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Safety and efficacy profiles of different commercial sodium hypochlorite/hypochlorous acid solutions (NaClO/HClO): antimicrobial efficacy, cytotoxic impact and physicochemical parameters *in vitro*

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Background: Sodium hypochlorite (NaClO, SHC)/hypochlorous acid (HClO, HCA) wound irrigation solutions have experienced a renaissance in the prevention and treatment of low-level wound infections. They are attributed with lower cytotoxicity and have therefore gained increasing attention in daily clinical practice.

Objectives: To determine the cytotoxicity and antimicrobial efficacy of six NaClO/HClO wound irrigation solutions.

Methods: For cytotoxicity evaluation (based on DIN EN 10993-5), human keratinocytes (HaCaT) and human skin fibroblasts (BJ) were used. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for antimicrobial efficacy evaluation (based on DIN EN 13727). Solutions were evaluated after 1, 5 and 15 min of exposure. Additionally, physicochemical properties (pH and oxidation–reduction potential values) were investigated.

Results: Efficacy and cytotoxicity varied significantly between solutions. Generally, increasing antimicrobial activity was associated with decreasing cell viability. Furthermore, a concentration- and time-dependent impact on pathogens and cells was observed: cytotoxic and antimicrobial activity increased with rising NaClO/HClO solution concentrations and extended exposure times. Based on these *in vitro* evaluations, the following ranking (lowest to highest microbicidal effect and cytotoxic impact) was found: Microdacyn60® (SHC/HCA-M) < Granudacyn® (SHC/HCA-G) < Veriforte™ (SHC/HCA-V) < KerraSol™ (SHC-K) < Lavanox® (SHC-L) ≪ ActiMaris®forte (SHC/SM-A).

Conclusions: The presented results indicate that microbicidal effects are almost always associated with certain negative side effects on cell proliferation. Efficacy and biocompatibility of NaClO/HClO solutions depend on their specific formulation and physicochemical properties. The investigations also underline the necessity for exact product- and application-specific efficacy profiles.

Introduction

Wound infection and development of microbial biofilms pose a serious threat to the wound-healing process. While the sole colonization of a wound with microorganisms that undergo limited proliferation does not generally lead to a host response, increasing microbial number, diversity, virulence and penetration of deeper tissue levels, can result in local infection, tissue damage and delayed healing.[1–3] Thus, infections of chronic wounds as well as acute wounds, such as surgical site infections (SSIs), are frequently encountered and cause serious consequences, such as impaired healing, prolonged treatment times, reduced quality of life and financial burden to the healthcare system. Up to 8% of surgical patients develop postoperative SSIs, which on average represent ∼20% of all hospital-acquired infections in Europe, depending on the area surveyed (ranging from 15.0% in the UK to 29.0% in Spain).[4–6] In terms of chronic wound infections, biofilm formation in particular represents a relevant factor, occurring in ∼78.2% of all chronic wounds.[7]

Generally, a holistic approach is needed to treat wound infections, and local cleansing with irrigation solutions and antiseptics is an important therapeutic aspect.[8,9] Over the years, several antimicrobials have been developed, of which octenidine dihydrochloride, polyhexanide and povidone-iodine (PVP-I) represent the most established agents.[8] Nonetheless, the search for alternative irrigation solutions and antimicrobials with more favourable biocompatibility continues.[10–13]

A group of agents known for some time are currently experiencing a renaissance: chlorine-based and -releasing agents [sodium hypochlorite (NaClO), hypochlorous acid (HClO) and hypochlorite

(OCl^-); Figure 1]. These agents are produced via electrolysis of sodium chloride (NaCl) in water and comparable electrochemical processes. In short, an electric current is applied, causing a redox reaction of the chemical substances and thereby transformation into NaClO and HClO.[14] Similar agents, often with only slight variations in the chemical composition or manufacturing process, are electrolysed water,[14] superoxidized water[15,16] and acid-oxidizing solution.[17] Their general antimicrobial efficacy has been described previously,[18–25] and HClO is known as an endogenous bactericidal substance from the human innate immune system, generated in particular by neutrophils via conversion from H_2O_2 by the enzyme myeloperoxidase during the defensive mechanism known as the 'oxidative burst'.[26]

In the past, these solutions faced a problem regarding stability and durability resulting in a rapid loss of efficacy. Recently it has become possible for production to be optimized to give increased durability, making the solutions a cheap, easy-to-produce and supposedly effective, biocompatible means of antimicrobial treatment. Nonetheless, claimed effects and biocompatibility of newly introduced NaClO/HClO products are mainly based on results obtained with previously available solutions and extrapolated to recent formulations. Critical evaluation of their specific efficacy and toxicity is lacking.

Therefore, this study aimed to provide such evaluations of six newly introduced NaClO/HClO wound irrigation solutions for common wound pathogens (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) as well as human skin fibroblasts (BJ cells) and keratinocytes (HaCaT cells).

