Fungicidal Effectiveness of Super-Oxidized Solution In Vitro: Potential Implications for Wound Care

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Disclosure: Bactiguard AB provided financial support for the study. Medical writing support was provided by Meridian HealthComms Ltd (Plumley, UK), funded by Bactiguard AB. R.K. is an employee of Bactiguard AB. S.M. discloses no financial or other conflicts of interest.

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Recommended Citation: Krishnen R, Muniandy S. Fungicidal effectiveness of super-oxidized solution in vitro: potential implications for wound care. Wounds. 2023;35(8):E243-E247. doi:10.25270/ wnds/23017

ABSTRACT

Introduction. Drug-resistant fungal infections in chronic wounds represent a major clinical challenge to clinicians. Fungal infections delay wound healing by prolonging inflammation and encouraging biofilm formation, which protects microbes against host defenses and anti-infective medications. As such, interventions that prevent and control nosocomial fungal infections without interfering with the wound healing process are increasingly required. Although conventional antiseptics can effectively exert fungicidal effects, they also have adverse effects on human cells. SOS is a well-known bactericidal agent that enhances the wound healing process, especially for chronic wounds. However, few studies have evaluated the antimicrobial activity of SOS on fungi. Objective. The objective of this study was to evaluate whether SOS exerts fungicidal activities against common fungal species. Materials and Methods. The efficacy of SOS was tested against 6 fungal species (Candida albicans, Candida auris, Candida tropicalis, Candida parapsilosis, Sporothrix schenckii, Trichophyton mentagrophytes) using an in vitro time-kill assay. Results. SOS achieved 99.9999% reduction of all tested fungi within 1 minute of exposure. Conclusions. This study shows that SOS may be an effective tool for the prevention and control of fungal infections.

Chronic wounds represent a growing public health concern.¹ Although the etiology of different types of chronic wounds varies, infections strongly influence the development of chronic wounds and exacerbate their nonhealing nature.¹ Infections impede the wound healing process by triggering the body's immune system to cause prolonged inflammation and further tissue damage.² Moreover, fungal infections are a challenging complication when treating chronic wounds such as venous ulcers, pressure injuries, and diabetic foot ulcers.³⁴ Although the exact prevalence of fungal infections in chronic wounds is unclear, a review published in 2023 noted that chronic wound-related fungal infections are often overlooked in clinical practice; fungal infection rates likely substantially exceed current estimates.1 Owing to variable presentations and definitions of fungal infections, as well as a lack of clinical awareness of diagnostic best practices, fungal infections are frequently misdiagnosed.5.6 Tissue cultures should ideally be used to diagnose and appropriately treat fungal infections.7 In addition, the choice of an appropriate agent depends on the infecting fungal strain and its drug susceptibility profile.^{8,9} Some clinicians may prefer to use more than 1 antifungal drug to treat fungal infections to ensure broad-spectrum coverage; however, overexposure to antifungals may promote the development of antifungal resistance.10 Moreover, fungi can contribute to polymicrobial biofilm development, which further protects any bacterial and fungal species in chronic wounds from antibiotics, antifungals, and host immune defenses.1

Keywords: antimicrobial, Candida albicans, Candida auris, Candida parapsilosis, Candida tropicalis, fungal infection, fungicidal, Sporothrix schenckii, super-oxidized solution, Trichophyton mentagrophytes

Abbreviations: HOCl, hypochlorous acid; SOS, super-oxidized solution.

Table 1. Effect of Super-Oxidized Solution Exposure Against Selected

 Fungi as per ASTM E2315-16

VARIABLE	INITIAL MICROBIAL COUNT (LOG10)	EXPOSURE TIME: 1 MINUTE		
		Final Microbial Count (Log ₁₀)	Log₁₀ Reduction	Percent Reduction
Candida albicans	9.4354	3.0000	6.4354	99.9999
Candida auris	6.5238	0.0000	6.5238	99.9999
Candida tropicalis	6.3222	0.0000	6.3107	99.9999
Candida parapsilosis	6.4771	0.0000	6.4226	99.9999
Sporothrix schenckii	9.0170	3.0000	6.0170	99.9999
Trichophyton mentagrophytes	8.8808	3.0000	5.8808	99.9999



Figure 1. Growth of *Candida albicans* colonies is completely abrogated following exposure to super-oxidized solution. The *C albicans* strain was cultured on sabouraud dextrose agar plates for 24 to 72 hours at 30 °C \pm 2 °C before incubation for 1 minute in (A) phosphate buffered saline or (B) super-oxidized solution.



Figure 2. Growth of *Candida auris* colonies is completely abrogated following exposure to super-oxidized solution. *C auris*, derived from CDC B11903, was cultured on MEA plates for 24 to 72 hours at 27.5 °C ± 2.5 °C before incubation for 1 minute in (A) phosphate buffered saline or (B) super-oxidized solution.

Abbreviations: CDC, Centers for Disease Control and Prevention; MEA, malt extract agar.

