Antibacterial and antibiofilm effects of sodium hypochlorite against *Staphylococcus aureus* isolates derived from patients with atopic dermatitis*

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**Summary**

Background Atopic dermatitis (AD) is characterized by an increased susceptibility to skin infections. *Staphylococcus aureus* is reported to dominate in AD lesions and reports have revealed the presence of staphylococcal biofilms. These infections contribute to aggravation of the eczema. Sodium hypochlorite is known to reduce bacterial load of skin lesions, as well as disease severity, in patients with AD, but the effect on biofilms is unknown.

**Objectives** To investigate the antimicrobial and antibiofilm effects of sodium hypochlorite against *S. aureus* isolates derived from patients with AD.

**Methods** Skin biopsies derived from patients with infected AD were examined by scanning electron microscopy (SEM). Using radial diffusion assays, biofilm assays and confocal laser scanning microscopy, we assessed the effect of sodium hypochlorite on *S. aureus* isolates derived from lesional skin of patients with AD. Results SEM revealed clusters of coccoid bacteria embedded in fibrin and extracellular substances at the skin of a patient with infected AD. At concentrations of 0.01–0.08%, sodium hypochlorite showed antibacterial effects against planktonic cells. Eradication of *S. aureus* biofilms in vitro was observed in concentrations ranging from 0.01% to 0.16%. Confocal laser scanning microscopy confirmed these results. Finally, when human AD skin was subjected to sodium hypochlorite in an *ex vivo* model, a dose of 0.04% reduced the bacteria derived from AD skin.

**Conclusions** Sodium hypochlorite has antimicrobial and antibiofilm effects against clinical *S. aureus* isolates. Our findings suggest usage of a higher concentration than currently used in bleach baths of patients with skin-infected AD.

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**What’s already known about this topic?**

- The association between atopic dermatitis (AD) and *Staphylococcus aureus* carriage is well established, and increasing attention has been given to the presence and impact of *S. aureus* biofilms in AD.
- Bleach baths are a commonly used treatment of infected AD; however, the effect of this treatment on biofilms formed by isolates from patients with AD is sparsely investigated.

**What does this study add?**

- This study revealed biofilm at the skin surface of a patient with infected AD.
- The current results show that sodium hypochlorite has both inhibitory effects on biofilm formation and the capacity to eradicate established biofilms of *S. aureus* isolates derived from the skin of patients with AD.
Microbial infections represent a broad disease spectrum and include many acute diseases, such as postoperative infections, as well as chronic inflammatory diseases. Atopic dermatitis (AD) is an inflammatory skin disease characterized by skin barrier impairment and aberrations in innate and acquired immunity. Staphylococcus aureus colonize lesional skin in > 90% of patients with AD. The recurrent skin infections seen among these patients are a crucial problem in the treatment of eczema. Recent publications have revealed that biofilm formation by S. aureus contribute to the pathogenesis of AD and the continuous inflammation of the skin.

Microorganisms, such as S. aureus and Staphylococcus epidermidis, have the ability to form biofilms under physiological conditions. Biofilm formation appears to be a survival strategy, protecting microorganisms from environmental conditions, antibiotics and phagocytosis. For those reasons, chronic biofilm infections, such as those seen in skin infections, resist antibiotic therapy and are resistant to host clearance mechanisms. In agreement, studies have shown that biofilms may have a greater than 100-fold increase in tolerance to antibiotics when compared with planktonic bacterial cells.

These infections represent significant challenges from a global perspective. A defective bacterial clearance, because of defects in the innate immune response, contributes to high-density S. aureus colonization in AD skin. Biofilms, and their complex mixture of extracellular polymeric substances, can serve as protective barriers for bacteria, such as S. aureus, by shielding them from antimicrobial defence mechanisms such as antimicrobial peptides, which are endogenously produced by the skin.

Although there is currently no clear evidence of the beneficial effects of antistaphylococcal therapies in patients with noninfected AD, antiseptic therapies may play a role in superinfected AD skin and are preferable to topical antibiotics owing to the risk of development of bacterial resistance. Bleach baths are a commonly used treatment of infected AD skin, and the effects of sodium hypochlorite to reduce both the bacterial load of skin lesions and disease severity have been shown in clinical trials. However, little is known about the effects of sodium hypochlorite on biofilm formation by S. aureus isolates derived from AD skin. Therefore, the aim of this study was to determine whether sodium hypochlorite has antibacterial and antibiotic effects on S. aureus isolates derived from AD skin.