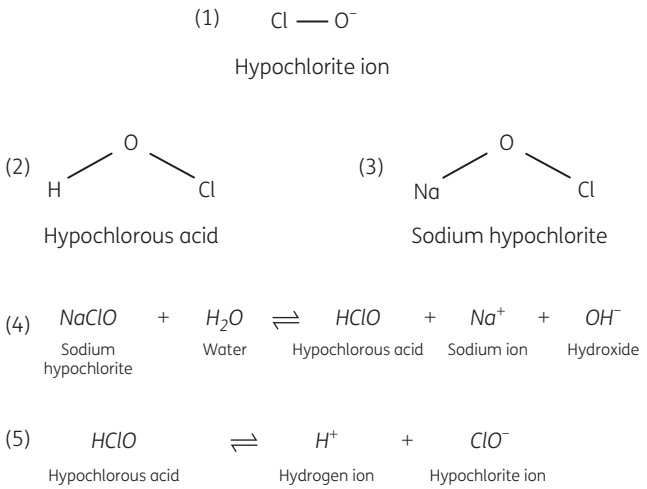

Figure 1. Main chemical structures and reactions. Structural formulas of hypochlorite [ClO^-; (1)], hypochlorous acid [HClO; (2)] and sodium hypochlorite [NaClO; (3)] as well as relevant chemical reactions in aqueous solutions. (4) Dissociation of sodium hypochlorite (NaClO) to hypochlorous acid (HClO), sodium (Na^+) and hydroxide (OH^-) in water (H_2O). (5) Equilibrium reaction of hypochlorous acid (HClO) to hydrogen (H^+) and hypochlorite (ClO^-) depending on pH value.

Materials and methods

Preparation of wound irrigation solutions

Six wound irrigation solutions based on sodium hypochlorite (SHC) alone, combined with hypochlorous acid (HCA) or sea salt (sal maris; SM) were investigated (Table 1): Lavanox® (SHC-L), KerraSol™ (SHC-K), Veriforte™ (SHC/HCA-V), Microdacyn60® (SHC/HCA-M), Granudacyn® (SHC/HCA-G) and ActiMaris® Forte (SHC/SM-A). NaClO/HClO solutions were diluted in double-distilled H_2O in order to maintain the product's pH value. Additionally, in *in vitro* cell culture assays, a concentrated medium consisting of 10% 10× DMEM (Biochrom, Berlin, Germany), 10% FBS (PAN-Biotech, Aidenbach, Germany) and 580 mg/L L-glutamine (Biochrom, Berlin, Germany) was used (as recommended in DIN EN ISO 10993-5[27]). Pre-diluted NaClO/HClO solutions were added to the concentrated DMEM/FBS/L-glutamine mixture, yielding final test concentrations of 75%, 50%, 25% and 10% (v/v).

Test organisms, cell lines and nutrient solutions

For cell culture tests, human keratinocytes [HaCaT; Cell Line Service (CLS), Eppelheim, Germany] and human skin fibroblasts (BJ, CRL-2522™; ATCC®, Manassas, VA, USA) were used. Both cell lines were cultured in DMEM supplemented with 10% FBS, 1 ng/mL basic fibroblast growth factor, 1 ng/mL epidermal growth factor (all from Pan-Biotech, Aidenbach, Germany) and 1% penicillin/streptomycin (Biochrom, Berlin, Germany) under humidified conditions with 5% CO_2 at 37°C.

For antimicrobial efficacy tests, *S. aureus* (DSM-799) and *P. aeruginosa* (DSM-2146, both from DSMZ, Braunschweig, Germany) were cultured according to standard protocols in sterile casein/soy peptone broth (CSB) and on casein/soy peptone agar (CSA) plates.

Cytotoxicity assay

In this study an application-orientated, modified test procedure, based on DIN EN 10993-5,[27] using short exposure times of 1, 5 and 15 min according to clinical utilization, was conducted. Cytotoxicity was evaluated using an XTT assay (CellTiter 96® AQ Proliferation Assay; Promega, Mannheim, Germany). Briefly, cells were seeded onto 96-well cell culture plates (Sarstedt, Nümbrecht, Germany) at a density of 7500 (BJ fibroblasts) or 30000 cells/well (HaCaT keratinocytes) to reach ∼90% confluency after 24 h of incubation in 5% CO_2 at 37°C. Subsequently, culture medium was replaced by 100 µL/well of prepared test solution dilutions. To maintain the influence of the different pH values of the NaClO/HClO solutions, experiments were conducted without buffer detergent. After rinsing with PBS, 100 µL XTT test reagent was added to each well and cells were incubated for 4 h under described conditions. Using a microplate reader (EON™; BioTeK Instruments, VT, USA), the percentage of surviving cells compared with an untreated control was evaluated by measuring the absorbance of each sample at 490 nm.

Antimicrobial efficacy assay

Antimicrobial efficacy of the NaClO/HClO solutions was evaluated by a quantitative suspension method based on DIN EN 13727[28] without organic load. Bacterial test suspensions were adjusted to 0.5 McFarland standard (∼1.5×10^8 cfu/mL). Briefly, 100 µL of bacterial test suspension was added to 900 µL of NaClO/HClO solution dilutions (100%, 75%, 50%, 25% and 10%). After 1, 5 and 15 min of exposure, 100 µL of the mixture was transferred into 900 µL of neutralizing solution for 5 min to stop antimicrobial activity. The neutralizing solution consisted of 3 g/L sodium thiosulphate, 30 g/L polysorbate 80 (Tween 80) and 3 g/L lecithin (all from Carl Roth, Karlsruhe, Germany). Subsequently, the neutralized test suspension was serially 10-fold diluted and 50 µL of each dilution step was plated onto CSA and incubated overnight at 37°C under aerobic conditions. Surviving microorganisms were counted (in cfu/mL) using a colony counter with a pen (Heathrow Scientific®, Vernon Hills, IL, USA).

