

Skin-protective effects of a zinc oxide-functionalized textile and its relevance for atopic dermatitis

Cornelia Wiegand¹
Uta-Christina Hipler¹
Sebastian Boldt²
Joachim Strehle²
Uwe Wollina²

¹Department of Dermatology, University Medical Center Jena, Jena, Germany; ²Department of Dermatology and Allergology, Academic Teaching Hospital Dresden-Friedrichstadt, Dresden, Germany

Abstract: Atopic dermatitis (AD) is a chronic inflammatory disease characterized by the impairment of the skin-barrier function, increased oxidative cellular stress, and bacterial colonization. Hence, medical therapies of AD aim to control infection, reduce inflammation, and restore skin-barrier function by use of topical and systemic antibacterial drugs, topical corticosteroids, topical calcineurin inhibitors, and moisturizers. Textiles have the longest and most intense contact with the human skin, and functional textiles with intrinsic properties such as antioxidative capacity and antibacterial activity have been gaining in importance in medical applications. Specially designed textiles may support AD treatment and improve quality of life of AD. Here, we investigated the role of ZnO-functionalized textile fibers in the control of oxidative stress in AD in vitro and in vivo. In addition, the antibacterial effect and biocompatibility of the Zn textile was evaluated in vitro. We observed a rapid improvement of AD severity, pruritus, and subjective sleep quality when AD patients wore the ZnO textiles overnight on 3 consecutive days. This is possibly due to the high antioxidative capacity of the ZnO textile, as well as the allocation of strong antibacterial activity. Moreover, it was shown that the ZnO textiles possess very good biocompatibility and were well tolerated by AD patients.

Keywords: atopic dermatitis, antibacterial activity, biocompatibility, functionalized textiles, oxidative stress

Introduction

The skin is the external barrier of the human body. It is the most versatile human organ, plays a key role in protecting the body against environmental influences, and participates in the regulation of homeostasis and metabolic processes as well as immunological reactions. Exposure to ultraviolet light induces the generation of free radicals in the cells, like reactive oxygen species (ROS) and reactive nitrogen species (RNS). Increased oxidative stress has been documented in affected skin of individuals suffering from atopic dermatitis (AD)¹⁻⁴ and seems to be involved in inflammation, skin-barrier impairment, and itch. Moreover, measurements of transepidermal water loss and corneometry have shown an inverse relation to AD severity.⁵ In addition, patients with AD display an augmented susceptibility to cutaneous bacterial, fungal, and viral infections.^{6,7} Microbial flora of atopic skin exhibits noticeable differences in contrast to normal skin, eg, more than 90% of patients with AD are colonized with *Staphylococcus aureus*, whereas it is found in less than 10% in healthy individuals.⁸ The carriage rate of AD patients for *S. aureus* was observed to be >90% in inflammatory lesions and approximately 76% in uninvolved skin.⁹ Furthermore, the quantity of *S. aureus* could be related to the severity of AD.^{9,10} Hence, medical therapies of AD

Correspondence: Uwe Wollina
Department of Dermatology and
Allergology, Academic Teaching
Hospital Dresden-Friedrichstadt,
Friedrichstrasse 41, Dresden 01067,
Germany
Tel +49 351 480 1685
Fax +49 351 480 1219
Email wollina-uw@khdf.de

aim to control infection, reduce inflammation, and restore skin-barrier function by the use of topical and systemic antibacterial drugs,¹¹ topical corticosteroids, topical calcineurin inhibitors, and moisturizers.^{12–14} Textiles have the longest contact with the human skin and have a major impact on its microenvironment. Hence, specially designed textiles may support medical treatment and improve quality of life of patients with AD.¹⁵

Here, we investigated the role of ZnO-functionalized textile fibers in the control of oxidative stress in AD in vitro and in vivo. In addition, the antibacterial effect and biocompatibility of the Zn textile was evaluated in vitro.