Materials and method

Microorganisms and skin biopsy

Staphylococcus aureus ATCC 29213 was from the American Type Culture Collection (Rockville, MD, U.S.A.). Staphylococcus aureus 1–11 were clinical isolates derived from skin of patients with AD. For identification of S. aureus bacteria, the samples were processed at the Department of Clinical Microbiology at Skåne University Hospital, Lund, Sweden, following standard routines for identification of S. aureus. Skin biopsies were taken from an AD lesional area of patients with infected AD and were processed as per the standard procedure for electron microscopy or subjected to sodium hypochlorite in an ex vivo model. The participants gave informed consent, complying with the Declaration of Helsinki, and the Regional Ethics Examination Board of Lund approved the study (permit numbers: 144/2010 and 82/2012).

Radial diffusion assay

Radial diffusion assays were performed as previously described. Staphylococcus aureus [3 × 10⁶ colony-forming units (CFU)] in mid-logarithmic phase were added to an underlay gel composed of 0.03% (w/v) tryptic soy broth (TSB), 1% (w/v) low-electroendosmosis type (low-EOE) agarose (Sigma Aldrich, St Louis, MO, U.S.A.) and 0.02% Tween-20, which was poured into 85-mm Petri dishes. After agarose solidification, 5 µL test sample (sodium hypochlorite 0.0075–0.06%) or control (dH₂O) were transferred to wells (4 mm in diameter) that were punched out in the gel. Plates were incubated for 3 h at 37 °C to allow diffusion of the samples, followed by covering of the underlay gel with 5 mL molten overlay (6% TSB and 1% low-EOE agarose in dH₂O). After 24 h of incubation at 37 °C, antibacterial activity of sodium hypochlorite was visualized as a clear zone around each well.

Biofilm assay

To investigate the effect of sodium hypochlorite on the biofilm formation of S. aureus isolates, abiotic solid surface assays were performed as described by Hell et al., with some minor modifications. Briefly, S. aureus were grown in 3% TSB in a rotary incubator at 37 °C, while shaking at 180 rpm, and then diluted 1: 1000 in 1.5% TSB supplemented with 0.3% glucose. Next, bacterial aliquots (5 × 10⁹ CFU) were added to each well of a 96-well round-bottom polystyrene microtitre plate (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) containing sodium hypochlorite (0.001–0.35%) and 1.5% TSB supplemented with 0.3% glucose, followed by incubation. After incubation for 20 h at 37 °C while shaking at 180 rpm, the biofilm wells were washed three times with phosphate-buffered saline (PBS), to remove the media and planktonic cells. Thereafter, biofilms were stained with 150 µL 0.1% (w/v) crystal violet solution (Sigma Aldrich) for 15 min at room temperature (RT). The plates were then washed three times in PBS, incubated in 99.5% ethanol for 15 min to solubilize the dye and, subsequently, 100 µL from each well was transferred to wells of a new, flat-bottomed, 96-well plate. The absorbance, which correlates to the amount of biofilm produced, was measured at OD₆₀₀. The different bacterial isolates were tested at least in three independent experiments.

Minimum inhibitory concentration and minimal biofilm eradication concentration

Bacterial susceptibility to sodium hypochlorite was determined using a modified version of the Calgary Biofilm Device.
method (MBEC method Biofilm Inoculator; Innovotech, Edmonton, Canada) following the manufacturer’s protocol. Briefly, in vitro biofilms were established in each well of an MBEC Biofilm Inoculator after incubation of 150 μL bacterial solution ($10^8$ CFU mL$^{-1}$) in 1:5% TSB supplemented with 0-3% glucose for 24 h in a rotary incubator at 37 °C and 180 rpm. Next, the lid from the MBEC plate, consisting of pegs with formed biofilms, was washed with PBS and transferred to a challenge plate (#167008; Thermo Scientific, Roskilde, Denmark) containing a range of sodium hypochlorite in 1:5% TSB supplemented with 0-3% glucose and incubated for 24 h at 37 °C while shaking at 180 rpm. The minimum inhibitory concentration (MIC) values, representing the concentration required to inhibit growth of planktonic bacteria, was determined from bacteria shed from the pegs of the lid.