Physicochemical parameters

Additionally, relevant physicochemical parameters such as pH value and oxidation–reduction potential (ORP) of the tested NaClO/HClO solutions were evaluated; pH values of the products and their dilutions were

Table 1. Product information and physicochemical properties of the tested NaClO/HClO solutions

Product	Manufacturer	Durability (months)	Composition	MDC	pH (mean ± SD)	Total chlorine (ppm)	ORP (mV)
Lavanox® (SHC-L)	Serag Wiessner GmbH & Co KG, Germany	3	H_2O, <0.08% NaClO	IIa	8.60 ± 0.09	670	806
KerraSol™ (SHC-K)	Crawford Healthcare GmbH, Germany	3	H_2O, <0.08% NaClO	IIa	8.76 ± 0.53	690	761
Veriforte™ (SHC/HCA-V)	Mediset Clinical Products GmbH, Germany	2	H_2O, NaCl, NaClO and HCIO	IIb	6.52 ± 0.17	93	882
Microdacyn60® (SHC/HCA-M)	Bamboo Health Care GmbH, Germany	2–3	H_2O, NaCl, NaClO and HCIO	IIb	6.88 ± 0.23	80	857
Granudacyn® (SHC/HCA-G)	SastoMed GmbH, Germany	2	H_2O, NaCl, NaClO and HCIO	IIb	6.78 ± 0.14	105	874
ActiMaris® Forte (SHC/SM-A)	ActiMaris AG, Germany, Switzerland	3	H_2O, 3% sal maris, 0.2% NaClO	IIb	9.51 ± 0.18	1315	747

Information regarding durability, composition and European Union medical device classification (MDC) was obtained from the manufacturer. pH and ORP values were determined continuously during experiments; total chlorine content is based on data received from the manufacturer or reported evaluations.[43]

measured using a pH meter (pH110; VWR, Darmstadt, Germany). The ORP was evaluated using a redox electrode (SenTix® ORP; all from WTW, Xylem Analytics, Weilheim, Germany).

Statistical analysis

For all experiments mean values ± SD were calculated from triplicates and differences considered statistically significant at $P < 0.05$. Microbial reduction rates were calculated for all tested NaClO/HClO solutions (in $\Delta \log_{10}$ cfu/mL). A high antimicrobial efficacy (reducing initial bacterial counts by 99.999%) was indicated by a reduction of at least 5 $\log_{10}$ within 1 min as specified in DIN EN 13727.[28]

Cytotoxic impact, based on cell viability, was assessed according to the cytotoxicity scale specified in DIN EN 10993-5.[27] In this scale, cell proliferation rates are set in relation to the negative control (100%) and classified into four cytotoxicity grades: 100%–81% is considered as non-cytotoxic (grade 0), 80%–71% as weakly cytotoxic (grade 1), 70%–61% as moderately cytotoxic (grade 2) and ≤60% as highly cytotoxic (grade 3).

Statistical evaluations of antimicrobial efficacy and cytotoxic impact, comparing between test solutions as well as with a control, were performed via two-way repeated-measures ANOVA with Tukey's HSD test as *post hoc* analysis for multiple comparisons using the statistics package GraphPad PRISM (GraphPad Software, La Jolla, CA, USA).

Results

Cytotoxicity

SHC/HCA-V, -M and -G (Figures 2b, d, e and 3b, d and e) demonstrated no cytotoxicity for human keratinocytes (HaCaT) and skin fibroblasts (BJ) within 15 min of exposure, while the remaining solutions (SHC-L, SHC-K and SHC/SM-A) partially exerted an increased cytotoxic impact.

Within the first minute, no tested SHC-L concentration showed a cytotoxic impact (Figures 2a and 3a). After 5 min, 75% SHC-L caused weak cytotoxicity in HaCaT cells and strong cytotoxicity in fibroblasts (30% proliferation reduction; $P \leq 0.0001$; Figure 3a). With increasing exposure (15 min), higher SHC-L concentrations (50% and 75%) caused grade 3 cytotoxicity in both cell lines (significant decrease in proliferation to 60% and 39% for keratinocytes and 17% and 13% for fibroblasts; Figures 2a and 3a).

SHC-K demonstrated markedly lower cytotoxicity compared with SHC-L ($P \leq 0.0001$; Figures 2c and 3c): except for 75% SHC-K causing grade 2 cytotoxicity in fibroblasts, no negative impact could be observed within 5 min of exposure. After 15 min, 75% SHC-K exerted moderate cytotoxicity in HaCaT cells and strong cytotoxicity in fibroblasts, while other tested concentrations again showed no negative impact ($P \leq 0.0001$; Figures 2c and 3c).

