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Research Article

# Antimicrobial and Tolerability Profile of a Sprayable Hypochlorous AcidOxidizing (AOS) Solution for Wound Care

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## Abstract

The presence of bacteria can slow the healing process and this is the reason for which an optimal cleansing is fundamental for supporting the healing process. The aim of the present publication is to describe the antimicrobial and tolerability profile of an innovative sprayable hypochlorous Acid Oxidizing solution (AOS) for wound care with several in vitro and in vivo models. The promotion of fibrin removal was significantly more effective ($p<0.01$) with AOS compared to saline, an octenidine-containing wound irrigation product or a betaine+PHMB-containing product while similar to NaClO+HClO-containing products. Antimicrobial efficacy has been confirmed versus several bacteria (including resistant strains), viruses and fungi. Tolerability has been confirmed with several tests (cytotoxicity, mutagenicity, photo toxicity, hypersensitivity, acute and a repeated skin, ocular and subcutaneous irritation, a systemic injection, intramuscular implantation and vaginal) and cytotoxicity index (CI) of 5 better than other tested cleanser and antiseptics. Good cleansing properties with associated a wide range of antimicrobial activity and high tolerability profile sustain the use of AOS as a valuable solution for an optimal WBP that may promote the restart of the healing process.

**Keywords:** Antimicrobial; AOS; Cell viability; Cleanser; Hypochlorous acid; Tolerability

## Introduction

Chronic wounds are wounds that fail to proceed through the normal phases of wound healing within an expected timeframe, and may persist for months or even years [1,2]. It is estimated that 1-2% of the population of industrialized countries will be affected by such wounds at some point in their lives [3,4]. Chronic wounds impose major restrictions on patients' activity and substantially reduce quality of life [5]. Thus, there is an urgent and largely unmet clinical need for novel, more effective strategies to both prevent and treat this significant and growing healthcare burden [6,7].

Wounds remain locked in an inflammatory state that precludes proliferation, where local tissue hypoxia, repetitive trauma and heavy bacterial burden, combined with impaired cellular and systemic host responses perpetuate a deleterious cycle that prevents progression into the proliferative phase of healing [8].

The presence of bacteria can slow the healing process by prolonging the inflammatory phase and, in addition to delaying wound repair, can also cause further damage. All chronic wounds are contaminated with microorganisms and the balance with the host defence is unstable leading a spectrum of bioburden from simple contamination, through colonization, critical colonization, to overt infection which impairs the healing.

Strategies for prevention of infection in both acute and chronic wounds begin with the principles of Wound Bed Preparation (WBP). If wounds are not managed well, such as

with ineffective WBP, bacteria will begin to replicate. This is the reason for which an easy diagnosis of the bioburden condition and an optimal cleansing are fundamental parts of a good WBP supporting the healing process.

A good cleansing of the wound bed is fundamental to avoid a delayed healing, having several benefits including removal of foreign bodies, necrotic or sloughy tissue and bacterial burden or biofilm [9] In this context, modern treatment strategies focus on the wound microenvironment, which is prominently influenced by the microbial load and the formation of bacterial biofilms [2,3,10].

A Cochrane revision identified the non-toxicity to human tissue and the ability to reduce bioburden as the most important features for a cleanser. The historical high use of antibiotics is causing a growing antibiotic resistance in public health and to block this course the use of antibiotics has to be minimized [11]. In recent years, various antiseptics, antimicrobial dressings and wound cleansers, designed to reduce the microbial load of chronic wounds, have been introduced into the market. [12] But a good balance between an efficient cleansing and a highly safety profile could be difficult for antiseptics and active antiseptic dressings and a frequent use of them may lead to resistance and even to a decrease of wound healing due to toxic side effects.

HClO is a potent broad-spectrum fast-acting antibacterial agent and shows a favourable safety profile [13,14], differently from NaHClO which shows increasing degrees of toxicity with the increase of its concentration in the solution [15]. Furthermore, HClO efficiently penetrates the negatively charged bacterial membrane due to its neutral charge, thereby exerting its antimicrobial action [16]. More precisely, the mechanism by which HClO exerts its biocidal effect includes the attack of the surface and plasma membrane proteins, interference with the transport of solutes and with the salt balance of bacterial cells (Pietersen et al. 1996).