Material and methods

In vivo pilot trial

We investigated the in vivo effects of a special AD textile, Benevit Zink+ (Benevit Van Cleve, Dingden, Germany), which consists of 74% Lyocell fiber, 19% SmartCell sensitive fiber, and 7% spandex. In this uncontrolled pilot trial, adult patients with moderate to severe AD were enrolled after informed consent. Three days after individualized topical treatment with corticosteroids, calcineurin inhibitors, or coal tar, the ZnO textile was used for overnight underclothes with trousers and long-sleeve shirts (Figure 1) for three subsequent nights. AD diagnosis was performed according to the clinical criteria of Hanifin and Rajka.¹⁶ Severity was calculated by the three item severity score index before and after use of the Zn textile.¹⁷ The severity of pruritus was scored by the 5D itch scale.¹⁸ Subjective quality of sleep was assessed by a modified sleep-habits questionnaire.¹⁹

In vitro study

Measurement of antioxidative capacity

The antioxidative capacity (AOC) of the ZnO textile against ROS was assessed by an Abel antioxidant test kit with Pholasin for superoxide and other free radicals (Knight Scientific, Plymouth, UK) and scavenging of RNS was determined by Abel antioxidant test kits specific for peroxynitrite anion (Knight Scientific). The assays were performed as described earlier.²⁰ In brief, to each ZnO-textile sample (0.25 cm² and 0.5 cm²) the assay solutions were added. Subsequent to injecting the solution, generating free radicals, luminescence intensity was measured using the Novostar Galaxy plate reader (BMG Labtech, Ortenberg, Germany). A control without sample was included for each assay. Antioxidant activity impedes the development of the luminescence peak (peroxynitrite assay) and decreases its



Figure 1 Benevit Zink+ clothing contains 74% Lyocell fiber, 19% SmartCell sensitive fiber (comprised of ZnO), and 7% spandex.

Notes: Adult patients with moderate to severe atopic dermatitis were enrolled after informed consent. This ZnO textile was used for overnight underclothes in the form of trousers and long-sleeve shirts.

light intensity (peroxynitrite assay and superoxide assay). AOC of a sample was calculated as percentage reduction of peak luminescence, as given in equation (1). All experiments were carried out twice with a triplicate sample set. Results are expressed as means \pm standard deviation. For statistical analysis, Student's *t*-test was performed. Statistical significance was defined as $P < 0.05$. Statistically significant results are identified by asterisk symbols: *** $P \leq 0.001$, ** $P \leq 0.01$, and * $P \leq 0.05$.

$$\% \text{ inhibition} = \frac{[(\text{Peak-control}) - (\text{Peak-probe})]}{(\text{Peak control})} \times 100. \quad (1)$$

Determination of antibacterial activity according to JIS L 1902

Testing for antibacterial activity was carried out in accordance with Japanese Industrial Standards L 1902: 2002, as reported previously.²¹ *Staphylococcus aureus* (American Type Culture Collection 6538) and *Klebsiella pneumoniae* were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen

(Braunschweig, Germany). For bacteria cultivation, special peptone and Lab-Lemco powder for fabrication of caso-bouillon and bacteriological agar were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Columbia agar plates with 5% sheep blood were acquired from BioMérieux (Marcy l'Etoile, France) and 0.9% NaCl solution from Fresenius Kabi (Bad Homburg, Germany). Test microbes were cultivated in caso-bouillon for 24 hours at 37°C under aerobic conditions. For experiments, 400 mg samples of the ZnO textile were inoculated with 200 µL test microbe solution and incubated for 24 hours at 37°C under aerobic conditions. Polyester material (ITS Textilhandels, Rutzenham, Austria) was used as growth control. For determination of the germ number, the incubated samples were extracted in 0.9% NaCl solution supplemented with 0.2% Tween 20. Serial dilutions were plated onto Columbia agar plates and incubated for 24 hours at 37°C. Subsequently, colonies were counted, total colony-forming units (cfu) determined, and growth reduction calculated according to equation (2). A logarithmic microbial growth reduction of less than 0.5 represented no antibacterial activity. Values between 0.5 and 1 were rated as slight, values greater than 1 and less or equal to 3 as significant, and a log reduction greater than 3 as strong antibacterial activity.

$$\text{Log growth reduction}_{(24 \text{ hours})} = \text{Log cfu (negative control)} - \text{Log cfu (sample)}_{(24 \text{ hours})} \quad (2)$$

Evaluation of biocompatibility according to DIN EN ISO 10993-5

For evaluation of biocompatibility, extracts of the ZnO textile were fabricated according to the standard used for evaluation of textile cytotoxicity (DIN EN ISO 10993-12). In brief, 1 g was incubated in 50 mL of Dulbecco's modified Eagle's medium (DMEM) in Erlenmeyer flasks (Greiner, Frickenhausen, Germany) at 37°C for 24 hours under shaking

