained from the participants before the start of the study. The diagnosis of AD patients has been established using a questionnaire based on UK Working party criteria (Williams et al., 1994). Participants did not use anti-inflammatory, anti-histamines and antibiotic medicine during, and 4 weeks prior to the study period. Furthermore, volunteers with systemic inflammatory, immunological and malignant diseases except AD were excluded. No sunbathing during, and 4 weeks prior to the study period was permitted. Also pregnant and lactating woman were excluded.

2.3. Estimation of the exposure concentration from the silver release test

Preceding the in vivo study, the release test was performed by soaking a sample of Silverlon[®] material in synthetic sweat for 8 h (2.5 cm²/ml). Synthetic sweat consisted of 0.5% (w/v) NaCl, 0.1% (w/v) lactic acid and 0.1% (w/v) urea in MilliQ water adjusted to pH 4.5 with 3.8% ammonia solution. The concentration of total silver released in synthetic sweat was determined by ET-AAS (4100 ZL, PerkinElmer, Waltham, MA, USA) with transversely heated graphite atomizer (THGA) and Zeeman background correction. The concentration of Ag released into sweat solution amounted to 300.3 µg/L which equals the release of 0.12 µg Ag/cm² of surface area. We assumed that in the in vivo dermal exposure all released silver will be transferred into the sweat layer on the skin. The volume of sweat has been estimated from literature data of sweat rate of 4.03 mg/cm² (Eishi et al., 2002). Assuming a density of sweat of 1 g/cm³, the calculated total silver concentration (i.e. exposure concentration), was 29.8 µg/cm³ (29.8 ppm) using the data as given above (0.12 µg Ag/cm²/4.03 mg/cm² × 10⁻³ g/cm³).

2.4. Tape stripping of the stratum corneum

The samples of the stratum corneum (SC) for the analysis of total silver mass have been collected on days 2 and day 5. The SC layers were sequentially removed with circular adhesive tapes (3.8 cm², standard D-Squame, Monaderm, Monaco, France). The tapes were consecutively attached to the volunteer's underarm and pressed for 10 seconds with a constant pressure (225 g/cm²) using a D-Squame pressure instrument D500 (CuDerm, Dallas, TX, USA). To normalize for the variable amount of SC material on tape strips, the optical density (OD) was measured using the D-Squame Scan 850A (Monaderm, Monaco, France). In this already validated method, SC material on the tape was calculated as the amount of protein using a procedure described elsewhere (Voegeli et al., 2007; Hahn et al., 2010). Assuming a SC density of 1 g/cm³ (Anderson and Cassidy, 1973) and uniform distributions of SC on the tapes and proteins within the SC, the protein mass removed was converted to a volume, enabling the calculation of depth of each strip in the SC(x) as described elsewhere (Pirrot et al., 1997).

2.5. Determination of silver in tape strips

To each tape strip 1 mL MilliQ water and 0.5 mL of ultrapure HNO₃ (69%) was added. Samples were sonicated for 15 mins and diluted to a final volume of 6.5 mL with MilliQ water. The total silver concentration in the tape strips was determined by an inductively coupled plasma mass spectrometry, ICP-MS 7500CE (Agilent Technologies, Santa Clara, CA, USA). The limit of detection of the method, LoD was 0.05 µg/L, RSD < 5%.

2.6. Determination of silver in urine

Urine samples were collected before and after ending of the 5-days exposure. As only a spot samples of urine were collected, the Ag concentrations were normalized with creatinine levels. The total silver concentration in urine was determined by a high resolution inductively coupled plasma mass spectrometry, HR-ICP-MS (Thermo Fisher Scientific, Bremen, Germany) (methodological limit of detection, LoD = 0.015 µg/L). For the quantification, an external calibration with internal standard correction by the element rhodium (Rh) was applied. The results which were under the detection levels of the method (0.015 µg/L) have been set to the 1/2 of the detection limit (0.008 µg/L).

