Stability and Antibiofilm Efficiency of PCS Hypochlorous Water (Hypochlorous Acid)

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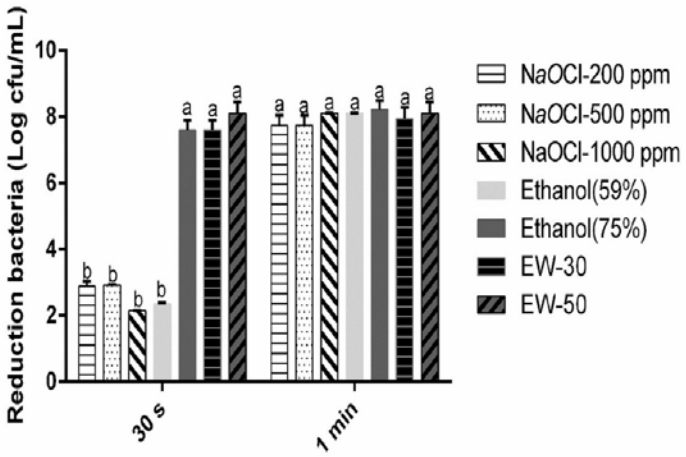
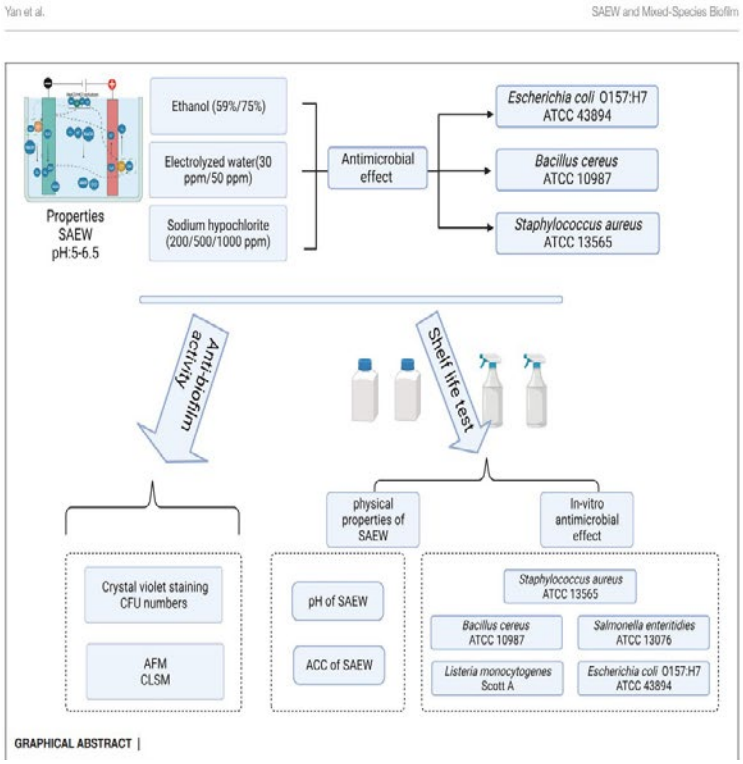
(1) Stability and Antibiofilm Efficiency of Slightly Acidic Electrolyzed Water Against Mixed-Species of Listeria monocytogenes and Staphylococcus aureus

In the natural environment, most microorganisms live in mixed-species biofilms, in which the metabolism and growth of organisms are different from that in single-species biofilms. Adhesive bacteria and their biofilms on the surface of food processing equipment are the sources of cross-contamination, leading to the risk for humans. Slightly acidic electrolyzed water (SAEW) has been proposed as a novel sanitizer in the food and agriculture industry. In this study, we investigated the changes in the physical properties of SAEW under different conditions and the disinfection abilities of SAEW against spore-forming and non-spore-forming pathogens. Furthermore, we examined the disinfection abilities of SAEW after 12 months of shelf life on a mixed-species biofilm of Listeria monocytogenes Scott A and Staphylococcus aureus. The results showed that SAEW at 30 and 50 ppm achieved all-kill of the spore-forming pathogen Bacillus cereus within 30 s. Changes in the ACC and pH of the produced SAEW were generally affected by the storage conditions. Both spore-forming and non-spore-forming pathogens were not detected under treatment with 50 ppm SAEW for 5 min under HDPE-closed conditions throughout the whole storage period. Moreover, 25 mg/L SAEW can inactivate L. monocytogenes Scott A and S. aureus biofilm cells in ~2.45 and 2.57 log CFU/mL in biofilms within 5-min treatment. However, the decline of the two bacteria in the mixed-species biofilm was 1.95 and 1.43 log CFU/mL, respectively. The changes in the cell membrane permeability of the mixed-species biofilm under treatment with SAEW were observed by using atomic force microscopy and confocal laser scanning microscopy. L. monocytogenes Scott A was more sensitive to SAEW in the mixed-species biofilm cells. These findings exhibited strong antibiofilm activities of SAEW in impairing biofilm cell membranes, decreasing cell density, and eliminating biofilm, which suggest that SAEW is an excellent antibacterial agent in the food processing industries.