SHC/SM-A demonstrated the highest cytotoxicity of all tested solutions ($P \leq 0.0001$; Figures 2f and 3f). Fibroblasts proved especially susceptible: within 1 min, 25% SHC/SM-A caused moderate cytotoxicity, while 50% and 75% SHC/SM-A exerted grade 3 cytotoxicity, with proliferation rates further decreasing over time for each tested concentration ($P \leq 0.0001$; Figure 3f). Keratinocytes proved less susceptible than fibroblasts, showing no cytotoxic impact for any tested concentration within the first minute of exposure. Extended exposure, however, also caused grade 3 cytotoxicity (5 min for 75% and 15 min for 50% SHC/SM-A; $P \leq 0.0001$), whereas 25% SHC/SM-A did not exceed grade 2 cytotoxicity.

Antimicrobial efficacy

The undiluted SHC/SM-A (100%), against both tested microorganisms as well as at a concentration of 75% against *S. aureus*, achieved >5 $\log_{10}$ reduction phases within 1 min (as defined for antiseptics in DIN EN 13727). Except for the lowest concentration (10%), all tested SHC/SM-A concentrations achieved a strong antimicrobial effect against both tested microbes within 15 min; concentrations of ≥50% produced complete eradication within 5 min (Figures 4f and 5f).

SHC-L and SHC-K both exerted strong microbicidal effects as well but needed a minimum of 5 min to achieve the required reduction of >5 $\log_{10}$. Overall, SHC-L and SHC-K demonstrated similar results against both tested bacteria (Figures 4a, c and 5a, c): undiluted, both solutions managed to completely eradicate both tested pathogens within 5 min, whereas a concentration of 75% needed extended exposure times and only managed to completely eradicate *P. aeruginosa*. Even though a 50% concentration of both solutions achieved >5 $\log_{10}$ reduction against *P. aeruginosa*, 50% SHC-K showed no effect on *S. aureus*, in contrast to SHC-L ($P \leq 0.0001$).

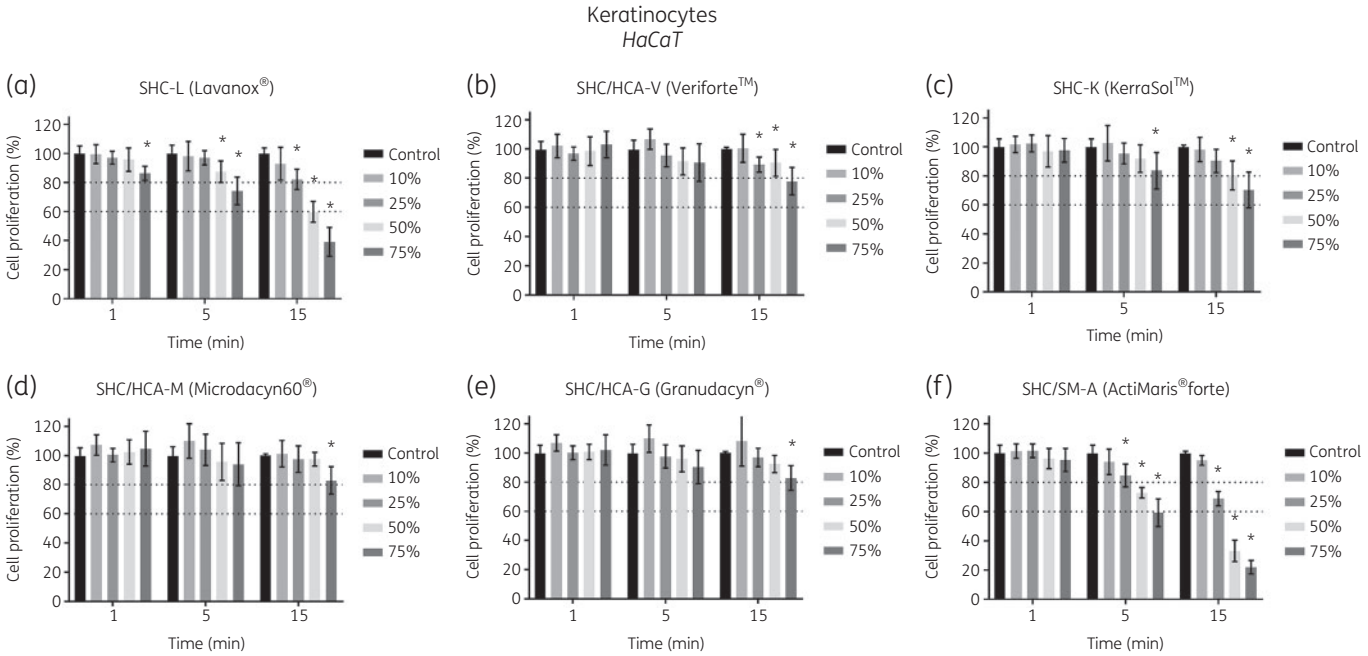

Figure 2. Cytotoxicity of tested NaClO/HClO wound irrigation solutions against keratinocytes (HaCaT cells). Cell proliferation rates of HaCaT cells treated with the different wound irrigation solutions (a–f) and their respective concentrations (10%–75%) after 1, 5 and 15 min of exposure. Dotted lines indicate cytotoxicity thresholds; the upper line represents the cut-off to non-cytotoxic (>81%) and the lower line indicates the cut-off to strongly cytotoxic (<60%). Values are expressed as percentage compared with an untreated control and as mean ± SD (*$P \leq 0.05$ versus untreated control).