2.7. Analysis of cytokines in tape strips

The cytokines interleukin IL-1α and its receptor antagonist IL-1RA were determined in the SC tapes collected after the 5th day from the exposed and placebo forearm. To determine the amount of soluble protein and cytokines, the tape strips were extracted in phosphate-buffered saline (pH 7.4) using ultrasound sonification (de Jongh et al., 2006). The concentration of soluble protein was determined with a Pierce Micro BCA protein assay kit (Thermo Scientific, The Netherlands) and cytokine concentrations were determined using ELISA assay (Bio-Source International, CA, USA) as described elsewhere (de Jongh et al., 2006).

2.8. Data analysis

To estimate the penetration parameters, an approach based on Fick's second law of diffusion, which applies to the non-steady state condition was used. The theoretical background and application of this method are extensively described in the studies of Crank (1975) and Pirrot et al. (1997). In this method, two parameters, K and D/L², are estimated by best-fit regression of the

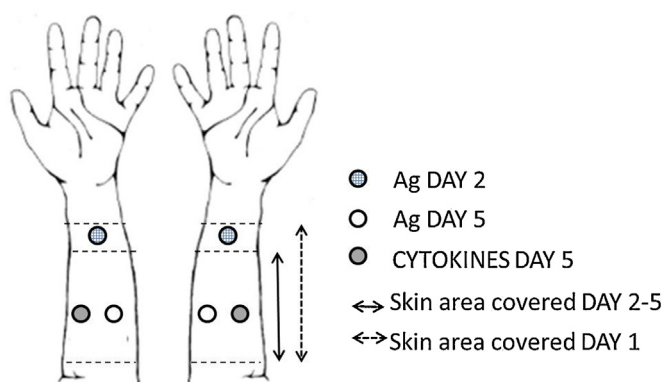


Fig. 2. Tape stripping skin sites and sampling schedule.

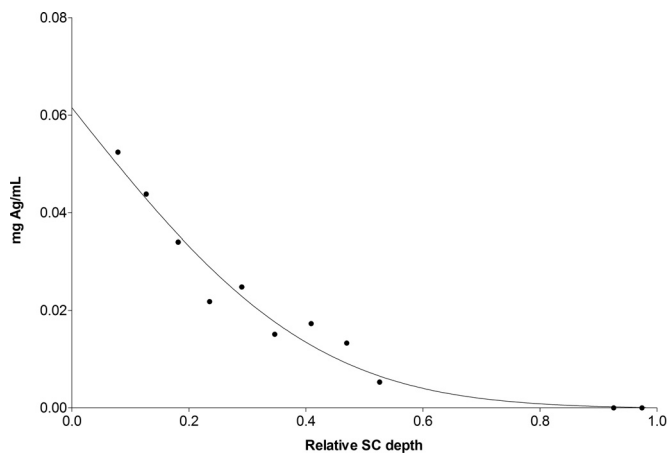


Fig. 3. Concentration decay of silver as a function of normalized position (x/L) in the SC after the first 8-h exposure to a Silverlon® sleeve.

concentration of silver as a function of relative SC depth (x/L) (Fig. 3) to the following equation:

$$c(x) = K \times c_{veh} \left(1 - \frac{x}{L}\right) - \sum_{n=1}^{\infty} \frac{2}{n\pi} K \times c_{veh} \sin\left(\frac{n\pi x}{L}\right) \exp\left(\frac{-Dn^2\pi^2 t}{L^2}\right) \quad (1)$$

where c_{veh} is the applied silver concentration (i.e. the concentration of silver in the sweat layer covering the skin surface) ($29.8 \mu\text{g}/\text{cm}^3$), c is the silver concentration (mg/cm^3) at SC depth x (cm), K is the SC/water partition coefficient of silver, L is the total thickness of the SC (cm), D is the apparent diffusion coefficient of silver through the pseudo-homogeneous SC (cm^2/h), D/L^2 (h^{-1}) is the diffusion rate constant, and t is the exposure duration (h). The fitted curve represents the solution to the Fick's second law of diffusion providing the estimates of K and D . In deriving the equation, K and D/L^2 are assumed to be constant throughout the SC. The apparent permeability coefficient (K_p , cm/h), which is commonly used for the risk assessment was estimated from the relationship $K_p = K \times D/L$. The values of K and D are obtained from the curve fitting and the thickness of the SC (L) is derived from the total amount of proteins removed by tape stripping. Dermal kinetics can be derived by this method only after a single exposure (Day 2). After day 5, only a cumulative amount of silver in the SC has been deducted from the area under the curve (AUC).