Following determination of the MICs for planktonic cells, the minimal biofilm eradication concentration (MBEC) pegs were washed once in sterile PBS and placed into a recovery plate containing 1:5% TSB supplemented with 0-3% glucose and universal neutralizer following the manufacturer’s protocol. The recovery plate was sonicated for 10 min at maximum settings in an ultrasonic bath (Elmasonic S 30H; Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany). MBECs were determined from three independent experiments by analysing the bacterial viability in the biofilm after 24 h of incubation at 37 °C, either by reading the turbidity at 600 nm in a 96-well plate reader (Victor3 1420 multilabel counter; PerkinElmer, Waltham, MA, U.S.A.) or by obtaining bacterial plate counts. The recorded MIC and MBEC represent range values from the results of at least three independent experiments.

Scanning electron microscopy

Scanning electron microscopy (SEM) imaging of biofilm in a skin biopsy derived from a lesional area of infected AD skin was performed after the specimen was fixed overnight at RT with 2-5% glutaraldehyde in cacodylate buffer. After washing with cacodylate buffer, the biopsy was dehydrated with an ascending ethanol series from 50% (v/v) to absolute ethanol, and subjected to critical point drying with carbon dioxide. The tissue sample was mounted on aluminium holders, sputtered with 20 nm palladium/gold and examined in a Philips/FEI XL 30 FESEM scanning electron microscope using an Everhart–Tornley secondary electron detector. Image processing was done with the Scandium software for simple image acquiring and autostorage into the Scandium database. All electron microscopy work was performed at the Core Facility for Integrated Microscopy, Panum Institute, University of Copenhagen. Contrast, brightness and pseudocolours were adjusted in Adobe Photoshop CS6 (Adobe, San Jose, CA, U.S.A.).

Biofilm imaging with confocal laser scanning microscopy

Bacteria were grown overnight in 10 mL 3% TSB (Becton Dickinson, Cockeysville, MD, U.S.A.). Next, the culture was suspended to $1 \times 10^7$ CFU mL$^{-1}$ in fresh 1:5% TSB supplemented with 0-3% glucose, and a 2 mL aliquot was used to grow S. aureus biofilms in sterile glass-bottomed Cellview$^{TM}$ cell-culture dishes (Greiner Bio-One, Frickenhausen, Germany) at 37 °C. After 24 h incubation the culture dishes were gently washed twice with PBS and the biofilms were treated with sodium hypochlorite (0-04%) for 1 h. The culture dishes were washed twice with PBS and the remaining adherent S. aureus biofilms at the bottom of the dishes was stained with LIVE/DEAD$^{TM}$ kit (BacLight$^{TM}$ bacterial viability kit L7012; Molecular Probes, Eugene, OR, U.S.A.) according to the instructions supplied by the manufacturer. The treated and untreated biofilms were examined by a Zeiss confocal laser-scanning microscope 510 (Carl Zeiss, Jena, Germany). Images were obtained using a 63 x /1.40 numerical aperture plan-apochromat oil-immersion lens. The image stacks collected by confocal laser scanning microscopy were analysed with Zeiss Efficient Navigation (ZEN) 2009 software (Carl Zeiss).

Ex vivo model to investigate effects of sodium hypochlorite on skin bacteria

Punch biopsies (4 mm) derived from lesional skin of patients with AD colonized with S. aureus were divided into four equal pieces and subjected to no, 0-02%, 0-04% and 0-16% solution of sodium hypochlorite in sterile, filtered tap water. The skin biopsies were incubated at 37 °C for 1 h and then removed from the solution and washed once in 100 μL PBS. Subsequently the skin biopsies were vortexed for 60 s and sonicated for 3 min at maximum settings in an ultrasonic bath (Elmasonic S 30H; Elma Hans Schmidbauer GmbH & Co. KG). The supernatants were plated on Todd–Hewitt (Becton, Dickinson, Sparks, MD, U.S.A.) agar plates and CFU were enumerated after overnight growth.

Lactate dehydrogenase assay

To determine the cytotoxic effect of sodium hypochlorite on keratinocytes the lactate dehydrogenase (LDH) assay was used. HaCaT keratinocytes were grown in 96-well plates at a density of 20 000 cells/well in Keratinocyte Cell Basal Medium (KBM-Gold) with supplements and growth factors (#00192152; Lonza Walkersville, MD, U.S.A.) for 22 h in CO$_2$ at 37 °C. Then, fresh medium and 0-005–0-04% sodium hypochlorite was added, and the cells were incubated for 24 h. The LDH Cytotoxicity Assay Kit (Pierce, Thermo Fisher, Rockford, IL, U.S.A.) was used to quantify the amount of LDH release from the cells. The absorbance was measured at 490 nm and the results represent the mean values of three independent experiments.