The importance of adopting alternative infection management measures, such as topical antiseptics, is increasingly recognized; however, conventional antiseptics, such as povidone-iodine, can interfere with wound healing.¹ SOS, also known as electro-activated water, is an antimicrobial agent composed of purified water and pure vacuum-dried sodium chloride, which breaks down into sodium hypochlorite and HOCl when an electric current passes through the water.^{11,12} HOCl is a nonselective oxidant well-known for its antimicrobial properties.^{13,14} Moreover, unlike conventional antiseptics, available evidence suggests that SOS-derived HOCl does not adversely affect human cells.15 Furthermore, there is evidence that HOCl plays a crucial role in wound management owing to its antimicrobial and anti-inflammatory properties.¹⁶ These properties have led to the therapeutic applications of HOCl in wound healing, pruritus, diabetic ulcers, and the management of several inflammatory cutaneous disorders.16,17

Existing research highlights the role SOS plays in wound management; studies have shown that SOS inhibits the growth of pathogenic microorganisms in wounds.^{12,16,18-20} Interestingly, SOS has also demonstrated antimicrobial activity against antibiotic-resistant species, including methicillin-resistant Staphylococcus aureus, in vitro.20 Nevertheless, there is limited evidence on the effect of SOS on fungi. In light of a recent increase in antifungal-resistant infections,9 the authors of the current study conducted an in vitro time-kill study to evaluate whether a specific SOS (Hydrocyn Aqua; Bactiguard) demonstrated fungicidal activities against common fungal species.

MATERIALS AND METHODS

Microbiological testing with timekill procedure (ASTM E2315-16) was conducted by 2 accredited laboratories (Industrial Biotechnology Research Centre, Selangor, Malaysia; BioScience Laboratories, LLC, Montana, United States) to assess the effect of SOS against 6 fungal species: *Candida* albicans, Candida auris, Candida tropicalis, Candida parapsilosis, Sporothrix schenckii, and Trichophyton mentagrophytes. The basic concept of the time-kill kinetic study is to establish the rate at which a microorganism is killed by a product as a function of survival data recorded at specific exposure time points.

A measured volume of each microorganism suspension (9 mL for C albicans, C auris, S schenckii, and T mentagrophytes; 10 mL for *C* tropicalis and *C* parapsilosis) was transferred into the SOS test solution for 1 minute. Following this, 0.1 mL of each test sample was transferred to agar plates (culture medium: sabouraud dextrose agar for *C albicans*, malt extract agar for Cauris, Emmon's sabouraud dextrose agar for S schenckii and T mentagrophytes, and tryptic soy agar for C tropicalis and *C parapsilosis*) and incubated for 24 to 72 hours at 30 °C \pm 2 °C for *C* albicans; 24 to 72 hours at 27.5 °C \pm 2.5 °C for *C* auris, *C tropicalis*, and *C parapsilosis*; 5 to 10 days at 25 °C ± 2 °C for *S schenckii*; and 2 to 10 days at $25 \,^{\circ}\text{C} \pm 2 \,^{\circ}\text{C}$ for *T* mentagrophytes. Microbial counts were recorded after incubation, and log and percent reductions were subsequently calculated using the following formulae:

 Log_{10} reduction (LR) = mean log_{10} (measured initial microbial population) – mean log_{10} (surviving microbial population)

and Percent reduction (%) = $100 \times (1-10^{-LR})$, respectively.

The same process was followed for the control solution, phosphate buffered saline.

In general, a 2 \log_{10} reduction is considered the minimum level of performance to demonstrate a substantive killing activity against a particular microorganism. Any reduction value less than 2 \log_{10} indicates that a relatively large number of microorganisms remain viable after treatment with the product. For example, a 1 \log_{10} reduction in a population of 1 million bacteria means that 100 000 bacteria re-

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SOURCE	MICROORGANISM	COMMON DISEASES
Sanglard and Odds, ²⁵ 2002 Whaley et al, ²⁶ 2016 Jeffery-Smith et al, ²⁸ 2017 Behzadi et al, ³⁰ 2015 Huppler et al, ³⁴ 2012	Candida albicans	Urinary tract infections, genital yeast infections, oral thrush, mucocutaneous candidiasis, bloodstream in- fections, invasive candidiasis, fungal endocarditis
Lee et al, ²⁹ 2011 Emara et al, ²⁷ 2015 Jeffery-Smith et al, ²⁸ 2017	Candida auris	Wound infections, otitis media, invasive candidiasis, respiratory tract infections, urinary tract infections, ear infections, bloodstream infections
Lima et al, ³² 2022	Candida tropicalis	Superficial mycosis, otomy- cosis, oral candidiasis, skin candidiasis, keratitis, genital tract infections
Trofa et al, ³¹ 2008 Whaley et al, ²⁶ 2016	Candida parapsilosis	Fungemia in infants and neonates, fungal endocarditis, fungal onychomycosis
de Lima Barros et al,²4 2011	Sporothrix schenckii	Sporotrichosis
Kaufman et al, ³³ 2007	Trichophyton mentagrophytes	Dermatophytosis

Table 2. Common Diseases Caused by Selected Fungi

main viable. The final microbial reduction should be greater than $2 \log_{10}$, or greater than 39% reduction, in a given exposure time for the product to be considered to have antimicrobial properties.