Prism 5 (Graph Pad Software Inc., San Diego, CA) for curve fitting and statistical calculations was used. Student's t -test (or Mann–Whitney test in case of non-Gaussian distribution of data) was used to test the differences between AD and healthy subjects. For cytokines, Wilcoxon matched pairs test was used to compare the values of the silver containing and placebo sleeves. A p -value of

<0.05 was considered significant. In the case of deviation from Gaussian distribution of data, median and interquartile ranges have been shown.

3. Results

3.1. Percutaneous penetration of silver as determined by tape stripping of the stratum corneum

Fig. 3 shows a typical concentration profile of silver across the SC obtained after the first 8 h of exposure. The average number of strips needed to remove the SC was 22 and 22 in healthy subjects and AD patients, respectively. The fitted curve represents the solution to the Fick's second law of diffusion providing the estimates of K and D . The curve fitting could not be performed for 3 healthy volunteers and 3 AD patients because of excessive variability of data points. The apparent permeability coefficient, K_p , has been estimated as described in Data analysis section. From K_p , the exposed skin surface and the exposure duration, the steady state flux has been estimated as follows: J ($\text{ng cm}^{-2} \text{h}^{-1}$) = $K_p \times c_{veh}$. Steady state dermal fluxes of silver estimated as described above are shown in Table 1. There was no significant difference in the fluxes between control subjects and subjects with AD (Table 1). By using the area under the concentration vs. depth curve (AUC) the amount of silver present in the entire stratum corneum has been estimated (Table 1). As seen from Table 1, the amount of silver in the SC was higher after Day 5 as compared to Day 2 which indicates accumulation of silver in the SC during repeated exposure. However, also for the absorbed amounts of silver in the SC there were no significant differences between healthy individuals and AD patients (Table 1).

3.2. Silver concentrations in urine

The concentrations of silver in urine sampled before and after 5 days of exposure are shown in Table 2. There was a large interindividual variation in the concentrations of silver, even after normalizing with creatinine and there was no significant increase of silver in urine after 5×8 h of dermal exposure either in healthy volunteers or in AD patients (as tested by a Wilcoxon matched pair test).

3.3. IL-1 Cytokines

The results of IL-1 cytokines in the SC collected on day 5 after the last 8-h exposure are shown in Fig. 4. There were no significant differences in the levels of investigated IL-1 cytokines between the silver- and placebo treated arm in both healthy volunteers (CTRL) and in AD patients, which indicates absence of any pro-inflammatory effect of silver in the garment. The values of IL-1RA were higher in AD patients compared to healthy volunteers,

Table 1

Dermal flux and absorbed amount of silver in the stratum corneum in healthy volunteers (Ctrl) and subjects with AD (AD) after 1st and 5th 8-h exposure. Results are expressed as median (25%; 75% quartiles).

	Ctrl <i>n</i> = 12	AD <i>n</i> = 12	Ctrl+AD <i>n</i> = 24
Dermal flux $\text{ng}/\text{cm}^2/\text{h}$	0.23 (0.12; 0.38)	0.20 (0.08; 0.41)	0.21 (0.11; 0.38)
Diffusion coefficient (D) $\text{cm}^2/\text{h} \times 10^{-10}$	9.08 (3.56; 13.62)	4.66 (2.93; 10.81)	8.09 (3.24; 12.44)
Permeability coefficient (K_p) $\text{cm}/\text{h} \times 10^{-6}$	7.79 (4.13; 12.71)	6.83 (2.67; 13.88)	7.11 (3.66; 12.71)
Ag (ng) absorbed in the SC 1st exposure	57.4 (26.1; 100.9)	73.9 (25.7; 115.0)	62.7 (26.1; 108.6)
Ag (ng) absorbed in the SC 5th exposure	105.0 (57.3; 156.6)	119.8 (65.7; 262.1)	116.3 (59.0; 186.0)

Table 2

Silver concentration in urine of control subjects (Ctrl) and subjects with AD (AD) before (Day 0) and after 5 days (Day 5) of exposure to Silverlon[®] sleeve. Results are expressed as median (25%; 75% quartiles).