3-(4,5-Dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide assay

To analyse cell viability after treatment with sodium hypochlorite the 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium
bromide (MTT) assay was used. Sterile filtered MTT (Sigma Aldrich) solution (5 mg mL\(^{-1}\) in PBS) was stored protected from light at \(-20^\circ\)C until use. HaCaT keratinocytes were grown in 96-well plates, as described above. After 24 h incubation with 0.005–0.04% sodium hypochlorite, 10 µL MTT was added to each well and incubated for 2 h in CO\(_2\) at 37 °C. The MTT containing medium was then removed. The blue formazan product generated was dissolved through addition of 100 µL 100% dimethylsulfoxide (Duchefa, Haarlem, the Netherlands) per well. The plates were always protected from light and gently swirled for 30 min at RT to resolve the precipitate. Absorbance was monitored at 550 nm and the results represent the mean values of three independent experiments.

Statistics

Data are presented as means ± SD. To describe the differences between groups, one-way ANOVA with Dunnett’s multiple comparisons test was used, and \(P < 0.05\) was considered significant. The statistical software used was GraphPad PRISM\(^{\text{\textregistered}}\) version 6.0c (GraphPad Software, La Jolla, CA, U.S.A.).

Results

Bacterial biofilms are present in infected atopic dermatitis lesional skin areas

To visualize bacterial biofilms on lesional skin, a tissue biopsy was taken from the skin of a patient with infected AD and analysed by SEM. The results revealed clusters of coccoid bacteria embedded in fibrin and extracellular substances on top of corneocytes at the skin surface (Fig. 1).

Antistaphylococcal activity of sodium hypochlorite

To investigate whether sodium hypochlorite exerts antibacterial activity, clinical S. aureus isolates, as well as the reference S. aureus strain ATCC 29213, were subjected to different concentrations of sodium hypochlorite in a radial diffusion assay. The results showed that sodium hypochlorite inhibited growth of the different clinical S. aureus isolates dose dependently and the reference strain in a similar fashion (Fig. 2), indicating that sodium hypochlorite has in vitro antibacterial effects against planktonic S. aureus. We then determined the MIC values for 11 S. aureus isolates derived from lesional skin of patients with AD. The MIC values ranged between 0.01% and 0.08% sodium hypochlorite (Table 1).

Sodium hypochlorite inhibits Staphylococcus aureus biofilm formation

To analyse biofilm formation, the bacterial isolates were grown in a 96-well microtitre polystyrene plate. The amount of biofilm formed on the plastic was determined by measuring the amount of crystal violet absorbed by the biofilm. The results showed that all tested isolates produced biofilm (Fig. 3). Next, the isolates were subjected to increasing concentrations of sodium hypochlorite. The results showed that biofilm formations by the S. aureus isolates were significantly inhibited when subjected to concentrations ranging from 0.0219% compared with control biofilms not subjected to sodium hypochlorite (Fig. 3).

Sodium hypochlorite eradicates established Staphylococcus aureus biofilms

To determine the potential of sodium hypochlorite to eradicate established biofilms, we used a slightly modified version of the Calgary Biofilm Device method, where bacterial biofilms are formed on plastic pegs for 24 h at 37 °C.\(^{15}\) The results showed that the MBEC values, representing the concentration of sodium hypochlorite necessary to eradicate formed biofilms, were similar for the various isolates, as well as the control strain, and ranged between 0.01% and 0.16% (Table 1).

Next, confocal microscopy and live/dead staining were used to further visualize the in vitro effect of sodium hypochlorite against S. aureus biofilms, formed on cover slide discs. The results showed that treatment of S. aureus biofilms with 0.04% sodium hypochlorite killed nearly all of the bacteria (red fluorescence; Fig. 4), whereas most of the cells in the untreated biofilms (controls) only stained green, indicating live bacteria with intact cell membranes.