CONCLUSION

To our knowledge, this is the first study to assess the effects of SAEW on the mixed-species biofilms of S. aureus and L. monocytogenes Scott A during a shelf life of 12 months.

The changes in physical properties and antimicrobial activity of SAEW are highly dependent on the storage time and conditions, including the material and open-closed environment. Neither spore-forming nor non-spore-forming pathogens were detectable for a 5-min reaction throughout the whole storage period under HDPE-closed conditions of 50 ppm SAEW.

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(2) In Vitro Antibacterial Activity of Hydrogen Peroxide and Hypochlorous Acid, Including That Generated byElectrochemical Scaffolds

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ABSTRACT Hydrogen peroxide (H2O2) and hypochlorous acid (HOCl) are biocides used for cleaning and debriding chronic wound infections, which often harbor drug resistant bacteria. Here, we evaluated the in vitro activity of H2O2 and HOCl against 27 isolates of eight bacterial species involved in wound infections. Minimum inhibitory concentrations (MICs) and minimum biofilm bactericidal concentrations (MBBCs) were measured. Compared to their respective MICs, MBBCs of isolates exposed to H2O2 were 16- to 1,024-fold higher, and those exposed to HOCl were 2- to 4-fold higher.These results suggest that HOCl has similar activity against planktonic and biofilm bacteria whereas the activity of H2O2 is less against biofilm than planktonic bacteria.

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TABLE 1 Susceptibility of bacterial isolates (planktonic and biofilm forms) to H_2O_2 and HOCl