	Ctrl <i>n</i> = 12	AD <i>n</i> = 12	Ctrl+AD <i>n</i> = 24
Day 0			
μg Ag/L of urine	0.014 (0.008; 0.02)	0.02 (0.008; 0.02)	0.02 (0.008; 0.02)
μg Ag/mol of creatinine	1.1 (0.57; 2.6)	2.3 (0.99; 3.04)	1.2 (0.67; 3.8)
Day 5			
μg Ag/L of urine	0.02 (0.011; 0.028)	0.02 (0.008; 0.028)	0.02 (0.008; 0.028)
μg Ag/mol of creatinine	2.0 (0.99; 3.04)	1.3 (0.64; 16.4)	1.9 (0.90; 16.4)

independently on the exposure (i.e. silver containing sleeve or placebo).

4. Discussion

Wearing of silver-containing sleeves resulted in low but measurable amounts of silver in the stratum corneum even after the first 8 h of exposure, revealing that silver is able to penetrate into and across the SC. The concentration-depth profiles of silver obtained from the silver concentrations in different layers of the SC after the first 8-h exposure period enabled determination of the steady state dermal flux which amounted to respectively 0.20 and 0.23 ng/cm²/h in the AD patients and healthy volunteers. There was no significant differences in dermal fluxes between healthy subjects and AD patients, although it has to be noted that the skin sites investigated for the percutaneous penetration were not visually affected by disease and the patients had only mild symptoms.

Literature data on *in vivo* silver absorption after dermal exposure are scarce and to our best knowledge this is the first study in which dermal flux has been estimated after repeated, controlled exposure. Several studies reported systemic uptake of silver by measuring an increase of silver plasma and urine levels in patients with burns treated with silver dressings, however the quantitative relationship between exposure to silver in these studies and the silver levels in biological samples is difficult to establish (Moimen et al., 2011).

The silver levels in the SC in the present study have been measured as total silver, so no characterization of silver species has been done. Several studies showed that silver is released from fabrics mainly in ionic form as Ag⁺, although dependently on the physico-chemical properties of used silver and method applied to treat the fibers, also metallic silver particles of nano or micro size can be released (Stefaniak et al., 2014; von Götz et al., 2013). Silver ions readily react with different constituents present in the sweat

on the skin surface and dependently on the solubility of these compounds, silver can be present in dissolved form or as particulate. Silver ions, its soluble forms, but theoretically also particles of several nm size could penetrate into the skin. Von Götz et al. (2013) investigated in an experimental setting the release of silver into artificial sweat from various textiles with claimed antimicrobial properties and found particles smaller than 450 nm in detectable amounts (23–74 μg/g/L).

The concentration of soluble Ag species which are able to penetrate the stratum corneum varied in that study from 43–45 μg/g/L. Stefaniak et al. (2014) found that the relative amount of ionic silver is also dependent on its concentration in sweat solution. At higher concentrations the silver rapidly reacted with sweat chloride ions to form poorly soluble silver chloride particles. In contrast, at lower silver ion concentrations measured in the use scenario experiment, silver was primarily in the ionic fraction. Clearly, the prediction of the concentration of bioavailable silver is the biggest challenge in determining the percutaneous penetration of silver and assess the health risk for a certain exposure scenario. In the present study we assumed that all silver which is released from the garment is available for penetration and further that silver present in the SC is available for systemic absorption.

