Beyond cleansing: harnessing HOCl as an effective wound treatment

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Background and Introduction

Pseudomonas aeruginosa is found in 48–52% of chronic wound biofilms, where its resistance to antimicrobials and host immunity presents a major clinical challenge. Hypochlorous acid (HOCl) is known to be an effective antimicrobial, as well as a wound healing and odor control agent, however the full scope of its various mechanisms of action has not been characterized. Here, we used tandem mass tag (TMT)-based quantitative proteomics to determine the effects of HOCl upon *P. aeruginosa* in a physiologically relevant chronic wound biofilm model, tested the efficacy of HOCl against a selection of clinically relevant strains of *P. aeruginosa*. The efficacy of HOCl was tested against the pro-inflammatory cytokine interleukin-6 (IL-6) which is elevated and persistent in chronic wounds. The impact of HOCl on dimethyl trisulfide (DMTS), a common volatile organic compound that is responsible for wound odor, was also studied.

P. aeruginosa Biofilm Inactivation and Inhibition by HOCl

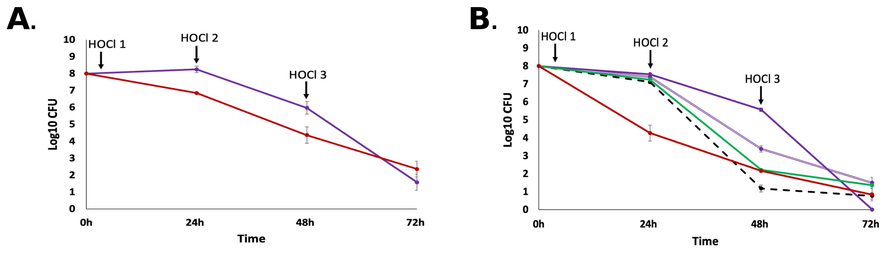


Figure 1. Efficacy of HOCl gel against chronic wound biofilms. Multiple doses of HOCl gel applied at 5, 24 and 48 h (indicated by arrows) to biofilms. A. Two-species biofilms of *Staphylococcus aureus* (purple) and *P. aeruginosa* (red) and B. Five-species of *Staphylococcus aureus* (purple); *Streptococcus pyogenes* (light purple); *Enterococcus faecalis* (black dotted); *Escherichia coli* (green) and *P. aeruginosa*

Table 1. Minimum Inhibitory Concentration (MIC) response of *P. aeruginosa* strains to hypochlorous acid (HOCl) following 5 min exposure. MIC assays were done in saline (pH 4) with 2.7 mM HOCl. HOCl was quenched after treatment and glucose was added post-treatment as a nutrient source. The MIC was determined resazurin fluorescence.

Bacterial strain	HOCl MIC (mM)
P. aeruginosa PAO1	0.66
ATCC 9027	0.33
PA14	0.16
Mucoid	0.33
Non-mucoid	0.33
Mi162	0.66
AMT006	0.66
AMT0023-34	0.66
LES-400	0.66
MHRA-1	0.33
MHRA-2	0.33
ULS-1	0.33
ULS-2	0.33

- HOCl gel is initially selective for *P. aeruginosa* in both two- and five-species biofilms
- Multiple applications of HOCl gel continually reduced all bacterial numbers
- HOCl gel shows promise as a new topical antimicrobial for wounds
- HOCl is effective at low concentrations against chronic wound *P. aeruginosa* strains

Effects of HOCl on *P. aeruginosa* Protein Levels

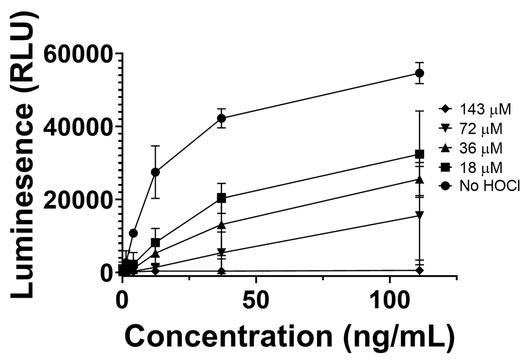
Table 2. Antibiotic target proteins depleted after HOCl treatment determined by TMT-based quantitative proteomics. HOCl gel (5.7 mM, pH 6) was applied to mature *P. aeruginosa* biofilms established in a