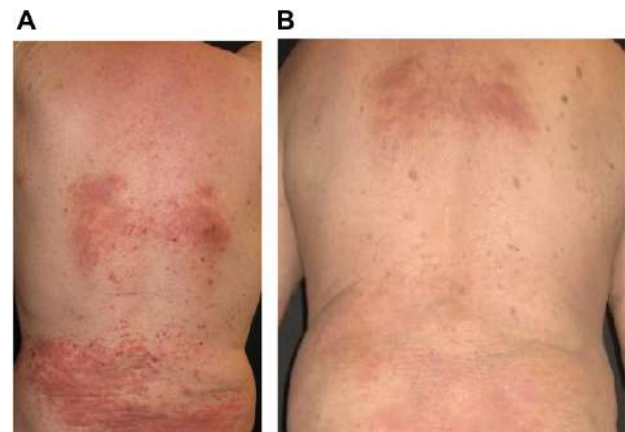


Figure 2 Clinical improvement of nummular atopic dermatitis with adjuvant ZnO textile overnight (A) before and (B) after 4 days.

(ThermoBath, GFL, Großburgwedel, Germany). At all times, samples were handled under sterile conditions and extra care was taken for avoiding cross-contamination. Subsequently, extracts were centrifuged at 1000 rpm over gauze to dispose of insoluble material residues. The resulting filtrate was sterilized afterwards by passage through a 0.2 µm filter and classified as original extract (100%).

Human HaCaT keratinocytes were a kind gift from NE Fusenig of the German Cancer Research Center, Heidelberg, Germany. The cells are routinely cultured in DMEM supplemented with 1% antibiotic-antimycotic solution (10,000 U/mL penicillin, 10,000 µg/mL streptomycin, 25 µg/mL amphotericin; PromoCell, Heidelberg, Germany) and 10% fetal calf serum (PromoCell) for 7 days in 75 cm² cell-culture flasks (Greiner) at 37°C in a humidified atmosphere containing 5% CO₂. For biocompatibility experiments, cells were harvested by trypsin-ethylenediaminetetraacetic acid (PromoCell) treatment, seeded into 96-well plates (Greiner) at a density of 40,000 cells/cm², and left for 48 hours. Then, culture medium was swapped for either fresh medium (negative control) or Zn-textile extracts in medium (original extract 100%,

Table I Summary of the results of the in vivo pilot study

	Before trial	After trial
Pruritus severity	3.5 ± 1.2	2.4 ± 1.1
Erythema	2.2 ± 0.8	1.0 ± 0.8
Edema and papules	1.9 ± 0.8	0.8 ± 0.6
Excoriations	1.7 ± 1.1	0.6 ± 0.5
Impairment by pruritus	3.7 ± 0.7	2.1 ± 1.1
Impairment of night sleep	1.7 ± 1.4	1.4 ± 0.7
Impairment at work	1.4 ± 1.5	0.7 ± 1.2
Impairment at leisure time	1.4 ± 1.6	0.7 ± 1.2
Impairment at housework	1.4 ± 1.5	1.0 ± 1.3

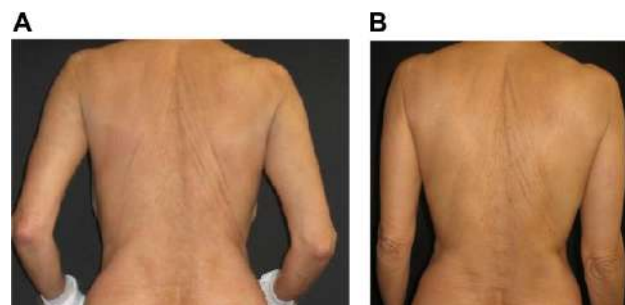


Figure 3 Clinical improvement of disseminated atopic dermatitis with adjuvant ZnO textile overnight (A) before and (B) after 4 days.

