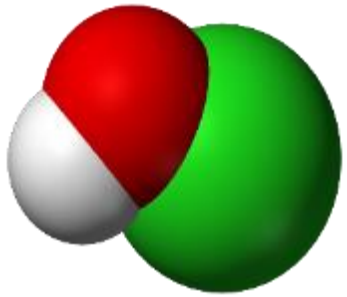
Hypochlorous acid (HOCl)

Its role in the management of Wounds



HOCl: General information

Whenever chlorine is used as a disinfectant, it is the free chlorine that acts as active ingredient.

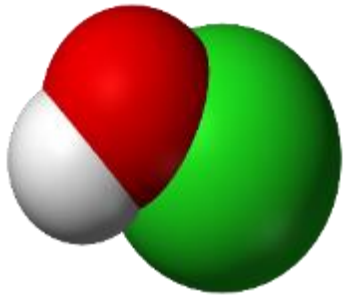
Three free chlorine molecules:

1. HOCl
2. OCl^- (bleach)
3. $CL_{2\,(g)}$

The relationship between the 3 free chlorine molecules in water, is governed by the pH of the solution

HOCl is 80 – 100 more bactericidal than bleach [1, 2]

1. LeChevallier MW, Cawthon CD, Lee RG. Inactivation of biofilm bacteria. Appl Environ Microbiol. 1988, 54(10)
2. Morris JC. Future of chlorination. J Am Water Assoc. 1966(11), 58:1475-1482. :2492-2499.