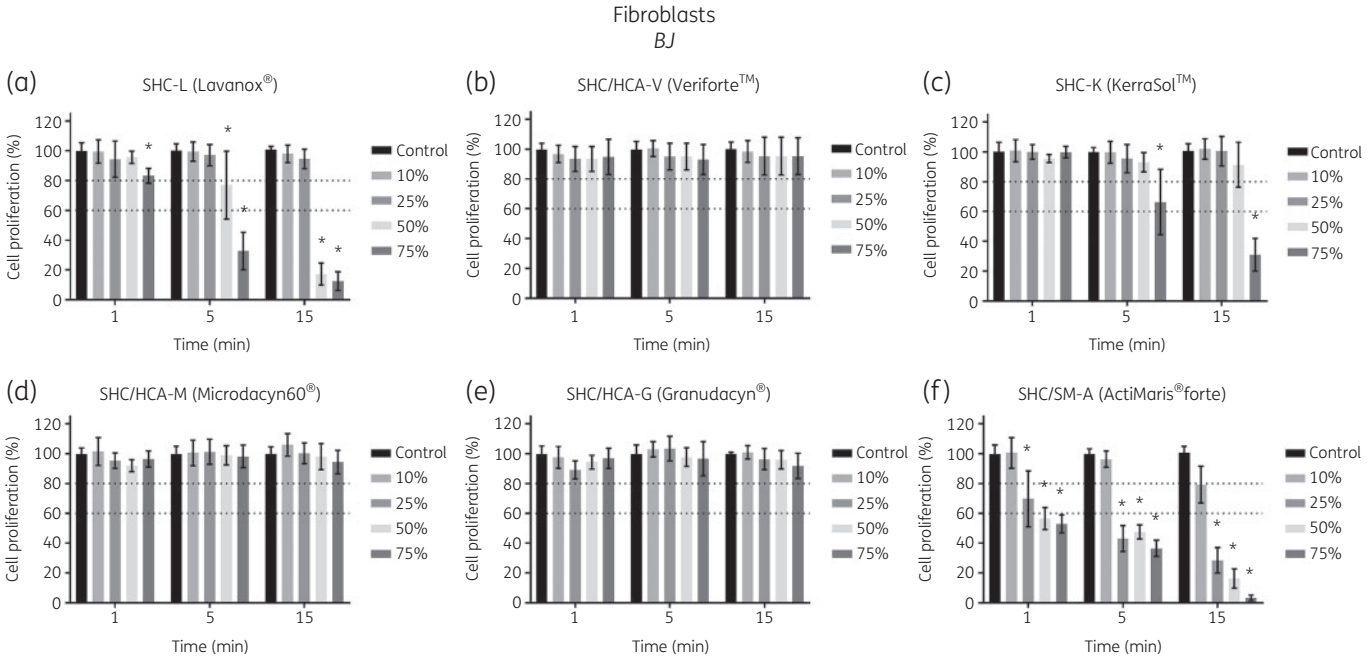

Figure 3. Cytotoxicity of tested NaClO/HClO wound irrigation solutions against skin fibroblasts (BJ cells). Cell proliferation rates of BJ cells treated with the different wound irrigation solutions (a–f) and their respective concentrations (10%–75%) after 1, 5 and 15 min of exposure. Dotted lines indicate cytotoxicity thresholds; the upper line represents the cut-off to non-cytotoxic (>81%) and the lower line indicates the cut-off to strongly cytotoxic (<60%). Values are expressed as percentage compared with an untreated control and as mean ± SD (*$P \leq 0.05$ versus untreated control).