Pure HClO has been described to be 80-100 times more potent as a germicide than the hypochlorite anion (ClO-). Notably, cells of the animal innate immune system produce high quantities of oxidants, including HClO, to eliminate invading pathogens [17]. This is the reason for which a solution containing HClO could be a safe and efficient support for WBP.

In this article we describe the antimicrobial and tolerability profile of an innovative sprayable hypochlorous AcidOxidizing solution (AOS) for wound care. The AOS is obtained with a patented method (Tehclo Technology™) that thanks to the use of nanomaterials for the electrolytic process, is able to produce a unique pure and stable Hypochlorous Acid (HClO) in a liquid carrier solution. Furthermore, this process allows to generate a solution with unique physico chemical characteristics in terms of pH (between 2.50 and 3.00), oxidative reduction potential (ORP) between 1000 and 1200 mV, free chlorine species (HClO is not less than 95% of Free Chlorine, ClO- is 0%, $Cl_2$ is less than 5%) having long-term stability properties. The characteristics of the AOS solution are unique and strictly related each other. There are several clinical evidences on the benefit of such solution to restart wound healing in chronic wounds by creating an ideal microenvironment to sustain the physiological healing process [5,18-22].

In addition to the mentioned properties of HClO, pH and ORP further support the antimicrobial properties. A low pH (2.5-3.0) is known to reduce bacterial growth by creating an unfavorable environment and promoting tissue oxygenation by the Bohr effect [23] while high oxidation reduction potential (> 1.000 mV) is known to further physically damage the cellular membrane of micro-organisms, resulting in inactivation of their defense mechanisms [24]. The aim of the present publication is to describe the antimicrobial and tolerability profile of AOS in pre-clinical *in vitro* and *in vivo* models.

## Materials and Methods

### Reagents

AcidOxidizing solution (AOS) was from APR (Nexodyn-APR Applied Pharma Research sa), NaClO+HClO-containing product #1 from Microdacyn 60, NaClO+HClO-containing product #2 from Puracyn plus, NaClO+HClO-containing product #3 from Veriforte Med, HClO-containing solution from WoundOx, Octenidine-containing product #1 from Octenilin, Octenidine-containing product #2 from Octenisept and a betaine+polyaminopropyl biguanide (PHMB)-containing product was from Prontosan.

### Wound Coating Detachment in vitro Model

To investigate and compare the efficacy of six wound rinsing solutions (AOS, one betaine+PHMB-containing product, three different NaClO+HClO-containing products and one octenidine-containing product) versus physiologic saline solution, an in vitro wound coating model was used. To form fibrin, plasma (810 µl) followed by 1.8 M $CaCl_2$ solution (90 µl) were applied to two sets of five diagnostic slides. After drying, each slide was weighed for baseline weight. Subsequently, fibrin slides were placed in petri dishes and filled with approximately 30 ml of each test washing solution. The slides were incubated at room temperature and manually moved slightly for 2.5 or 5 minutes. After incubation and removal of test solution, the slides were dried for 24 hours at room temperature and at 37 ˚C for 1 hour and then weighed. The percent difference of slide weight, corresponding to the ability to detach fibrin protein from the slide, was then determined for each test solution.

### In vitro Bactericidal, Fungicidal and Virucidal Efficacy

The in vitro efficacy of AOS against different microorganisms was assessed using a time-kill assay, an ex vivo skin model and a virucidal efficacy test as outlined below:

### Time-Kill Assay

To test the performance of AOS against several types of microorganisms, including antibiotic-resistant bacteria and fungi, a time-kill assay was derived from the American Society for Testing and Materials (ASTM) International Method E2315-03 [25]. Results are expressed in percent and log10 reduction of viability for each test microorganism.