HOCl

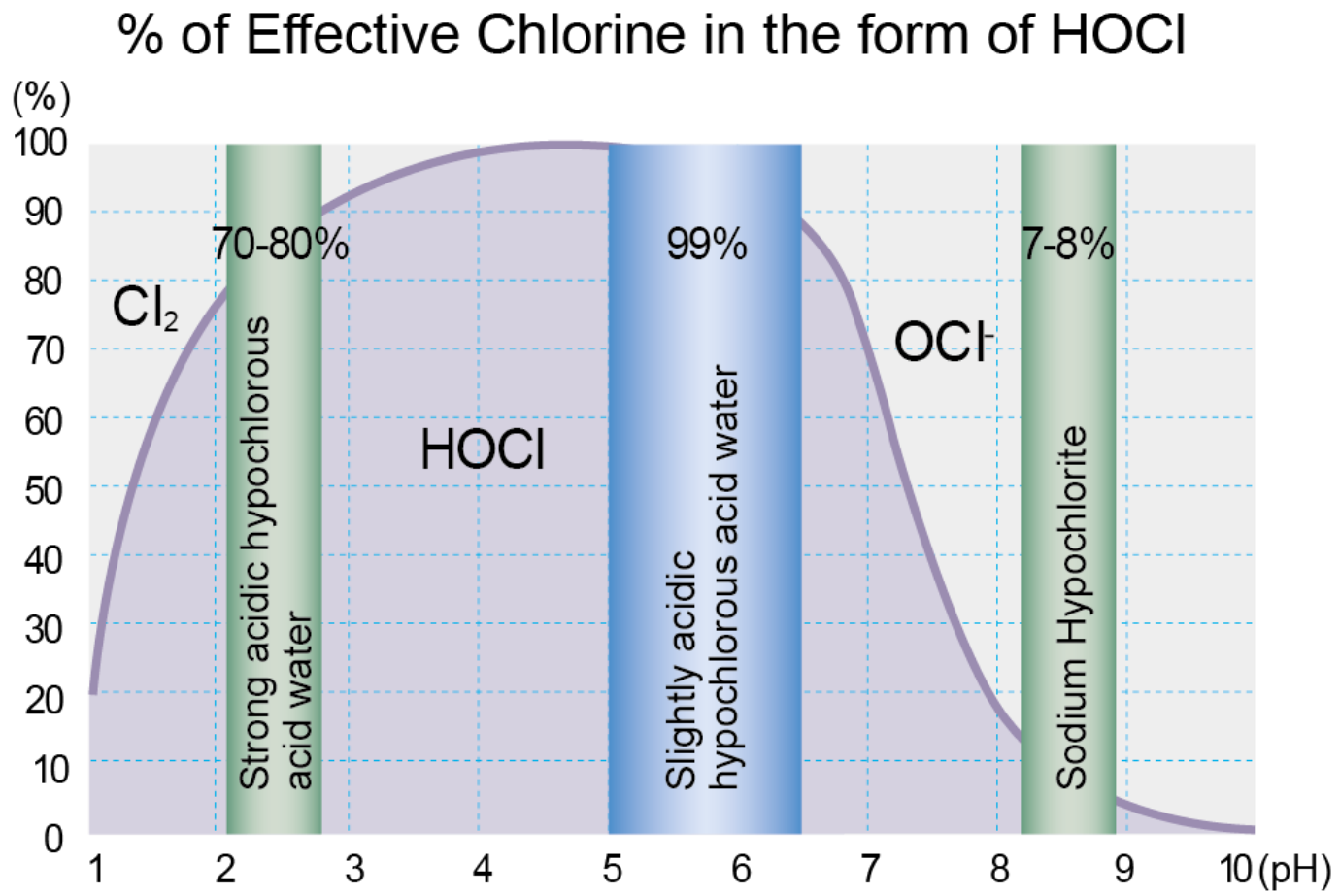
HOCl is a weak acid

Dissociation constant of well-known acids:

HCl		< 1
Lactic	3.86	
Ascorbic	4.10	
Citric		4.74
HOCl		7.5
H_2O_2		11.62
H_2O		14

What this means is that HOCl, when uncontaminated, should have a pH of 4.5-5.5

The relationship between the 3 free chlorine molecules in water, is governed by the pH of the solution.



Correlation between pH and purity

pH of Electrolyzed water	FAC Composition (%)		
	HOCl	Cl2	*OCl⁻
2.7	70	30	0
3.0	80	20	0
3.5	90	10	0
4.0	95	5	0
4.5	100	0	trace
5.0	100	0	trace
5.5	100	0	trace
6.0	98	0	2
6.5	95	0	5
7.0	78	0	22
7.5	50	0	50

HOCl vs. its salts

NaOCl, $Ca(OCl)^{2,}$ Chlorine dioxide, $Cl^{2\,(g)}$

All have respiratory, skin and eye side effects.

HOCl is colorless, tasteless, and only has a slight chlorine smell

No side effects

Strong Bactericidal Effect

How does HOCl kill pathogens?

The antimicrobial activity of HOCl has been extensively studied.

It causes respiratory loss in bacterial cell membranes due to an irreversible reaction with "sulfur" and "heme" containing membrane enzymes and structural proteins[1] leading to cell death and nonviability[2]

1. Vissers MC, Winterbourn CC. Oxidation of intracellular glutathione after exposure of human red blood cells to hypochlorous acid. Biochem J 1995;307:57−62 .

1. McKenna SM, Davies KJ. The inhibition of bacterial growth by hypochlorous acid. Possible role in the bactericidal activity of phagocytes. Biochem J 1988;254:685−92

Strong Bactericidal Effect

- HOCl has long history as an anti-microbial agent [1,2] Efficacy against bacteria, viruses and fungi.
- 10^6 neutrophils stimulated in vitro can produce 0.1µM HOCl and this can kill 1.5 x 10^7 *E. coli* in less than 5 minutes[3]
- Has fast onset of action against a wide range of pathogens: Usually less than 2 minutes (Exception is *Streptococcus pyogenes* – 0.169 ppm: 10 minutes)[4]
- Very low MBC (Minimum Bactericidal Concentrations) of less than 3 ppm needed. Only exception *Aspergillus niger* 86.6 ppm[4]
- HOCl is 80 – 100 x more deadly against microorganisms than bleach. Far outperforms both NaOCl and H_2O_2 [4]
- No build-up of resistance against its use has been demonstrated.
- In the mouth, stronger reaction against Gram-negative than Gram-positive bacteria[5] (bactericidal against pathogenic bacteria)