			Value (means ± SD, in mM) for[a]:					
			H_2O_2			HOCl		
Bacteria	Isolate designation	Isolate characteristics	MIC	MBIC	MBBC	MIC	MBIC	MBBC
S. aureus	USA100	Clinical isolate, resistant to methicillin	0.40 ± 0.00	0.40 ± 0.00	85 ± 29	1.65 ± 0.57	1.32 ± 0.57	1.32 ± 0.57
S. aureus	USA200	Clinical isolate, resistant to methicillin	0.27 ± 0.11	0.40 ± 0.00	85 ± 29	1.65 ± 0.57	0.99 ± 0.00	1.32 ± 0.57
S. aureus	USA300	Clinical isolate, resistant to methicillin	0.40 ± 0.00	0.66 ± 0.23	68 ± 29	1.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
S. aureus	IDRL-6169	Prosthetic hip isolate; resistant to methicillin and mupirocin	0.40 ± 0.00	0.66 ± 0.23	51 ± 0.00	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00
S. aureus	Xen 30	Clinical isolate; resistant to methicillin	0.66 ± 0.23	0.53 ± 0.23	119 ± 78	1.32 ± 0.57	1.32 ± 0.57	1.32 ± 0.57
S. aureus	IDRL-4284	Clinical isolate; resistant to methicillin	0.66 ± 0.23	0.66 ± 0.23	170 ± 59	1.99 ± 0.00	1.32 ± 0.57	0.99 ± 0.00
S. epidermidis	ATCC 35984	Catheter sepsis isolate; resistant to methicillin	0.53 ± 0.23	0.53 ± 0.23	170 ± 59	1.65 ± 0.57	1.32 ± 0.58	1.65 ± 0.57
S. epidermidis	IDRL-6461	Prosthetic knee infection isolate; susceptible to methicillin	0.53 ± 0.23	0.66 ± 0.23	136 ± 59	1.32 ± 0.57	0.99 ± 0.00	0.99 ± 0.00
S. epidermidis	Xen 43	Catheter isolate; susceptible to methicillin	0.40 ± 0.00	1.06 ± 0.46	102 ± 0.00	1.32 ± 0.57	1.32 ± 0.58	1.32 ± 0.57
E. faecalis	ATCC 29212	Urine isolate	3.19 ± 0.00	1.86 ± 1.22	136 ± 59	0.66 ± 0.29	1.32 ± 0.57	1.65 ± 0.57
E. faecalis	IDRL-8618	Prosthetic hip infection isolate	0.53 ± 0.23	1.33 ± 0.46	102 ± 0.00	0.50 ± 0.00	1.32 ± 0.57	1.99 ± 0.00
E. faecalis	IDRL-7107	Prosthetic knee infection isolate	3.19 ± 0.00	4.25 ± 1.84	170 ± 59	0.50 ± 0.00	1.99 ± 0.00	2.32 ± 1.52
E. faecium	IDRL-11790	Abscess isolate; resistant to vancomycin and penicillin, and susceptible to linezolid	0.80 ± 0.00	0.80 ± 0.69	55 ± 45	0.99 ± 0.00	0.82 ± 0.28	1.32 ± 0.57
E. coli	IDRL-10366	bla_{KPC}-positive isolate; resistant to ceftolozane-tazobactam, imipenem, meropenem, ertapenem, ceftriaxone, and cefepime	1.33 ± 0.46	0.66 ± 0.23	170 ± 59	0.99 ± 0.00	1.32 ± 0.57	1.32 ± 0.57
E. coli	IDRL-7029	Prosthetic hip infection isolate	1.59 ± 0.00	1.86 ± 1.22	340 ± 118	0.99 ± 0.00	1.32 ± 0.57	3.31 ± 1.15
E. coli	IDRL-6199	Prosthetic knee infection isolate	2.13 ± 0.92	1.59 ± 0.00	408 ± 0.00	0.99 ± 0.00	1.65 ± 0.57	3.31 ± 1.15
E. coli	IDRL-8110	Blood isolate	2.66 ± 0.92	3.72 ± 2.44	340 ± 118	0.99 ± 0.00	1.65 ± 0.57	3.97 ± 0.00
P. aeruginosa	IDRL-7262	Prosthetic hip infection isolate	0.66 ± 0.23	170 ± 58.89	408 ± 0.00	0.99 ± 0.00	1.65 ± 0.57	1.65 ± 0.57
P. aeruginosa	Xen 5	Blood isolate	2.13 ± 0.92	170 ± 58.89	612 ± 353	0.99 ± 0.00	>3.97	>3.97
P. aeruginosa	PAO1, ATCC 47085	Wound isolate; type strain	2.66 ± 0.92	153 ± 88.33	680 ± 236	0.99 ± 0.00	1.65 ± 0.57	1.99 ± 0.00
P. aeruginosa	PA14	Wild-type laboratory strain	3.19 ± 0.00	85 ± 29.44	408 ± 0.00	0.99 ± 0.00	1.65 ± 0.57	1.65 ± 0.57
P. aeruginosa	PA14 Δ*katAB*	*katA* and *katB* double knockout of PA14	0.20 ± 0.00	3.72 ± 2.43	51 ± 0.00	0.99 ± 0.00	1.32 ± 0.57	1.65 ± 0.57
P. aeruginosa	IDRL-11442	Groin isolate; resistant to piperacillin-tazobactam, cefepime, ceftazidime, meropenem, aztreonam, ciprofloxacin, levofloxacin; susceptible to colistin	0.60 ± 0.34	51 ± 0.00	170 ± 59	0.99 ± 0.00	1.65 ± 0.57	1.32 ± 0.57
A. baumannii	ATCC 17978	Meningitis isolate	0.80 ± 0.00	2.13 ± 0.92	85 ± 29	0.83 ± 0.29	0.99 ± 0.00	1.32 ± 0.57
A. baumannii	ATCC BAA-1605	Sputum isolate; resistant to ceftazidime, gentamicin, ticarcillin, piperacillin, aztreonam, cefepime, ciprofloxacin, imipenem and meropenem	0.80 ± 0.00	2.12 ± 0.92	68 ± 29	0.83 ± 0.29	1.32 ± 0.57	0.83 ± 0.29
A. baumannii	ARLG-1268	Wound isolate; resistant to amikacin, ampicillin, cefepime, ceftazidime, ciprofloxacin and tobramycin	1.06 ± 0.46	2.66 ± 0.92	102 ± 0.00	0.66 ± 0.29	0.66 ± 0.29	0.66 ± 0.29
K. pneumoniae	IDRL-10377	bla_{KPC}-positive isolate; resistant to ceftolozane-tazobactam, imipenem, meropenem, ertapenem, ceftriaxone and cefepime	0.40 ± 0.00	2.12 ± 0.92	102 ± 0.00	0.99 ± 0.00	0.66 ± 0.29	0.99 ± 0.00

[a]Susceptibility data values (i.e., MIC, MBIC, and MBBC) are represented as means ± SD ($n = 3$). All experiments were performed in triplicates. *S. aureus* USA100, USA200, and USA300 strains were provided by Henry Chambers III (University of California, San Francisco). Xen 30, Xen 43, and Xen 5 strains were provided by Caliper Life Sciences. *P. aeruginosa* PAO1, PA14, and PA14 Δ*katAB* strains were provided by Daniel Hassett (University of Cincinnati). *A. baumannii* ARLG-1268 was provided by the Antibacterial Resistance Leadership Group of the National Institutes of Health.