### Ex vivo skin model

The effectiveness of AOS against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Trichophyton rubrum* was evaluated using an ex vivo skin model (VITRO-Skin®, IMS Inc., Portland, USA). Human skin model carriers were inoculated with a suspension of the selected test microorganisms and dried at specific temperatures and relative humidity for specified times. Afterwards, carriers were sprayed or covered with test substances and remained in contact with the inoculated skin at specified conditions and times. Following the exposure time, each carrier was individually neutralized and assayed for survivors. Results are presented in percentage and $\log_{10}$ reduction of viability for each test microorganism.

### Virucidal Efficacy

To evaluate its virucidal properties, AOS was tested against the Human immunodeficiency virus type 1 (HIV-1) as well as the Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). For testing against HIV-1, glass slide carriers were inoculated, dried and then treated with AOS according to the test methods required by the United States Environmental Protection Agency (U.S. EPA) for registration of a product as a virucide [26]. Percentage and log10 reductions were calculated by determining the $TCID_{50}$ (50% tissue culture infective dose) per carrier before and after treatment with AOS.

A modified protocol of the Standard test method of efficacy of antimicrobial agents against viruses in suspension was used to evaluate the antiviral properties of AOS against HSV-1 and -2 [27]. AOS was mixed with the virus suspension in a sterile tube and held for the remainder of the specified 5-minute exposure time at 35.0 °C. Immediately after the exposure time, a 0.1 ml aliquot was removed and the mixture was titrated by 10-fold serial dilutions and assayed for the presence of virus.

## Biocompatibility

The biocompatibility of AOS was assessed in several in vitro and in vivo experiments; all of which were conducted in compliance with good laboratory practice (GLP). The following testing procedures were performed according to standard operating (ISO) procedures: cytotoxicity with the agar diffusion method [28]; a reverse mutation assay [29]; a phototoxicity test [30]; a test for delayed-type hypersensitivity [31]; an acute and a repeated skin irritation test [31]; an acute and a repeated ocular irritation test [31]; pyrogen test and a systemic injection test [32]; intramuscular implantation test [33], acute and repeated subcutaneous test [34,35] and vaginal test [36].

### MTT Cell Viability Assay

The cell viability preservation of AOS was compared to six wound cleansers and antiseptics (two NaClO+HClO-containing products, one HClO-containing solution, two octenidine-containing products and one betaine+PHMB-containing product) using murine fibroblasts (L929 NCTC cells). Each tested product was examined in triplicate with 5 scalar dilutions in 100 µL phosphate buffer: $10^0$, 1:2, 1:5, $10^{-1}$, $10^{-2}$ and $10^{-3}$. After a 30-minute incubation time, cell viability was tested using the cytotoxicity MTT test (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) as described elsewhere [6]. The MTT cell viability assay is defined as the dilution required to generate experimental cell viability to be 85% of blank (phosphate buffered saline [PBS], pH 7.4). Cells treated with only PBS were used as blank control. For example, a non-toxic dilution of 1:1,000 corresponded to a cytotoxicity index of 1,000.

### Rabbit Cornea Infection Model

The purpose of this experiment was to evaluate the antimicrobial efficacy of AOS in an animal model with bilateral cornea infection. Therefore, six New Zealand White rabbits, following local anaesthesia, received a 10 µL injection containing a suspension with 500 colony forming units (cfu) of Staphylococcus aureus (ATCC 25923) into the corneal stroma of each eye. Starting from the day of infection, the animals were subjected to 2 to 4 washes daily per eye (Day 1: 2 washes; Day 2 and 3: 4 washes; Day 4: 3 washes). These washings were performed with 15 ml (x 15 sec) of AOS or saline solution in 3 animals each. After 4 days of treatment, the animals were sacrificed and the entire corneas of both eyes per animal were removed. Subsequently, the number of viable bacteria (cfu) was quantified in corneal homogenates.

For statistical analyses, an independent-samples t-test was run to determine existing differences in the number of countable viable bacteria isolated from cornea, reported as cfu/cornea, between the AOS- and saline-treated group.

The animals used in this study were treated in agreement with the European Directive 86/609/EEC regarding the protection of animals, enforced by the Italian act Decreto Legislativo no. 116 (January 27, 1992). The least number of animals required was used in compliance with current regulations and scientific integrity.