1. Dychadala 1991 Disinfect, Steril and Pres 4th ed (Ed SS Block) Lea and Febiger 131-151
2. Sang, PS et al. Antimicrobial Effect of Hypochlorous Acid on Pathogenic Microorganisms. J Prev Vet Med Vol. 37, No. 1: 49-52, March 2013
3. Lapenna D, Cuccurullo F. Hypochlorous acid and its pharmacological antagonism: an update picture. Gen Pharmacol. 1996;27(7):1145-1147.
4. Wang L, Bassiri M, Najafi R, et al. Hypochlorous Acid as a Potential Wound Care Agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds. 2007;6:65-79.
5. Chong-Hou, Sam Hsein-Kun Lu. The Role of Hypochlorous Acid As One of the Reactive Oxygen Species in Periodontal Disease. *J Dent Sci* 2009; 4(2):45 -54

Antibacterial reports

Effective anti-microbial activity of HOCl at various concentrations

Bacteria
Fungi

Shin et al., 2013 J. Prev. Vet. Med. **37**: 49-52

Other references

Eryilmaz et al., 2013 Trop. J. Pharm Res. **12**: 123-126

Tanaka et al, (http://www.aquaox.net/uploads/antimicrobial_activity_of_EAW.pdf)

Table 1. Antimicrobial effect of hypochlorous acid according to concentration

Bacterial species (ATCC[1] Number)	Initial concentration	20ppm	40ppm	80ppm
Bacillus cereus (11778)	3.26×10^6	NE[2]	1.21×10^3	<10
Bacillus cereus (21366)	4.18×10^6	NE	NE	<10
Bacillus circulans (9500)	2.72×10^6	NE	NE	NDC[3]
Bacillus thuringiensis (10792)	5.17×10^6	NE	3.57×10^3	<10
Candida albicans (10259)	4.26×10^6	7.80×10	NDC	NDC
Candida albicans (10261)	7.12×10^6	4.68×10^2	NDC	NDC
Candida albicans (10231)	1.21×10^6	NDC	NDC	NDC
Candida albicans (18804)	3.97×10^6	5.12×10^2	NDC	NDC
Candida albicans (11006)	1.77×10^6	NDC	NDC	NDC
Candida albicans (18814)	3.24×10^6	4.10×10	NDC	NDC
Candida albicans (22972)	5.11×10^6	2.45×10^2	NDC	NDC
Candida albicans (28471)	5.32×10^6	3.11×10^2	NDC	NDC
Enterococcus faecalis (29212)	1.74×10^6	NE	NDC	NDC
Enterococcus faecalis (19433)	4.25×10^6	NE	NDC	NDC
Escherichia coli (25922)	6.11×10^6	NE	NDC	NDC
Escherichia coli (11105)	3.43×10^6	NE	NDC	NDC
Listonella anguillarum (19264)	2.82×10^6	NDC	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33591)	5.39×10^6	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33593)	3.06×10^6	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (6538)	2.52×10^6	NE	NDC	NDC
Mycobacterium chelonae subsp. *chelonae* (35752)	1.39×10^6	NDC	NDC	NDC
Mycobacterium fortuitum subsp. *fortuitum* (6841)	2.11×10^6	NDC	NDC	NDC
Mycobacterium smegmatis (19420)	2.26×10^6	NDC	NDC	NDC
Paenibacillus alvei (6344)	4.15×10^6	NE	NE	NDC
Pseudomonas aeruginosa (27853)	2.02×10^6	NE	NDC	NDC
Pseudomonas aeruginosa (25619)	3.55×10^6	NE	NDC	NDC
Salmonella enterica subsp. *enterica* (*typhimurium*) (29629)	5.76×10^6	NE	NDC	NDC
Shigella flexneri (29903)	3.42×10^6	NE	NDC	NDC
Shigella sonnei (25931)	5.79×10^6	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (29213)	8.15×10^6	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (25923)	5.68×10^6	NE	NDC	NDC

[1]ATCC : American type culture collection.
[2]NDC : not detected colony.
[3]NE : no effect.