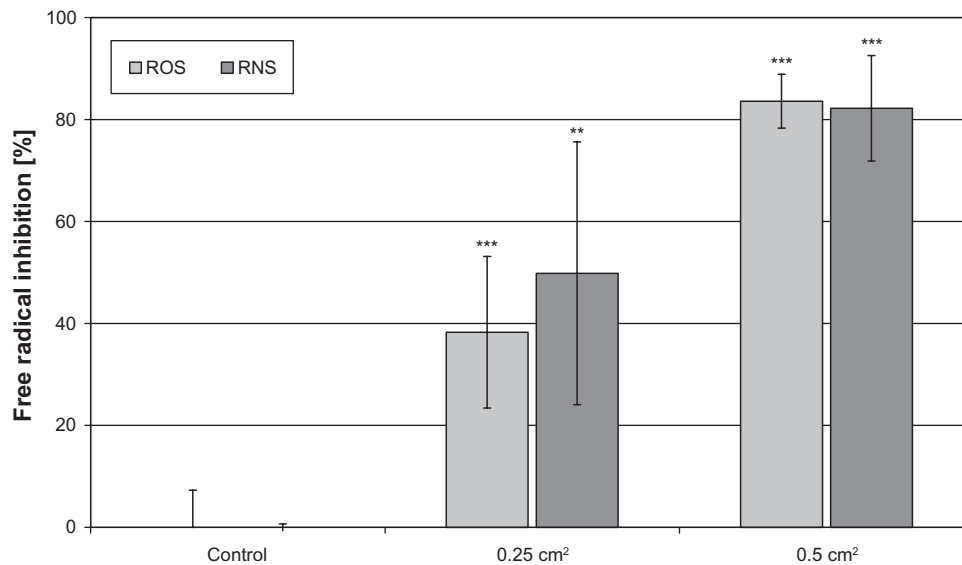


Figure 4 A high antioxidative capacity was determined for the ZnO-textile.

Notes: It was equally effective in inhibiting reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation in vitro. ** $p \leq 0.01$; *** $p \leq 0.001$.

extraction ratio 1 g:50 mL) and serial dilutions of the original extract (75% at 0.75 g:50 mL, 50% at 0.5 g:50 mL, 25% at 0.25 g:50 mL, and 10% at 0.1 g:50 mL). As positive control for cytotoxicity, a Triton-X 100 (Merck, Darmstadt, Germany) was utilized. The HaCaT cells were afterwards incubated for 1, 24, and 48 hours followed by determination of cell viability and proliferation, which was performed on the basis of a luminometric adenosine triphosphate (ATP) assay (ATPLite M Assay; PerkinElmer, Waltham, MA, USA), as reported previously.²² Briefly, lysis solution was added to each well, followed by the substrate solution (luciferase/D-luciferin). After

incubation, luminescence was determined using the Lumistar Galaxy microplate luminometer (BMG Labtech). Cellular ATP content was calculated on the basis of a standard curve. For illustration, the number of viable cells was expressed as a percentage of the control cells at the respective time point. Cytotoxic effects were analyzed by measurement of lactate dehydrogenase release using a cytotoxicity detection kit (Roche, Basel, Switzerland). The assay was run according to the manufacturer's instructions. Optical density was determined at 490 nm with a reference measurement at 620 nm (Fluostar; BMG Labtech). Afterwards, cytotoxicity was evaluated by

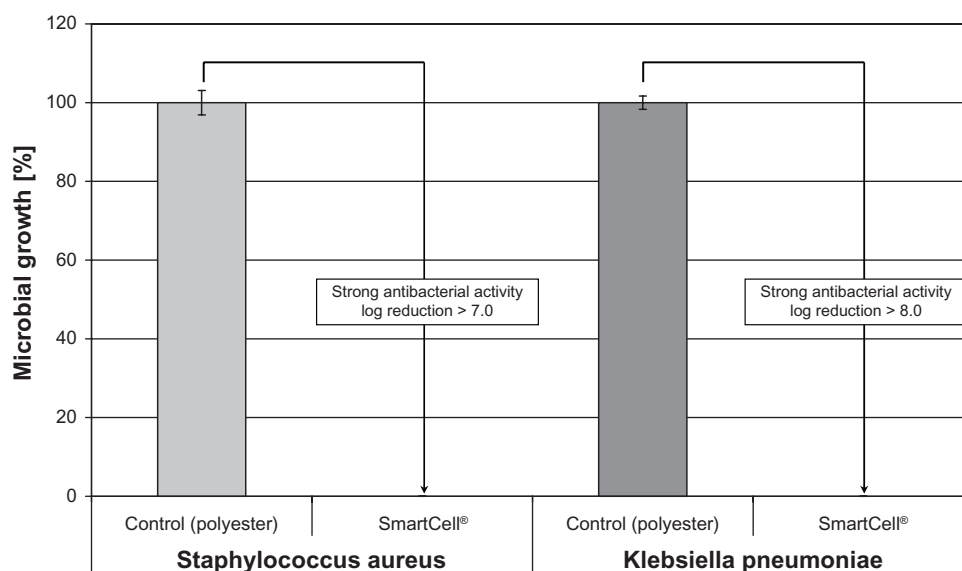


Figure 5 The ZnO textile possesses a strong antibacterial effect (log reduction > 3) against both *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Note: Complete inhibition of microbial growth was achieved.

calculation of the percentage of the optical density of treated cells compared to control cells.