## Results

### Effect of AOS on Wound Coating Detachment

The promotion of fibrin removal, which is a central step in wound debridement, was significantly more effective ($p<0.01$) after exposure to AOS compared to saline, an octenidine-containing wound irrigation product or a betaine+PHMB-containing product at both time points measured (Figure 1). An overall comparable effectiveness in terms of protein detachment was shown for AOS, NaClO+HClO-containing products #1 and #2, indicating similar cleansing properties of these products.

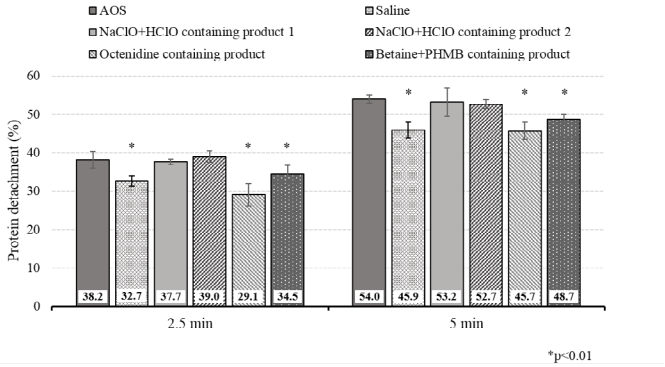


**Figure 1:** Relative protein-detachment abilities of different wound cleansers.

Efficacy of AOS on protein detachment measured on fibrin-coated slides (n=5 for each product at each evaluation time) compared to saline, two different NaClO+HClO containing products, one octenidine-containing product and one betaine+PHMB-containing product at both time points (*$p<0.01$, one-way ANOVA followed by Bonferroni post-hoc test).

AOS, AcidOxidizing solution; HClO, hypochlorous acid; NaClO, sodium hypochlorite; PHMB, polyaminopropyl biguanide. poly-[hexa-methylene]-biguanidehydrochloride.

### *In vitro* Bactericidal, Fungicidal and Virucidal Efficacy of AOS

AOS was highly active against a wide range of bacteria, including resistant strains, as well as fungi and viruses (Table 1). The antimicrobial activity on several bacterial strains including numerous drug-resistant strains showed a substantial bacterial reduction already at 15 seconds after application. Successful activity against viruses and fungi was also demonstrated.

| **Activity Against Bacteria** | | |
|---|---|---|
| **Time-Kill Assay** | **Reduction after 15 sec exposure time, % ($n_{log10}$)** | |
| Pseudomonas aeruginosa | >99.9999% (>6.11 $log_{10}$) | |
| Escherichia coli | >99.999% (>5.55 $log_{10}$) | |
| Staphylococcus aureus | 99.9992% (5.11 $log_{10}$) | |
| Staphylococcus pyogenes | 99.9958% (4.38 $log_{10}$) | |
| Staphylococcus epidermidis | 99.9499% (3.30 $log_{10}$) | |
| **Time-Kill Assay** | **Reduction after 1 min exposure time, % ($n_{log10}$)** | |
| Propionibacterium acnes | >99.9999% (>6.9 $log_{10}$) | |
| **VITRO-SKIN (ex vivo) model** | **Reduction after 30 min exposure time, % ($n_{log10}$)** | |
| Methicillin resistant staphylococcus aureus | **AOS**<br>99.99% (4.68 $log_{10}$) | **Mupirocin**<br>60.1% (0.40 $log_{10}$) |