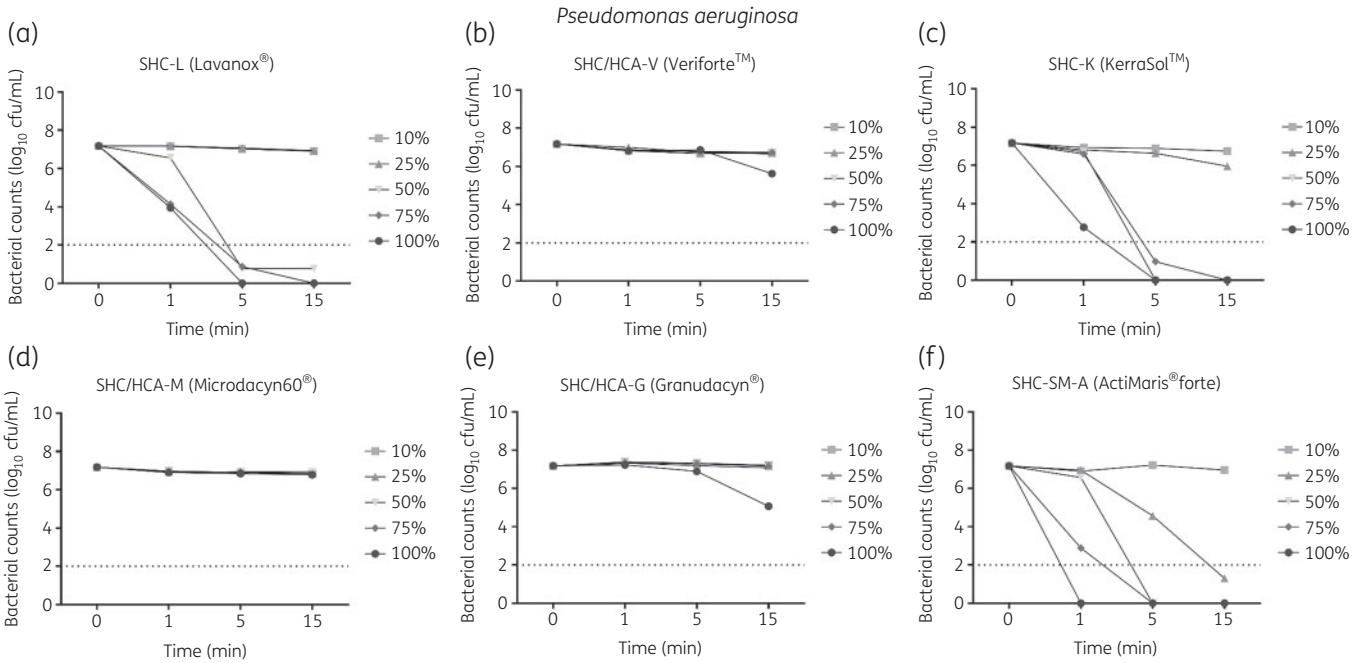

Figure 4. Time-kill curves of the tested NaClO/HClO wound irrigation solutions against *P. aeruginosa*. Reduction of bacterial counts (in $\log_{10}$ cfu/mL) over time (1, 5 and 15 min) for all tested wound irrigation solutions (a–f) and their respective concentrations (10%–100%). The dotted line represents a reduction of >5 log phases (high antimicrobial effect).

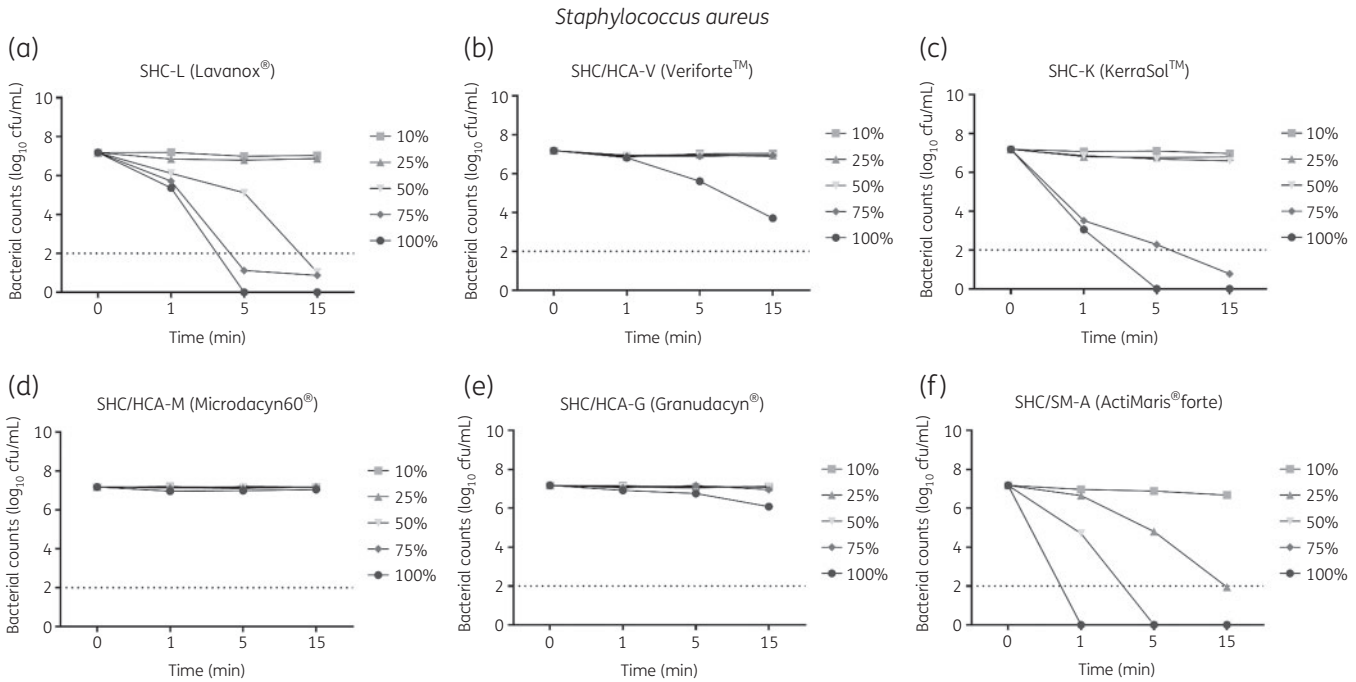

Figure 5. Time-kill curves of the tested NaClO/HClO wound irrigation solutions against *S. aureus*. Reduction of bacterial counts (in $\log_{10}$ cfu/mL) over time (1, 5 and 15 min) for all tested wound irrigation solutions (a–f) and their respective concentrations (10%–100%). The dotted line represents a reduction of >5 log phases (high antimicrobial effect).