Results

In vivo results

The results of the in vivo pilot trial are summarized in Table 1. Twelve adult AD patients were included

(five women, seven men) with a mean age of 60.6 ± 15.1 years (range 28–78 years). Before the use of the Zn textile, the average pruritus duration was 6–24 hours a day. Only four patients reported less than 6 hours of pruritus. After three nights with the Zn textile, ten patients reported less than 6 hours of pruritus. None of the patients suffered from pruritus all day long. Pruritus severity dropped from

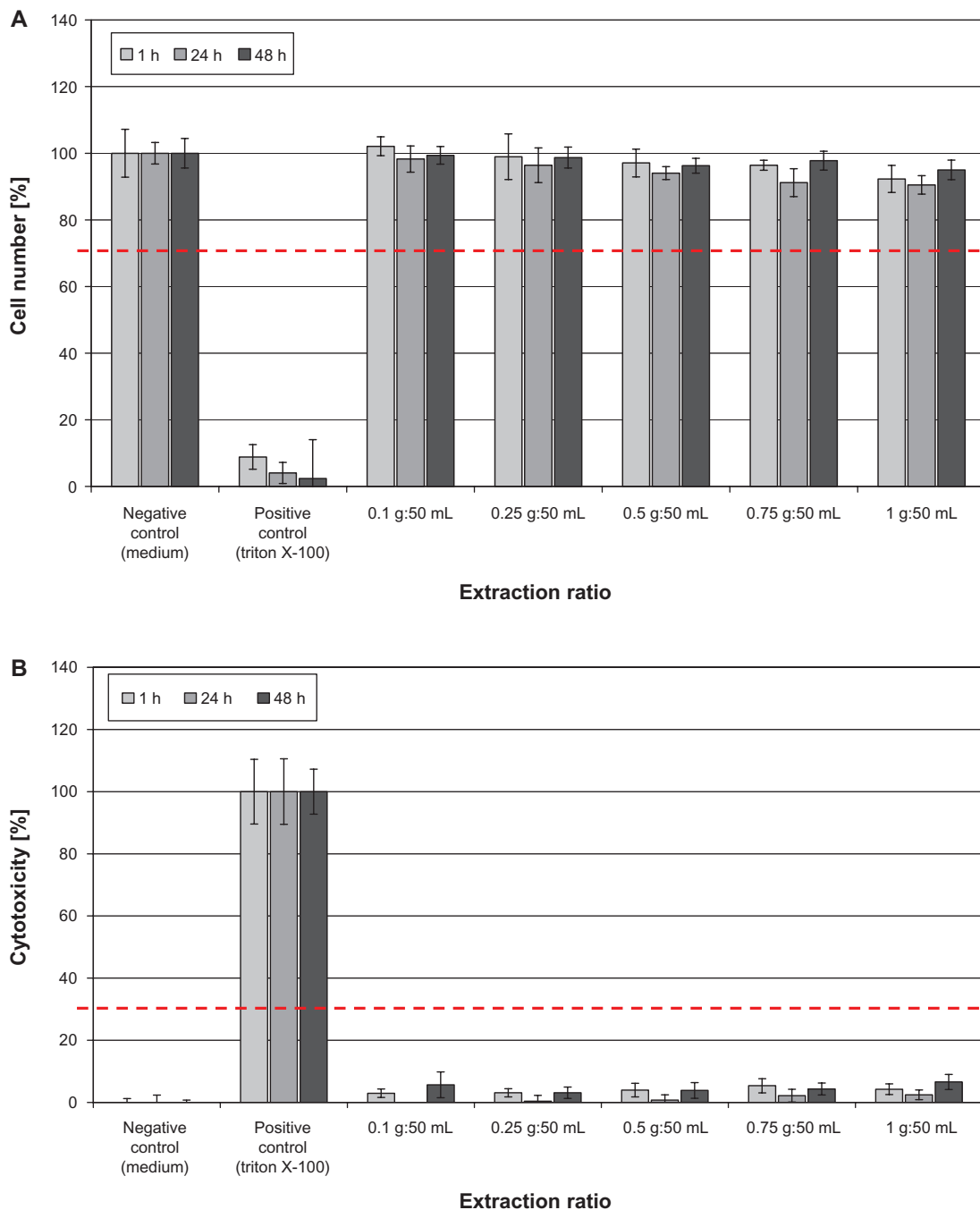


Figure 6 (A) Determination of HaCaT keratinocyte cell viability and cell proliferation after 1, 24, and 48 hours of incubation with ZnO-textile extract. No negative effect on the cells was observed in vitro. At no time point or extraction ratio tested did cell numbers drop below the 70% threshold (red dotted line). **(B)** A significant release of lactate dehydrogenase was observed only for the positive control Triton X-100.