| ACTIVITY AGAINST RESISTANT STRAINS | |
|---|---|
| **Time-Kill Assay** | **Reduction after 15 sec exposure time, % ($n_{log10}$)** |
| ESBL-producing Enterobacteriaceae | >99.9999% (>6.23 $log_{10}$) |
| ESBL-producing Proteus mirabilis | >99.999% (>5.99 $log_{10}$) |
| MDR Escherichia coli | >99.999% (>5.92 $log_{10}$) |
| VR Enterococcus faecalis | >99.999% (>5.87 $log_{10}$) |
| VISA | >99.999% (>5.84 $log_{10}$) |
| MDR Staphylococcus aureus | >99.999% (>5.44 $log_{10}$) |
| MDR + OXA-48 producing Klebsiella pneumoniae | >99.999% (>5.32 $log_{10}$) |
| **Activity Against Fungi** | |
| **Time-Kill Assay** | **Reduction after different exposure times, % ($n_{log10}$)** |
| Trichophyton mentagrophytes | >99.999% (>5.5 $log_{10}$) reduction after 5 min. exposure time |
| Candida albicans | >99.999% (>5.01 $log_{10}$) reduction after 15 sec. exposure time |
| **VITRO-SKIN (ex vivo) model** | **Reduction after 10 min exposure time, % ($n_{log10}$)** |
| Trichophyton rubrum | >99.99% (>4.8 $log_{10}$) reduction |
| **Virucidal Efficacy** | **Inactivation after 10 min exposure time, % ($n_{log10}$)** |
| Human immunodeficiency virus type 1 | 100% |
| **Antiviral Properties** | **Inactivation after 5 min exposure time, % ($n_{log10}$)** |
| Herpes simplex virus type 1 | ≥99.9997% (≥5.5 $log_{10}$) |
| Herpes simplex virus type 2 | 99.994% (4.25 $log_{10}$) |
| AOS, acid-oxidizing solution; ESBL, extended-spectrum β-lactamase; MDR, multi-drug resistant; OXA-48, Ambler class D beta-lactamase enzyme; VISA, vancomycin intermediate resistant Staphylococcus aureus; VR, vancomycin resistant. | |

**Table 1:** Antimicrobial tests conducted on different bacterial and virus strains.

### AOS Biocompatibility

In diverse in vitro and in vivo experiments examining the potential toxicity of AOS, the cleansing solution was shown to possess cytotoxicity Grade 1 which is below the threshold for mild cytotoxicity (i.e., Grade 2 cytotoxicity or more). AOS was also found to be non-irritant versus different tissues, non-mutagenic, and non-sensitizing. (Table 2).

| Tests | Results |
|---|---|
| ***General toxicology*** | |
| Cytotoxicity | Not cytotoxic [Grade 1* (0-4 scale)] |
| Hypersensivity | Non sensitizing |
| Genotoxicicty | Not mutagenic |
| Phototoxicity | Not phototoxic |

| ***Irritation studies*** | |
|---|---|
| Skin (acute & repeated) | Not irritant |
| Ocular (acute & repeated) | Not irritant |
| Subcutaneous (acute& repeated) | Not irritant |
| Vaginal | Not irritant |
| Intramuscolar implantation test | Not different from physiologic solution (control) |
| ***Toxicity studies*** | |
| Intraperitoneal (acute) | Not toxic |
| Pyrogenicity | Not-pyrogenic |
| * A numerical grade greater than 2 is considered a cytotoxic effect<br>Oral and nasal tolerability have been already published [37]. | |

**Table 2:** Biocompatibility results.

**Effects of AOS on the Preservation of Cell Viability**

AOS showed a similar effect on cell viability compared to the two reference products NaClO+HClO-containing product #1 and #2, with a dilution of 1:5 generating experimental cell viability of more than 85% of the blank. This corresponded to a cytotoxicity index (CI) of 5 (Table 3). In contrast, a CI of 10 was determined for NaClO+HClO-containing product #3 and a CI of 100 for an HClO-containing solution, octenidine-containing products #1 and #2 and for the betaine+PHMB-containing comparator product.