For SHC/HCA-V, -M and -G no antimicrobial effect could be detected, except for a low antimicrobial activity of the undiluted SHC/HCA-V against *S. aureus* and SHC/HCA-G against *P. aeruginosa* after 15 min (both ~3 $\log_{10}$ reduction; Figures 4b, d, e and 5b, d, e).

Comparing the undiluted solutions, SHC/SM-A demonstrated a significantly faster microbicidal effect than the other tested solutions ($P \leq 0.0001$). SHC-K showed a significantly stronger effect than SHC-L within the first minute ($P \leq 0.05$), but with increasing

exposure time no significant difference in efficacy could be detected. SHC/HCA-V, -M and -G proved significantly less effective in general ($P \leq 0.0001$).

Physicochemical parameters

Three of the tested NaClO/HClO wound irrigation solutions (SHC-L, SHC-K and SHC/SM-A) demonstrated alkaline pH values (pH >8), whereas for the remaining solutions (SHC/HCA-V, -M and -G) slightly acidic pH values were measured (pH 6.5–7.0; Table 1). In the unbuffered cell culture experiments, the pH values of solutions decreased (in the case of alkaline solutions) or increased (in the case of acidic solutions) stepwise, eventually resulting in a physiological pH range (pH 7.5–7.7; Tables S2 and S3, available as Supplementary data at *JAC* Online).

The ORPs of the investigated solutions generally ranged between 747 and 882 mV; those with higher antimicrobial efficacy and cytotoxic impact displayed lower ORP values (Tables 1 and S4).

Discussion

Sodium hypochlorite/hypochlorous acid wound irrigation solutions are chlorine-based and -releasing agents. Their effect mainly arises from the freely available chlorine species present and/or formed within a solution on contact with water, organic and inorganic material. The main active chlorine species are HClO and OCl^-; the ratio of the prevalent chlorine ion species depends on the pH value and temperature.[26,29] NaClO is hydrolysed upon addition to water, forming HClO, which itself partly dissociates into OCl^- and hydrogen ions (H^+) (Figure 1). Depending on pH, temperature and storage, a reaction equilibrium is established, maintaining a certain ratio of chlorine species.

The investigated NaClO/HClO solutions are intended for wound irrigation, rinsing and cleansing of moderately infected wounds and general prevention of wound infections. Contact with the wound as well as the microbial pathogens that are present is mostly short in clinical practice, and therefore short exposure times of 1, 5 and 15 min were tested, to adequately mimic clinical utilization. These exposure times are also recommended by the manufacturers. To take account of the possible effect of the varying pH values of the solutions, no buffer detergent was used in the experiments (additional data can be found in Table S1).

The presented results demonstrate an indirect proportional correlation: an increasing antimicrobial effect is associated with decreasing viability of keratinocytes and fibroblasts. Consequently, microbicidal effects are almost always accompanied by a certain negative impact on cell proliferation and viability. As expected, a concentration- and time-dependent impact on microorganisms and cells could be observed: both cytotoxic and microbicidal effects increased with rising concentrations of the NaClO/HClO solutions and extended exposure times. Additionally, significant differences between the investigated solutions were found. No relevant cytotoxicity of any tested concentration of SHC/HCA-V, -M and -G was observed, verifying a high biocompatibility. However, these NaClO/HClO solutions also did not demonstrate a considerable antimicrobial effect, compared with SHC-L, SHC-K and SHC/SM-A ($P \leq 0.0001$).

Of the investigated solutions, SHC/SM-A was the most effective microbicidal agent (>5 $\log_{10}$ reduction within 1 min), with an efficacy comparable to that of established antiseptics such as octenidine dihydrochloride, PVP-I and polyhexanide.[30] In terms of cytotoxicity, SHC/SM-A demonstrated the highest negative effect, highlighting the inseparable relationship between microbicidal and cytotoxic impact. Nevertheless, no cytotoxic effect on keratinocytes was detected for any tested SHC/SM-A concentration within the first minute of exposure, and within 5 min a moderate (grade 2) cytotoxicity was not exceeded. Therefore, SHC/SM-A exhibits an excellent balance of beneficial and negative effects with good biocompatibility within 5 min of exposure (Figures 2f, 4f and 5f). On fibroblasts, however, SHC/SM-A caused a strong negative impact within 1 min of exposure (Figure 3f). Overall, cells remained considerably more viable under treatment with SHC/SM-A than with established antiseptics in earlier studies (0% for 10% Octenisept and 7.5% PVP-I; <20% for polyhexanide).[11,12]