The question arises whether dermal absorption under real life exposure scenarios can lead to adverse health effects. For this purpose we estimated the hypothetical uptake of silver in a baby who wears a comparable silver-containing garment for 8 h/day. There is currently no reference dose for dermal exposure but US Environmental Protection Agency (EPA) has established a chronic oral Reference Dose (RfD) for silver ingestion of 5 μg per kg of body weight per day based on a review of 70 cases of argyria that were associated with oral and other uses of silver compounds (EPA, IRIS). Argyria is a medically benign but permanent bluish-gray discoloration of the skin. To calculate the uptake associated with dermal exposure the following equation has been used:

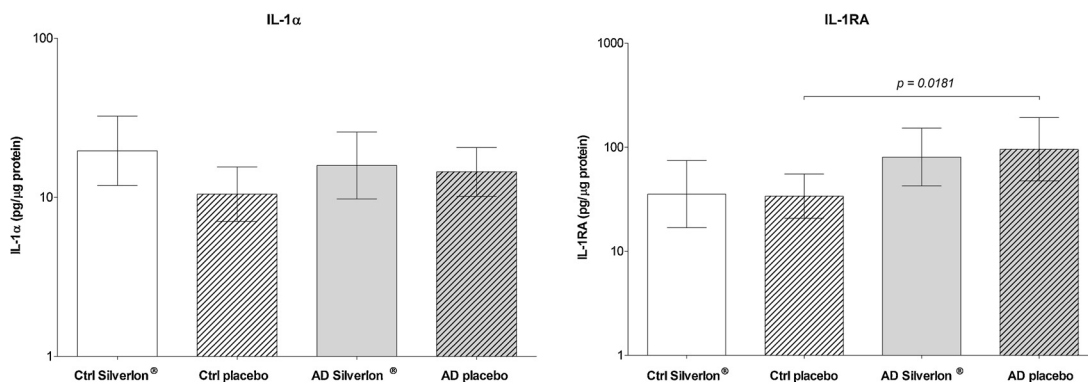


Fig. 4. The levels of IL-1 cytokines in the stratum corneum after 5 × 8-h dermal exposure to respectively Silverlon[®] and placebo sleeves (geometric mean ± 95% CI). The differences in cytokine concentrations between Silverlon[®] and placebo sleeve were tested by a Wilcoxon matched paired test and between AD patients and healthy controls by Mann–Whitney test.

Dermaluptake(mg/kg/day)

$$= \frac{J(\text{mg}/\text{cm}^2/\text{h}) \times \text{Exposure area}(\text{cm}^2) \times \text{Exposure time}(\text{h})}{\text{Body weight}(\text{kg})} \quad (2)$$

The skin surface area of a child from three to six months of age is estimated to be 0.38 m² (EPA, Exposure factor handbook, 2011) with average body weight of 7.4 kg. Contribution of trunk, arms and legs to the total skin surface is 70% and corresponds to 0.266 m² (EPA, 2011). The estimated flux of 2.1 mg/cm²/h has been used for the calculation (Table 1). Assuming that a child (average body weight of 7.4 kg) is exposed to silver coated textile covering only trunk, legs and arms (with skin surface area of a 0.266 m²) during 8 h and that all textile is in close contact with the skin, the average dermal uptake can be calculated using the equation:

$$\begin{aligned} \text{Dermaluptake}(\text{mg}/\text{kg}/\text{day}) &= \frac{2.1 \times 10^{-7} \text{ mg}/\text{cm}^2/\text{h} \times 2660 \text{ cm}^2 \times 8 \text{ h}}{7.4 \text{ kg}} \\ &= 6.01 \times 10^{-4} \text{ mg}/\text{kg}/\text{day} \end{aligned} \quad (3)$$

This equals to the uptake of 0.6 µg/kg of body weight/day. This value is well below the RfD value of 5 µg per kg of body weight per day. For comparison, the current norm for drinking water in the USA is 0.1 mg/L (EPA, 2012). Assuming daily drinking water consumption for a 10 kg child to be 1 L/day, the uptake via water is approximately 0.01 mg/day/kg which is higher than dermal uptake 0.6 µg/kg of body weight/day calculated above. It has to be noted that the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. In general, silver containing garments are used only in periods with disease exacerbations and furthermore AD clears up during infancy or childhood in the majority of children (Garmhausen et al., 2013) so the exposure to silver will be intermittent and over a limited period during a lifetime.

