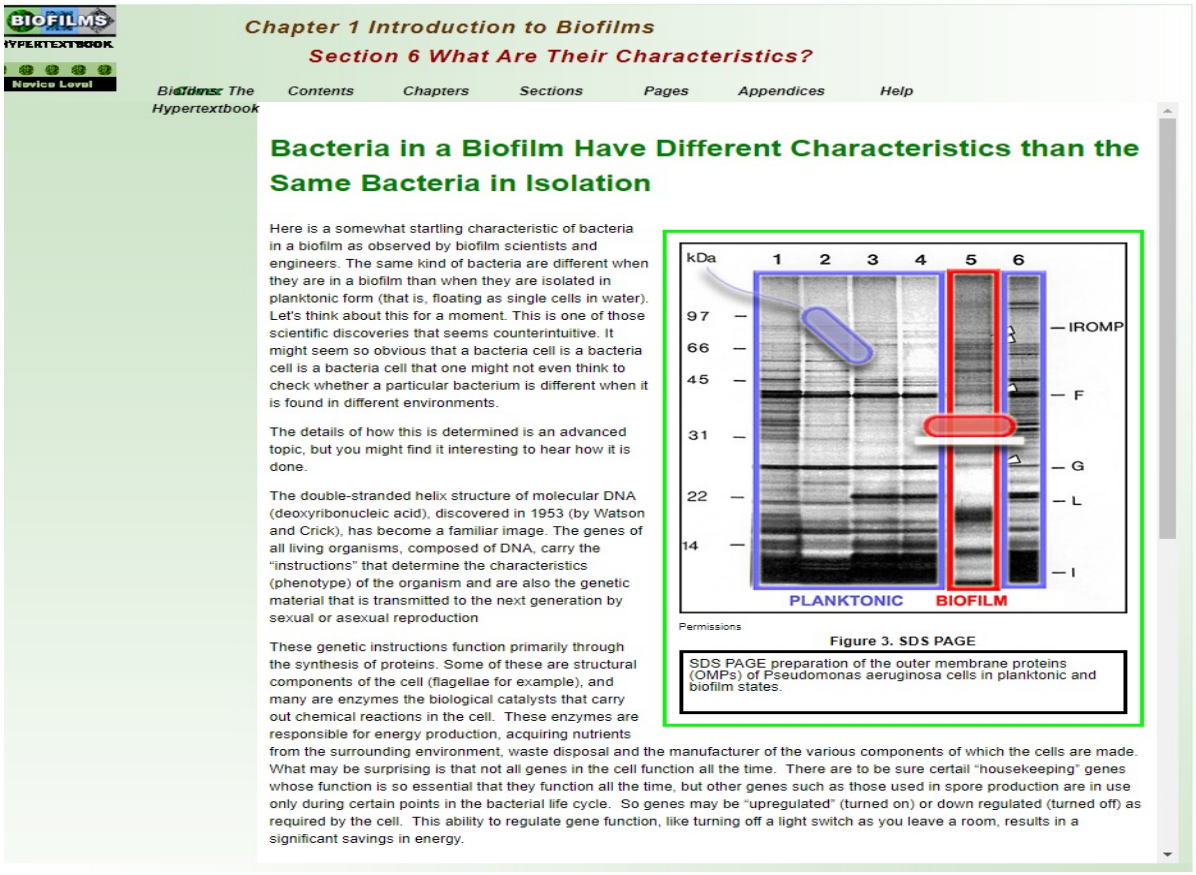
Planktonic bacteria are more susceptible to antimicrobial chemicals designed to kill them than are biofilm bacteria



BIOFILMS HYPERTEXTBOOK
Novice Level

Chapter 1 Introduction to Biofilms
Section 6 What Are Their Characteristics?

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Bacteria in a Biofilm Have Different Characteristics than the Same Bacteria in Isolation

Here is a somewhat startling characteristic of bacteria in a biofilm as observed by biofilm scientists and engineers. The same kind of bacteria are different when they are in a biofilm than when they are isolated in planktonic form (that is, floating as single cells in water). Let's think about this for a moment. This is one of those scientific discoveries that seems counterintuitive. It might seem so obvious that a bacteria cell is a bacteria cell is a bacteria cell that one might not even think to check whether a particular bacterium is different when it is found in different environments.

The details of how this is determined is an advanced topic, but you might find it interesting to hear how it is done.

The double-stranded helix structure of molecular DNA (deoxyribonucleic acid), discovered in 1953 (by Watson and Crick), has become a familiar image. The genes of all living organisms, composed of DNA, carry the "instructions" that determine the characteristics (phenotype) of the organism and are also the genetic material that is transmitted to the next generation by sexual or asexual reproduction

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Figure 3. SDS PAGE

SDS PAGE preparation of the outer membrane proteins (OMPs) of Pseudomonas aeruginosa cells in planktonic and biofilm states.

These genetic instructions function primarily through the synthesis of proteins. Some of these are structural components of the cell (flagellae for example), and many are enzymes the biological catalysts that carry out chemical reactions in the cell. These enzymes are responsible for energy production, acquiring nutrients from the surrounding environment, waste disposal and the manufacturer of the various components of which the cells are made. What may be surprising is that not all genes in the cell function all the time. There are to be sure certail "housekeeping" genes whose function is so essential that they function all the time, but other genes such as those used in spore production are in use only during certain points in the bacterial life cycle. So genes may be "upregulated" (turned on) or down regulated (turned off) as required by the cell. This ability to regulate gene function, like turning off a light switch as you leave a room, results in a significant savings in energy.

So what? Beyond the intellectual interest this holds for biofilm scientists and engineers, what practical use does this knowledge have?

One example is in the development of antibiotics. These drugs traditionally have been developed to kill planktonic bacteria under the assumption that they would kill the same bacteria wherever they were found. We now know, however, that Planktonic bacteria are more susceptible to antimicrobial chemicals designed to kill them than are biofilm bacteria, and Many of the infections plaguing humans are actually caused by bacteria in the biofilm mode of growth, not the planktonic mode of growth.

Put these two things together with the fact that traditional antibiotics have been designed for and tested on bacterial cells in their relatively unprotected, planktonic state and we can begin to understand why it is that antibiotics don't work well on these same bacteria when they exist in a biofilm—the same bacterium is different in the biofilm state than in the planktonic state for which the antibiotic was designed and tested!

Disinfectants and cleaning agents like antibiotics are validated with test on Planktonic single cell bacteria and can not be relied upon to be effective against biofilm bacteria.

Many research studies are now testing both Planktonic bacteria and Biofilm bacteria providing institutions with more information on efficacy on both vegetative Planktonic bacteria and biofilm bacteria.

PCS hypochlorous water group of cleaning agents and disinfectants all contain hypochlorous acid (HOCL) one the very few chemistries effective against planktonic and biofilm bacteria at very low concentrations.

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

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American Society for Microbiology- Accepted 23 February 2021
(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.