| **Product** | **Cell viability, % (mean ± SD)** | | | | | | |
|---|---|---|---|---|---|---|---|
| Dilutions | **$10^0$** | **1:2** | **1:5** | **$10^{-1}$** | **$10^{-2}$** | **$10^{-3}$** | CI |
| AcidOxidizing solution (AOS) | 2.82 ± 1.01 | 42.02 ± 3.68 | 97.48 ± 3.34 | 96.82 ± 6.03 | 97.10 ± 9.09 | 104.73 ± 1.92 | 5 |
| NaClO+HClO-containing product #1 | 3.38 ± 0.47 | 37.52 ±3.15 | 96.86 ± 7.94 | 103.20 ± 9.75 | 97.69 ± 14.88 | 97.27 ± 12.24 | 5 |
| NaClO+HClO-containing product #2 | 1.89 ± 1.47 | 3.19 ± 1.47 | 94.46 ± 3.79 | 105.91 ± 3.65 | 104.51 ± 3.65 | 107.26 ± 2.49 | 5 |
| NaClO+HClO-containing product #3 | 2.87 ± 1.34 | 14.81 ± 6.79 | 76.03 ± 8.49 | 100.70 ± 5.48 | 96.93 ±0.49 | 96.03 ± 4.98 | 10 |
| HClO-containing solution | 2.79 ± 1.16 | 2.45 ± 1.16 | 9.34 ± 3.69 | 71.34 ± 8.88 | 103.05 ± 5.39 | 105.49 ± 4.09 | 100 |
| Octenidine-containing product #1 | 4.56 ± 1.52 | 5.64 ± 1.61 | 4.45 ± 0.87 | 29.02 ± 5.33 | 106.51 ± 2.89 | 107.30 ± 5.52 | 100 |
| Octenidine-containing product #2 | 5.97 ± 0.96 | 5.19 ± 1.47 | 3.74 ± 0.73 | 12.06 ± 3.62 | 103.53 ± 15.88 | 116.49 ± 5.22 | 100 |
| Betaine+PHMB-containing product | 3.86 ± 0.45 | -- | -- | 4.30 ± 0.34 | 97.09 ± 5.74 | 94.46 ± 3.57 | 100 |
| CI, cytotoxicity index; HClO, hypochlorous acid; NaClO, sodium hypochloride; PHMB, polyaminopropyl biguanide; SD, standard deviation. | | | | | | | |

**Table 3:** Cell viability test of different cleansing and antimicrobial solutions selected as representative products present on the markets. n=3 replicates at each dilution.

### *In vivo* Control of Wound Infection by AOS

After washing inoculated corneas with AOS for four days, infection has been controlled where only 28 (0.6%) cfu's could be retrieved, compared with 2950 (59%) cfu's retrieved from corneas washed with saline (Figure 2).

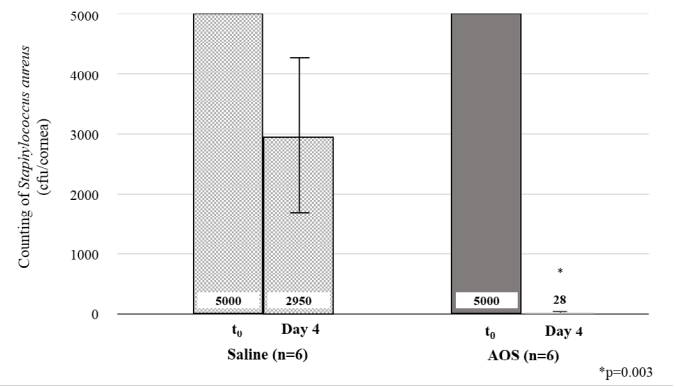


**Figure 2:** Average number of Staphylococcus aureus cfu in an in vivo rabbit cornea infection model after 4 days of treatment with AOS or saline. AOS treatment significantly (p=0.003) reduced the cfu number at Day 4 (28 [95%CI: XX to XX] compared with saline (2950 [95% CI: -4,304 to 1,539]).

AOS, AcidOxidizing solution; cfu, colony forming units; t0: time 0 (day of inoculation); CI, confidence interval.

## Discussion

For successful treatment of chronic wounds, cleansing is a fundamental step of WBP and the use of an appropriate cleanser is vital not only for the cleansing itself but also as a good preparation for debridement (if any). To identify the best antimicrobial agent, two are features to be taken into consideration: efficacy and tolerability. For this purpose, the effects of the newly developed AOS were evaluated in a wide array of pre-clinical in *vitro* and in *vivo* experiments, with the objective of exploring the correspondence with the requested features for an optimal cleanser. These results might give an indication on how AOS features support an efficacious cleansing and ancillary antimicrobial activity properties with a good tolerability profile.