Low systemic uptake as calculated from the estimated dermal flux is consistent with low amounts of silver detected in urine samples. The concentrations of silver in urine before and after exposure were within the concentration range (Table 2) reported in the literature for the general population which is caused by environmental exposure to silver, e.g. via drinking water and food (Rodushkin and Ödman, 2001; Rodushkin et al., 2001, 2004) (Fung and Bowen, 1996). Clinical studies in patients treated with silver medical products for severe burns have shown that elevated silver levels were detectable in urine within 24 h, thus the exposure time of 5 days in the present study would have been long enough to detect an increase of silver in urine (Wan et al., 1991). Boosalis et al. (1987) found the mean urinary silver excretion after treatment for severe burns with silver sulfadiazine was found to reach approximately 1100 µg/L; much higher than the average levels detected in the present study of 0.02 µg/L.

Exposure to silver did not lead to increased expression of IL-1 cytokines in the SC as there was no significant difference in the measured values before and after wearing of silver containing sleeves neither in AD patients or in the healthy volunteers. These cytokines are highly abundant in the SC and are released after damage of the skin barrier or inflammation as e.g. shown after exposure to a skin irritant (de Jongh et al., 2006). IL-1α, when released, mediates local inflammation, which is prevented by IL-1 RA. Though, in placebo treated skin IL-1RA was higher in AD patients as compared to healthy volunteers which can be explained by an increased inflammatory status in atopic dermatitis (Kezic et al., 2012).

The performed study has several uncertainties which should be considered in the interpretation of the results. Several assumptions have been made to calculate the exposure concentration. It has been assumed that the total amount of silver found in the release test is available for dermal absorption. However, as addressed previously, released silver ions in contact with sweat form complexes and salts which limits the amount of dissolved silver available for penetration. Furthermore, silver can be present as particulate (von Götz et al., 2013) that might have different penetration kinetics dependently on the particle size. As the speciation of silver in the present study has not been done we assumed a worst case scenario, i.e. the total amount of silver which is released from the garment into sweat is available for penetration.

Another assumption made in the present study is that silver present in the SC is available for systemic absorption. However, silver ions once in the SC can react with different functional groups of the present proteins and free amino acids, especially those containing sulfur, which might reduce the amount of silver available for the penetration and systemic uptake (Liu et al., 2012).

The value for the volume of sweat on the skin surface needed for the calculation of the exposure concentration has been taken from the literature (Eishi et al., 2002). Possible changes in the sweat volume due to absorption into the garment or occlusion have not been taken into account.

In conclusion, the in vivo data based on the percutaneous penetration and excretion of silver in urine revealed that dermal absorption of silver after wearing a garment containing 13% silver is low and not likely to lead to systemic effects. Furthermore, dermal exposure to silver from the investigated garment does not lead to altered expression of inflammatory IL-1 cytokines in the skin, indicating absence of a local pro-inflammatory effect.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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References

- Anderson, R.L., Cassidy, J.M., 1973. Variation in physical dimensions and chemical composition of human stratum corneum. *J. Invest. Dermatol.* 61, 30–32.
- Boosalis, M.G., McCall, J.T., Ahrenholz, D.H., Solem, L.D., McClain, C.J., 1987. Serum and urinary silver levels in thermal injury patients. *Surgery* 101 (1), 40–43.
- Mathematics of Diffusion. In: Crank, J. (Ed.), Oxford University Press, Oxford, pp. 50–51.
- Eishi, K., Lee, J.-B., Bae, S.-J., Takenada, M., Katayama, I., 2002. Impaired sweating function in adult atopic dermatitis: results of the quantitative sudomotor axon reflex test. *Br. J. Dermatol.* 147, 683–688.
- EPA Integrated Risk Information System (IRIS) (<http://www.epa.gov/iris/subst/0099.htm>).
- Exposure Factor Handbook. National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460, 2011 Edition.
- Edition of the drinking water: Standards and Health Advisories, EPA 822-S-12-001, Office of Water U.S. Environmental Protection Agency Washington, D.C.
- Fung, M.C., Bowen, D.L., 1996. Silver Products for Medical Indications: Risk-Benefit Assessment. *Clin. Toxicol.* 34 (1), 119–126.