Cytotoxic effect of sodium hypochlorite and cell viability of keratinocytes

To investigate the cytotoxic effect of sodium hypochlorite against keratinocytes LDH assays were performed.
The results showed that 1 h of incubation with 0.1% sodium hypochlorite on adherent bacteria, biopsies were first eradication (Fig. 6, white bars). To investigate the effect of bacteria released from the biopsy in the incubation solution, whereas 0.02% sodium hypochlorite did not kill attached bacteria, whereas 0.04% did reduce bacterial numbers after both washing and vortexing/sonication. Total eradication of weakly attached bacteria was observed with a concentration of 0.16% sodium hypochlorite, whereas approximately 10% of the strongly attached bacteria survived.

**Discussion**

The association between AD and *S. aureus* carriage is well established, and an increasing body of data suggests the importance of *S. aureus* as a possible causal factor for exacerbations in AD and disease severity in general. Moreover, increasing attention has been given to the presence and impact of *S. aureus* biofilms in AD. In agreement, using SEM we showed the presence of biofilm at the skin surface of a patient with infected AD. Bleach baths are a commonly used treatment of infected AD; however, the effect of this treatment on biofilms formed by isolates from patients with AD is sparsely investigated. Hence, it is essential to evaluate effects of sodium hypochlorite on planktonic *S. aureus*, biofilm formation and established biofilms.

Here we report the *in vitro* antibacterial activity of sodium hypochlorite against AD skin-derived *S. aureus* isolates and biofilms, when subjected to concentrations comparable with those found in bleach baths used in clinical practice. Although antistaphylococcal activity of sodium hypochlorite has been reported previously, to our knowledge no such in vivo studies have been performed on *S. aureus* isolates derived from the skin of patients with AD. Notably, in a previous study of susceptibility testing of sodium hypochlorite against *S. aureus* strains of different origins, variation among the strains giving a MIC value range from 0.0128% to 0.82% was presented. The slightly higher MIC values observed in the present study might be explained by differences in the origin of the bacterial isolates, as well as differences in the method of analysis.

Although biofilm is an excellent strategy for *S. aureus* to be protected from environmental conditions, such as antimicrobial agents and antiseptic substances like sodium hypochlorite, the current results show that sodium hypochlorite has

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<th>S. aureus isolate</th>
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<th>MBE range (%)</th>
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<td>0.02–0.16</td>
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Concentrations of 0.005% and 0.01% sodium hypochlorite showed no significant increased cell toxicity compared with the nontreated cells (Fig. 5a). However, as indicated in Figure 5(b), the viability of the keratinocytes, analysed by utilizing the MTT assay, is significantly affected when concentrations of sodium hypochlorite reached 0.01%.

**Ex vivo effects of sodium hypochlorite on skin bacteria**

Although sodium hypochlorite is effective *in vitro*, it should be considered that the antibacterial and antifilm effects of sodium hypochlorite are not guaranteed *in vivo*. Therefore, we investigated the effect of sodium hypochlorite (0.0–0.16%) on bacteria on punch biopsies derived from lesional skin of patients with AD (total amount of bacteria in the untreated biopsies ranged from 9.9 × 10^5 to 45.7 × 10^5 CFU cm^-2). The results showed that 1 h of incubation with 0.02% or 0.04% sodium hypochlorite resulted in > 90% killing of bacteria released from the biopsy in the incubation solution, whereas 0.16% sodium hypochlorite resulted in complete eradication (Fig. 6, white bars). To investigate the effect of sodium hypochlorite on adherent bacteria, biopsies were first washed in PBS (Fig. 6, black bars), to remove weakly attached
both inhibitory effects on biofilm formation and the capacity to eradicate established biofilms. In agreement, concentrations from 0.02% inhibited the formation of biofilm in all the tested S. aureus strains. Moreover, confocal microscopy studies showed efficient killing of bacteria within biofilms by sodium hypochlorite in vitro.

From a clinical perspective, our findings suggest that the use of a higher concentration of sodium hypochlorite than currently used in bleach baths for the treatment of patients with infected AD would be more beneficial to combat S. aureus biofilms. Although several reports on the beneficial effects of bleach baths in the treatment of AD are available,10–12,18 including a trend of decreased bacterial load of treated patients with AD,12 others have failed to find any advantageous effect of additional bleach baths to standard topical steroid treatment concerning clinical improvement or normalization of the microbiome in AD.21,22 Thus, although effective in vitro, it should be considered that the antibacterial and antibiofilm effects of sodium hypochlorite are not guaranteed in clinical practice, and a recent study failed to prove any superior effect of bleach baths as compared with water baths in reducing S. aureus and improving AD.23 Correspondingly, our ex vivo data revealed that 0.02% of sodium hypochlorite, the tested concentration closest to the clinical used solution, failed to kill bacteria attached to the skin. In contrast, 0.04% sodium hypochlorite resulted in >80% reduction of strongly attached bacteria. Recently published data showing that S. aureus penetrates human AD skin might be a survival mechanism for bacteria,24 making it very difficult to eradicate these remaining bacteria. However, by reducing the predominance of S. aureus, although eradication is not achieved, the in vitro data presented herein suggest that a higher concentration of a sodium hypochlorite solution vs. the 0.005% solution used for clinical bleach baths would be more beneficial in combating S. aureus biofilms. In a recent study it was shown that bleach baths do not impair skin barrier function more than water bath