Data presented in this study suggest that AOS has at least comparable and sometimes better cleansing properties in vitro compared with other wound cleansers. With the mechanical action of the cleanser being ensured, additional features of AOS, primarily the ancillary antimicrobial properties, complete the profile of this product in order to contribute to a multi-faceted approach to wound healing.

Chronic wounds are often accompanied by microbial infections, which are typically abundant and extremely diverse, thus representing one of the key factors that impair wound healing [37]. Our antimicrobial experiments demonstrate that AOS was highly active against a variety of bacteria (including drug-resistant strains), fungi and viruses. Furthermore, a recent publication demonstrates the efficacy of AOS also against the *Mycobacterium ulcerans* causing persistent open wounds called Buruli ulcer. [38] In addition, wound biofilm formation prevents wound decontamination by many products since the structural and physico-chemical characteristics of biofilms can prevent diffusion of locally applied products [39]. In vitro, AOS has recently been shown to induce morphological changes of biofilms needed to facilitate the release and elimination of bacteria from the EPS matrix of human epidermis [39]. As biofilms can rapidly reform and repeated debridement alone is unlikely to prevent biofilm regrowth, an effective topical antimicrobial application within this time-dependent window may therefore suppress biofilm reformation. By lowering the overall bacterial load, the wound microenvironment is improved, thus setting the basis for resumption of the wound healing process [40].

The progress of epithelialization requires the activity of fibroblasts and keratinocytes, which may be impaired by aggressive and toxic wound cleansers and antimicrobials [6]. Severing et al [15] have nicely shown how different free chlorine-based solutions retain different performance and safety profiles based on the physio-chemical features of the solution. Based on the pH-dependent relative content of NaHClO and HClO, the oxidation-reduction potential (ORP) and the total chlorine content, solutions present different abilities to exert an antimicrobial action and different degrees of ability to preserve cell viability. A favourable balance is obtained with AOS, consistently demonstrating antimicrobial activity whilst at the same time ranking among the most efficient products in preserving cell viability. Additionally, in several pre-clinical tests, AOS has demonstrated good biocompatibility. Topical application did not irritate the ski, oral and nasal mucosa [41] and eyes, and it was not sensitizing [42-50]. Our findings are in line with the latest reports in the literature clarifying the properties of the various free chlorine solutions and sustain the favourable tolerability profile of AOS.

Limitations of this study include the pre-clinical nature of the in vitro and in vivo experiments, and that the results of these experiments cannot be directly translated into clinical outcomes but need to be interpreted as indicators of a potential activity in each specific setting. Evidence from clinical experiences evaluating the safety and efficacy of AOS in chronic wounds suggest confirmation of the features observed in the pre-clinical setting. In a clinical study, chronic ulcers, infected at enrolment, showed a rate of complete healing of 37% at Week 5, and a significant average wound size reduction of 71.7% vs baseline ($p<0.0001$) of AOS and inert dressings solely used as the therapeutic strategy. Moreover, a very satisfactory improvement of the clinical signs of local

infection was shown by a median score of 8 at baseline reduced to 1.5 after 5 weeks ($p<0.0001$) [18]. In another clinical experience, the addition of AOS to standard of care (SOC), consisting of use of saline for cleansing and advanced dressings for the medication, in the treatment of diabetic foot patients with post-surgical, non-ischemic, non-infected lesions showed statistical superiority in protection from infections (12% infections with AOS+SOC vs 48% with SOC; $p<0.05$), speed of healing (65 days average healing time with AOS+SOC vs 147 days with AOS+SOC; $p<0.01$) and avoidance of debridement procedures (4% needed debridement procedures with AOS+SOC vs 40% with SOC; $p<0.05$) when compared to SOC alone [19].

## Conclusion

The use of AOS for treatment of chronic wounds may represent an effective strategy to reduce risk of infection in a safety way. Good cleansing properties with associated a wide range of antimicrobial activity sustain the use of AOS as a valuable solution for an optimal WBP that may promote the restart of the healing process.

At the same time, a reassuring safety profile sustains the use of the solution in different conditions and phases of the wound bed without harming cell viability or tissue integrity.

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### Transparency Declarations

NG and GR are employees of APR Applied Pharma Research S.A. (Balerna/ Switzerland).

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