- Garmhausen, D., Hagemann, T., Bieber, T., Dimitriou, I., Fimmers, R., Diepgen, T., Novak, N., 2013. Characterization of different courses of atopic dermatitis in adolescent and adult patients. *Allergy* 68, 498–506.
- Götz von, N., Lorenz, C., Windler, L., 2013. Migration of Ag- and TiO₂-(Nano) particles from Textiles into Artificial Sweat under Physical Stress: Experiments and Exposure Modeling. *Environ. Sci. Technol.* 47 (17), 9979–9987.
- Hahn, T., Hansen, S., Neumann, D., Kostka, K.H., Lehr, C.M., Muys, L., Schaefer, U.F., 2010. Infrared densitometry: a fast and non-destructive method for exact stratum corneum depth calculation for in vitro tape-stripping. *Skin. Pharmacol. Physiol.* 23, 183–192.
- Jongh de, C.M., Verberk, M.M., Withagen, C.E., Jacobs, J.J., Rustemeyer, T., Kezic, S., 2006. Stratum corneum cytokines and skin irritation response to sodium lauryl sulfate. *Contact Dermatitis* 54, 325–333.
- Kezic, S., O'Regan, G.M., Lutter, R., Jakasa, I., Koster, E.S., Saunders, S., Caspers, P., Kemperman, P.M., Puppels, G.J., Sandilands, A., Chen, H., Campbell, L.E., Kroboth, K., Watson, R., Fallon, P.G., McLean, W.H., Irvine, A.D., 2012. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J. Allergy Clin. Immunol.* 129, 1031–1039.
- Kezic, S., Novak, N., Jakasa, I., Jungersted, J.M., Simon, M., Brandner, J.M., Middelkamp-Hup, M.A., Weidinger, S., 2014. Skin barrier in atopic dermatitis. *Front. Biosci. (Landmark Ed.)* 19, 542–556.
- Liu, J., Wang, Z., Liu, F.D., Kane, A.B., Hurt, R.H., 2012. Chemical transformations of nanosilver in biological environments. *ACS Nano* 6, 9887–9899.
- Moiemen, N.S., Shale, E., Drysdale, K.J., Smith, G., Wilson, Y.T., Papini, R., 2011. Acticoat dressings and major burns: systemic silver absorption. *Burns* 37, 27–35.
- Pirot, F., Kalia, Y.N., Stinchcomb, A.L., Keating, G., Bunge, A., Guy, R.H., 1997. Characterization of the permeability barrier of human skin in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 94, 1562–1567.
- Rodushkin, I., Ödman, F., 2001. Application of inductively coupled plasma sector field mass spectrometry for elemental analysis of urine. *J. Trace Elem. Med. Biol.* 14, 241–247.
- Rodushkin, I., Ödman, F., Olofsson, R., Burman, E., Axelsson, M.D., 2001. Multi-element analysis of body fluids by double-focusing ICP-MS. *Transworld Res. Network. Recent Res. Devel. Pure & Applied Chem.* 5, 51–66.
- Rodushkin, I., Engström, E., Stenberg, A., Baxter, D.C., 2004. Determination of low-abundance elements at ultra-trace levels in urine and serum by inductively coupled plasma-sector field mass spectrometry. *Anal. Bioanal. Chem.* 380, 247–257.
- Stefaniak, A.B., Duling, M.G., Lawrence, R.B., Thomas, T.A., LeBouf, R.F., Wade, E.E., Abbas Virji, M., 2014. Dermal exposure potential from textiles that contain silver nanoparticles. *Int. J. Occup. Environ. Health* 20 (3), 220–234.
- Voegeli, R., Heiland, J., Doppler, S., Rawlings, A.V., Schreier, T., 2007. Efficient and simple quantification of stratum corneum proteins on tape strippings by infrared densitometry. *Skin. Res. Technol.* 13, 242–251.
- Wan, A.T., Conyers, R.A.J., Coombs, C.J., Masterton, J.P., 1991. Determination of silver in blood, urine and tissue of volunteers and burn patients. *Clin. Chem.* 37, 1683–1687.
- Williams, H.C., Burney, P.G., Hay, R.J., et al., 1994. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br. J. Dermatol.* 131, 383–396.
- Windler, L., Height, M., Nowack, B., 2013. Comparative evaluation of antimicrobials for textile applications. *Environ. Int.* 53, 62–73.