Notes: None of the ZnO textile-extraction ratios tested exhibited a cytotoxic potential. In accordance, cytotoxicity did not reach the threshold of 30% (red dotted line).

3.5 ± 1.2 to 2.4 ± 1.1 . Impairment by pruritus was scored 3.7 ± 0.7 and decreased to 2.1 ± 1.1 .

The impairment of night sleep improved from 1.7 ± 1.4 to 1.4 ± 0.7 . Impairment during professional activities improved from 1.4 ± 1.5 to 0.7 ± 1.2 . Impairment of leisure time was reduced from 1.4 ± 1.6 to 0.7 ± 1.2 . Housework impairment also decreased from 1.4 ± 1.5 to 1.0 ± 1.3 .

Overall, sleep quality seemed to be better after use of the Zn textile. The number of patients suffering from wakefulness was reduced from five to two. Problems falling asleep were reported by six patients before use of the Zn textile and reduced to four patients after the use of the Zn textile overnight. Wake-up periods were noted by nine patients before and only six patients after the test period. Sleeping duration remained unchanged.

In addition, clinical cutaneous symptoms improved distinctly. Erythema decreased from 2.2 ± 0.8 to 1.0 ± 0.8 , edema and papules decreased from 1.9 ± 0.8 to 0.8 ± 0.6 , and excoriations reduced from 1.7 ± 1.1 to 0.6 ± 0.5 (Figures 2 and 3).

In vitro effects

The Zn textile exhibited a significant antioxidant capacity against ROS as well as RNS in vitro (Figure 4). As illustrated, it exerted a dose-dependent effect. Moreover, it was shown that it is equally effective against ROS and RNS.

In addition, strong antibacterial activity against both *Staphylococcus aureus* and *Klebsiella pneumoniae* was observed, yielding log reductions > 3 (Figure 5). Bacterial growth was completely inhibited after a 24-hour incubation period.

Figure 6 shows the results of the biocompatibility study performed according to DIN EN ISO 10993-5, used for evaluation of cytotoxic effects of textiles. Treatment of human HaCaT keratinocytes with Zn-textile extract had no negative influence on cell viability or cell proliferation (Figure 6A). In accordance, no cytotoxic potential, inducing cell death by necrosis, was observed (Figure 6B).

Discussion

AD is a chronic inflammatory disease characterized by the impairment of the skin-barrier function, increased oxidative cellular stress, and bacterial colonization. The development of new textiles with intrinsic properties such as antioxidative capacity and antibacterial activity presents a promising path in the treatment and maintenance of AD. Here, we observed a rapid improvement of AD severity, pruritus, and

subjective sleep quality when AD patients wore the Benevit Zink+ clothing overnight. This textile comprises a specially coated ZnO fiber with high AOC against ROS and RNS, as well as strong antibacterial activity. In general, natural fibers like silk or cotton are preferred for individuals with sensitive skin or AD.²³ These fibers can be functionalized by incorporation of silver or chitosan to reduce colonization of AD skin by staphylococci for improvement of disease severity.^{24–26} However, negative effects on cells of both silver ions^{21,27} and chitosan formulations^{22,28,29} have been observed in vitro. Moreover, the use of silver compounds has been associated with the risk of inducing bacterial adaptation.^{30–32} Hence, there is a demand for alternative effective compounds. ZnO may present an interesting option, as it provides sun protection³³ and has been shown to improve hypertrophic scars.³⁴ ZnO helps to restore the disturbed skin-barrier function in eczematous diseases³⁵ and enhances wound healing.³⁶ Moreover, ZnO is considered safe to use, since it does not penetrate the skin, even with disturbed barrier function.^{37,38} In accordance, high biocompatibility of the Benevit Zink+ was proven in this study in vitro. In addition, we were able to demonstrate that short time usage of this ZnO textile can contribute to improvement of night sleep, pruritus, and AD severity when used adjuvant to medical treatment. Hereby, an improvement of quality of life can be achieved for AD patients.

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Disclosure

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