It is our view that to ideally use H2O2 as an antibiofilm agent, a high working concentration of H2O2 along with a long surface contact time are likely to be needed.

The mean MICs of HOCl against the bacteria studied ranged from 0.50 to 1.99 mM. In contrast to H2O2, we did not observe large variations in MIC, MBIC, or MBBC ranges. The mechanism of action of HOCl is incompletely defined, and how bacterial molecular stress mechanisms respond to it are also poorly understood. It has been proposed that the transport of free chlorine into biofilms is a significant factor in imparting resistance (35). In work done by Castillo et al., HOCl was used as oral rinses to remove dental plaque (36). HOCl was a more effective antibacterial agent than chlorhexidine and reduced bacterial viability of different periodontopathic bacteria found in biofilms. The authors suggested that HOCl can oxidize taurine, an amino acid, promoting the formation of chlorine-taurine complexes that have antibacterial activity. In another study, 0.018% HOCl (2.72 mM) removed lipopolysaccharides found in Porphyromonas gingivatis biofilms. The authors suggested that HOCl forms chlorohydrins, which attack acyl chains in unsaturated fatty acids, causing cell membrane damage along with cytolysis (37). HOCl has been found to interact with sulfur-containing amino acids, aromatic amino acids, nitrogen-containing compounds, and lipids (38). Various ATP-independent HOCl-sensing chaperones, like Hsp33, RidA, CnoX, etc., have been found to be activated as part of the immediate counter-response to HOCl, especially in Gram-negative bacteria.

In conclusion, our data suggest that HOCl has similar activity against planktonic and biofilm bacteria, whereas H2O2 is substantially less active against biofilm than planktonic bacteria. We did not observe the emergence of antibiofilm resistance with repeated exposure to either H2O2- or HOCl-producing e-scaffolds under the conditions studied.

Aherne et al. BMC Oral Health (2022) 22:415
(3) Effects of stabilized hypochlorous acid on oral biofilm bacteria
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Abstract

Background: Caries and periodontitis are amongst the most prevalent diseases worldwide, leading to pain and loss of oral function for those affected. Prevention relies heavily on mechanical removal of dental plaque biofilms but for populations where this is not achievable, alternative plaque control methods are required. With concerns over undesirable side-effects and potential bacterial resistance due to the use of chlorhexidine gluconate (CHX), new antimicrobial substances for oral use are greatly needed. Here we have investigated the antimicrobial effect of hypochlorous acid (HOCl), stabilized with acetic acid (HAc), on oral biofilms and compared it to that of CHX. Possible adverse effects of stabilized HOCl on hydroxyapatite surfaces were also examined.

Methods: Single- and mixed-species biofilms of six common oral bacteria (Streptococcus mutans, Streptococcus gordonii, Actinomyces odontolyticus, Veillonella parvula, Parvimonas micra and Porphyromonas gingivalis) within a flow-cell model were exposed to HOCl stabilized with 0.14% or 2% HAc, pH 4.6, as well as HOCl or HAc alone. Biofilm viability was assessed in situ using confocal laser scanning microscopy following LIVE/DEAD® BacLight™ staining. In-situ quartz crystal microbalance with dissipation (QCM-D) was used to study erosion of hydroxyapatite (HA) surfaces by stabilized HOCl.

Results: Low concentrations of HOCl (5 ppm), stabilized with 0.14% or 2% HAc, signifcantly reduced viability in multi-species biofilms representing supra- and sub-gingival oral communities, after 5 min, without causing erosion of HA surfaces. No equivalent antimicrobial effect was seen for CHX. Gram-positive and Gram-negative bacteria showed no significant deferential susceptibility to stabilized HOCl.
Conclusions:
At low concentrations and with exposure times which could be achieved through oral rinsing, HOCl
stabilized with HAc had a robust antimicrobial activity on oral biofilms, without causing erosion of HA surfaces or affecting viability of oral keratinocytes. This substance thus appears to offer potential for prevention and/or treatment of oral biofilm-mediated diseases.
Keywords: Biofilm control, Oral disease, Caries, Periodontitis, Oral infection

In summary, this study shows that at low concentrations and with short exposure times, HOCl stabilized with HAc has a robust antimicrobial activity against biofilms of a range of different oral bacteria, without causing erosion of HA surfaces or affecting keratinocyte viability. In the light of concerns regarding development of resistance to antibiotics and even CHX, this substance appears to offer potential for the prevention and treatment of oral biofilm-mediated diseases. Further studies are now required to investigate the efficacy of stabilized HOCl in vivo, where factors such as substantivity and the role of saliva can be assessed.