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Fig 3. Effect of sodium hypochlorite on biofilm formation. (a) Staphylococcus aureus (S.a.) biofilms grown in the presence of a range of sodium hypochlorite. Results are reported as mean absorbance (OD600) ± SD that correlates to the amount of biofilm produced. Uppermost dashed line indicates nontreated growth control, and bottom dashed line indicates sterile control. Values indicated with an asterisk are significantly (*p < 0.05, **p < 0.01, ***p < 0.001) different from the nontreated controls as analysed by one-way ANOVA with Dunnett’s multiple comparisons. (b) Illustration of a typical 96-well plate with S. aureus biofilms grown in the presence of a range of concentrations of sodium hypochlorite. After 20 h, biofilms were stained with crystal violet, washed and the dye was dissolved in ethanol. Clin. isolate, clinical isolate.

Fig 4. Effect of sodium hypochlorite on Staphylococcus aureus biofilms. Biofilms were grown for 24 h on sterile Cellview™ cell-culture dishes with a glass bottom, followed by treatment with 0.04% sodium hypochlorite for 1 h. Representative confocal laser scanning microscopy images are shown of control and treated biofilms of a clinical S. aureus isolate and the ATCC 29213 strain stained using the LIVE/DEAD® kit (Molecular Probes, Eugene, OR, U.S.A.). The top row shows dead bacteria stained with propidium iodide (red fluorescent); the bottom row shows both live and dead bacteria stained with green fluorescent SYTO9. The confocal images show a plan view (squares) looking down the biofilm and side views through the biofilm (right and above the squares). Magnification ×63, scale bar 20 μm.
exposure, and are safe and well tolerated among patients with AD. However, when in vitro assays were performed on keratinocytes to evaluate the cytotoxic effects and impact of cell viability of sodium hypochlorite, the findings indicated that concentrations of ≤ 0.02% adversely affected the cells.

Clearly, as we only show the in vitro results of the antibiofilm effects of sodium hypochlorite, there is a need for further in vivo experiments to investigate these effects in AD.

Therefore, the exact in vivo relevance of our data has to be further evaluated in clinical trials to determine the most advantageous concentration of sodium hypochlorite to gain the best clinical effects, without causing skin irritation and/or hypersensitivity reactions. Taken together, the presented results indicate that sodium hypochlorite has potent antimicrobial and antibiofilm effects when tested in vitro and ex vivo, and has a potential role in treatment regimens of patients with infected AD.

Acknowledgments

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Fig 5. In vitro effects of sodium hypochlorite on keratinocytes. (a) The cytotoxic effect of sodium hypochlorite against HaCaT keratinocytes was evaluated in a lactate dehydrogenase (LDH) assay. Results are indicated as mean values of absorbance after sodium hypochlorite treatment, corresponding to amount of released LDH, and are compared with the control representing nontreated cells (NT). Lysis buffer yielded 100% lysis of the cells. (b) Cell viability of HaCaT keratinocytes was analysed using a 3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide assay. Results are indicated as mean absorbance values after sodium hypochlorite treatment, which corresponds to the amount of living cells, and are compared with the control representing nontreated cells. Lysis buffer yielded 100% lysis of the cells. **p < 0.01, ***p < 0.001.

Fig 6. Ex vivo effects of sodium hypochlorite on skin bacteria. Skin biopsies were subjected to different concentrations of sodium hypochlorite and incubated for 1 h; total bacterial count of the incubation solution was measured (white bars) and compared with untreated control. Subsequently, the biopsies were washed in phosphate-buffered saline (black bars), and then vortexed and sonicated (gray bars); the supernatants were plated and colony-forming units were enumerated after overnight growth. Error bars represent SEM, n = 3.