RESULTS

SOS met the minimum threshold required to be considered fungicidal ($2 \log_{10}$ reduction) against all 6 fungal species and reduced microbial counts by 99.9999% (**Table 1**). Log₁₀ reduction was highest for *C auris* (**Table 1**). As seen in **Figure 1** and **Figure 2**, respectively, *C albicans* and *C auris* colonies were nearly nonexistent after 1-minute exposure to SOS; however, there continued to be a substantial colony presence in the controls.

DISCUSSION

These results are in line with those of previous studies indicating that SOS, and HOCl in general, has the potential to be the antimicrobial agent for wound disinfection, irrigation, and dressing.¹⁶⁻²²

The presence of fungi in chronic wounds delays wound healing by prolonging inflammation, perduring infection, and, in certain cases, contributing to biofilm formation.¹ The prevalence of fungal infections varies across different types of chronic wounds, and fungal infection rates are likely to be substantially higher than reported in clinical practice.1 Although incidence rates vary, the available literature suggests that the prevalence of chronic wounds complicated with fungal infections ranges between 6% and 40%, across a variety of chronic wounds such as diabetic foot ulcers, nonhealing surgical wounds, and pressure ulcers.1 While the majority of fungi are harmless, certain species can cause serious infections.23

The fungal species tested in the current study can cause a variety of diseases, including life-threatening systemic infections (**Table 2**).^{24:34} *Candida* species are the most common fungi found in chronic wounds.³⁵ In particular, *C auris* is resistant to multiple antifungal drugs used to treat *Candida* species infections.²⁶ Remarkably, in the current study SOS was able to kill 99.9999% of *C auris* in 1 minute. *C auris* has been found to exhibit resistance to fluconazole and variable susceptibility to other azoles, amphotericin B, and echinocandins, and is associated with mortality rates as high as 72%.^{36,37} Alarmingly, clonal inter- and intra-hospital transmission of this difficult-to-treat yeast has become widespread in several countries, causing a broad range of health care-associated invasive infections in a short period of time.³⁸

Most fungal and bacterial infections can be treated with drugs. However, for drug-resistant fungi and bacteria, their genes evolve so quickly that the treatment becomes ineffective and allows the dangerous disease to spread.³⁹ Use of SOS for wound infection prevention and control may reduce the need to perform confirmatory diagnostic procedures when faced with a suspected fungal infection and subsequently decrease the burden on laboratories, the time from infection onset to treatment, and the use of inappropriate antifungal therapies.

The findings of the current study suggest that SOS displays broad-spectrum fungicidal activity against C albicans, C auris, C tropicalis, C parapsilosis, S schenckii, and *T mentagrophytes*. These results are likely to be related to the presence of HOCl (0.003%) in SOS. Accordingly, previous research findings have suggested that HOCl is the main antimicrobial component of SOS.^{17,21,40} In 1981, Albrich et al⁴¹ reported that antimicrobial activity of HOCl was possibly contributed by its ability to oxidize nucleotides, activate latent enzymes, or even inactivate enzymes and electron transport systems, thereby disrupting basement membranes or cell membranes of pathogens. HOCl is a nonselective oxidant that reacts with biomolecules, including nucleotides and enzymes, to exert its antimicrobial effects.13,14

LIMITATIONS

This study has several limitations. Only 6 fungal species were tested, so it is

unclear whether SOS would display the same fungicidal activity for all fungi. Nevertheless, SOS displayed substantial fungicidal activity against Candida species, the most common fungal agent in wound infections.35 Additionally, the effectiveness of SOS was not compared with that of commonly administered antifungals. SOS should be tested in humans, and compared with antifungal medications, to confirm whether the in vitro results of this study can be translated to the clinical setting. Although the results of this preliminary in vitro study are promising, fungi were cultured on agar plates which do not effectively mimic the physiological conditions in chronic wounds. As a result, potential fungal defenses that may have been activated in a chronic wound setting may not have been available in vitro. In addition, the SOS used in this study is one of multiple commercially available SOS products.

CONCLUSIONS

The potent in vitro fungicidal activity shown by SOS against the selected fungi suggests that SOS may be an effective tool to treat and prevent fungal infections in hospital- and health care-associated settings. Furthermore, SOS may be an ideal choice for wound irrigation and debridement owing to its antimicrobial effects, which help to prevent wound infections and accelerate the wound healing process. These preliminary in vitro results warrant further investigation to determine whether the fungicidal activity of SOS is maintained in humans. Future studies could compare the effectiveness of SOS with that of topical antifungals to investigate whether SOS has enhanced activity against biofilms. Alternatively, future clinical studies could compare the effectiveness of SOS and conventional antiseptics on chronic wound infections and monitor wound healing progression in these patients. W

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