SHC-L and SHC-K needed prolonged exposure times to achieve a strong antimicrobial effect, but caused significantly less cytotoxicity than SHC/SM-A ($P \leq 0.001$). Again, keratinocytes were less susceptible, with only a weak impact (grade 1) of SHC-L at most, and no impact of SHC-K within 5 min. At that time, however, SHC-L as well as SHC-K had already achieved a complete eradication of both tested pathogens (Figures 2a, c, 4a, c and 5a, c). In fibroblasts, a higher cytotoxic impact was detected for both solutions, whereby with increasing concentration and exposure time SHC-L was more cytotoxic than SHC-K ($P \leq 0.0001$; Figure 3a, c). Overall, SHC-L and SHC-K caused considerably less cytotoxicity in the present work than established antiseptics, while achieving similar microbicidal effects.[11,12,30,31]

Several earlier *in vitro* studies on NaClO, HClO and OCl^-, investigating their general efficacy,[18,20,26,29] reported favourable microbicidal effects against a variety of microbes,[32] while exerting a comparably low cytotoxicity.[8,33] All investigated solutions are classified as cleansing and irrigation solutions, suited for decontamination and treatment of acute and chronic wounds.[3,8] Additionally, they are frequently advertised as highly effective against microbes and non-cytotoxic for humans due to their natural occurrence in the human innate immune system.[26,34] However, as the presented results demonstrate, careful differentiation between the generally reported effect of an antimicrobial agent and the actual efficacy of a product based on such an agent is important: out of six investigated solutions, half demonstrated no relevant antimicrobial effect at all, while the rest could not be verified as highly effective and without cytotoxic side effects.

These discrepancies result from the physicochemical properties of the solutions. Even though all these solutions contain certain amounts of NaClO, HClO and/or OCl^-, they differ significantly in terms of total chlorine content, pH value and ORP (Table 1). While in general HClO is the most potent microbicidal chlorine species, its availability depends on the solution's pH value: at a pH of 4–6, HClO is predominant; with rising pH, increasing OCl^- is formed until the two species are in equilibrium at a physiological pH range. At a pH of >8.5, OCl^- exceeds HClO.[26,29] Linking the pH value to the predominant chlorine species, the relevance of the ORP value becomes clear: HClO and OCl^- are strong oxidizing agents, exerting a high interaction potential with other molecules in a redox reaction. This results in the formation of reactive oxygen species, such as peroxides (mainly hydrogen peroxide; H_2O_2), superoxide ($^{\cdot}O_2^-$), hydroxyl radicals (^{-}OH) and singlet oxygen ($^1O_2$),[29] protein denaturation,[35–38] oxidation of lipids in cell membranes/walls,[39]

oxidative enzyme deactivation[40,41] and DNA damage.[42] The higher the redox potential, the higher the ORP value; solutions with a high antimicrobial potential generally range above 650 mV, as all the investigated solutions did (Table 1). However, contrary to what was expected, the ORP values correlated inversely with the antimicrobial and cytotoxic effects: more effective solutions showed lower ORP values. We postulate that this can be explained by the total chlorine content probably being the most important physicochemical property of the investigated NaClO/HClO solutions. The most effective product, SHC/SM-A, has the highest content (1315 ppm), followed by SHC-K and SHC-L (690 and 670 ppm), while SHC/HCA-V, -G and -M only contained around 100 ppm (Table 1). Considering that the ORP is a relative potential, a solution can present a higher redox potential but an overall lower net reactive efficacy due to the simply insufficient absolute amount of readily available reactive species. Also, those solutions with no antimicrobial effect ranged around a physiological pH (Table 1), where less of the more reactive and pH-dependent HClO is available and where also a relatively high decomposition rate of the undissociated HClO has been described.[29]

In summary, the presented investigations outline significant differences between certain NaClO/HClO-based solutions. Their efficacy and biocompatibility depend on specific physicochemical properties, concentrations and exposure times. Especially, the solutions' pH values and total chlorine contents are the main drivers of reactivity. Based on the evaluated cytotoxicity and microbicidal efficacy, the solutions can be ranked as follows (lowest to highest microbicidal effect and cytotoxic impact): Microdacyn60® (SHC/HCA-M) < Granudacyn® (SHC/HCA-G) < Veriforte™ (SHC/HCA-V) < KerraSol™ (SHC-K) < Lavanox® (SHC-L) ≪ ActiMaris® Forte (SHC/SM-A). SHC/HCA-V, -G and -M can be considered as non-cytotoxic and therefore suitable as biocompatible irrigation solutions, but without verifiable antimicrobial efficacy based on the presented *in vitro* results. SHC/SM-A, SHC-L and SHC-K demonstrated relevant microbicidal effects accompanied by a certain level of cytotoxicity (ranging from grade 0 to 3) in keratinocytes and fibroblasts depending on concentration and exposure time. These investigations highlight the necessity for accurate product- and application-specific safety and efficacy profiles of newly introduced wound-care products, rather than extrapolating results from similar products.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 [containing additional data regarding cytotoxicity evaluations strictly conducted according to DIN EN 10993-5 (24 h exposure and in a buffered test procedure), changes in pH value in buffered and unbuffered test procedures (for all tested dilutions) as well as ORP values and corresponding pH values] are available as Supplementary data at *JAC* Online.

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