Family of Antibiotics	Known targets depleted after HOCl treatment
Aminoglycosides	Ribosomal 30s
Quinolones	DNA gyrase and synthesis via topoisomerase II and IV
B-lactams	Penicillin binding proteins
Polymyxins	Lipid/LPS biosynthesis
Macrolides	Ribosomal 50s
Macrocyclic peptide (MCP)	LPS transport

- HOCl treatment significantly reduced biofilm viability
- Depletion of proteins involved in biosynthesis, virulence, antibiotic resistance, and biofilm formation
- Proteins shift toward survival phenotypes and weakened pathogenicity.
- HOCl disrupts multiple pathways essential for *P. aeruginosa* survival and virulence.

Anti-inflammatory Effects of HOCl

Figure 3. *In vitro* IL-6 bioassay with engineered human cells. Signal transduction detection by luminescence of cells incubated with IL-6 treated with HOCl.[4]



- HOCl directly modifies IL-6 and prevents binding to the IL-6 receptor responsible for initiating an inflammatory cascade
- HOCl may help mitigate proinflammatory processes that interfere with wound healing

Odor Control by HOCl

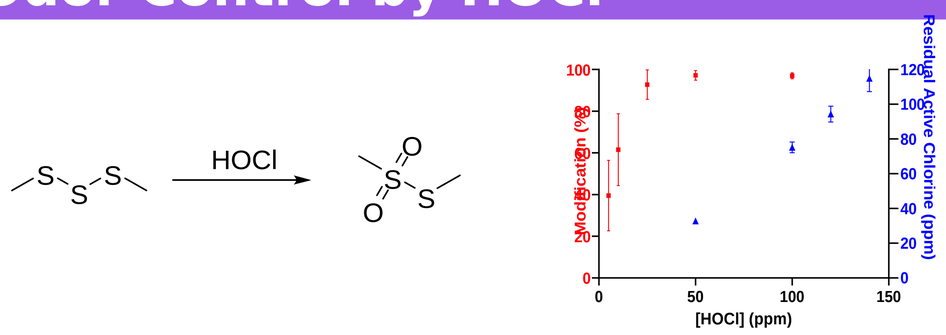


Figure 4. Modification of DMTS by HOCl detected by HPLC.[5]

- HOCl eliminates odor compounds from chronic wounds
- Sensory studies confirm HOCl alters the perception of DMTS and other wound VOCs (e.g., indole) found in chronic wounds
- Improves patients' and caregivers' quality of life

References

1. Duckworth PF et al. A novel flow-system to establish experimental biofilms for modelling chronic wound infection and testing the efficacy of wound dressings. Microbiol Res. 2018;215:141-7.
2. Nedelea AG, Plant RL, Robins LI, Maddocks SE. Testing the efficacy of topical antimicrobial treatments using a two- and five-species chronic wound biofilm model. J Appl Microbiol. 2022;132(1):715-24.
3. Robins LI et al. Dissecting HOCl Action in Chronic Wound Biofilms: Proteomic Insights From a Host-Relevant Model of Pseudomonas aeruginosa. MicrobiologyOpen. 2025 Dec;14(6):e70181.
4. Robins LI et al. Modifications of IL-6 by Hypochlorous Acids: Effects on Receptor Binding. ACS Omega. 2021 Dec 14;6(51):35593-35599. doi: 10.1021/acsomega.1c05297. PMID: 34984290; PMCID: PMC8717532.
5. Dennler-Church TE et al. Modification of Major Contribfutors Responsible for Latrine Malodor on Exposure to Hypochlorous Acid: The Potential for Simultaneously Impacting Odor and Infection Hazards to Encourage Latrine Use. Am J Trop Med Hyg. 2020 Dec;103(6):2584-2590.

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