HOCl vs. viruses

Effective anti-microbial activity of HOCl at low concentrations[1, 2] (0.02 ppm)

Viruses

Adenovirus type 4
Bacteriophage MS2
Hepatitis A & B (HAV)
Herpes virus type 1
HIV-1
Human and Animal Influenza (including H5N1 Avian Influenza)
MS2 virus
Human Norovirus (Norwalk)
Murine Norovirus
Orthopoxvirus
Poliovirus type 1
Poliovirus type 2
http://www.hsp-usa.com/faq/
Rhinovirus,

1. Yu et al., The effect of a low concentration of hypochlorous acid on rhinovirus infection of nasal epithelial cells. Am J Rhinol Allergy. 2011 25(1):40-4.
2. G. Sykes S.K. Kapoor "Disinfection and Sterilization" THE UNIVERSITY OF ILLINOIS STUDY

Comparative bactericidal vs toxicity effect

1. This antiseptic profile of NaOCl resemble some of the over-the-counter antiseptics: silver nitrates or silver ions, Betadine, and acetic acid.

2. Moreover, there are other antiseptics that are less toxic to mammalian cells but at the same time have *lower antimicrobial activity* (ethanol, hydrogen peroxide, and 5% mafenide acetate solution) as compared to HOCl[1]

1. Wang L, Bassiri M, Najafi R, et al. Hypochlorous Acid as a Potential Wound Care Agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds. 2007;6:65-79.

Safety aspects of HOCl

Reports that HOCl kill bacteria without cytotoxic effect:

1. McKenna, SM. & Davies, KJ. (1988): Inhibition of bacterial growth by hypochlorous acid. Possible role in the bactericidal activity of phagocytes. The Biochemical Journal, 254 (3): 685-92.

2. Dahlgren, A. & Karlsson. (1999): Respiratory burst in human neutrophils. J. Immunol Methods 232: 3-14.

3. Babior, BM. (1978): “Oxygen-dependent microbial killing by phagocytes (first of two parts)”, N Engl J Med. Mar 23; 298(12):659-68.

4. Wang L. (2007): “Hypochlorous Acid as a Potential Wound Care Agent. Part I. Stabilized Hypochlorous Acid: A Component of the Inorganic HOCL Armamentarium of Innate Immunity”, J Burns Wounds. 2007; 6: 69-69.

5. Selkon JB. Cherry GW. Wilson JM. Hughes MA. (2006): Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers; Journal of wound care; 15 (1); 33-37.

HOCl: Inflammation Modulation Effect

1. Inflammation is modulated through its effect on nuclear factor $_{k}$B and activator protein-1 of monocytes[1]

2. After chlorination of taurine by HOCl, taurine chloramine is mostly anti- inflammatory[1]

3. HOCl neutralizes various pro-inflammatory cytokines and chemokines (IL-1^{beta} , IL-2, IL-6)[1]

4. HOCl promotes an innate immune response against Gram-negative bacteria, via chlorination of antigens[2]

1. Chong-Hou, Sam Hsein-Kun Lu. The Role of Hypochlorous Acid As One of the Reactive Oxygen Species in Periodontal Disease. *J Dent Sci* 2009; 4(2):45 -54
2. Marcinkiewicz J et al.. Differential effects of chlorination of bacteria on their capacity to generate NO, TNF-% and IL-6 in macrophages. Immunology 1994;83:611−6.

HOCl: Healing Effect

1. Healing is promoted by regulating cytokines and growth factors[1]

2. After chlorination of taurine by HOCl, taurine chloramine enhances healing[2]

3. Enhanced healing because of neutralization and inhibition of pro-inflammatory factors[3]

4. The successful wound healing effect of HOCl was reported on a series of chronic leg ulcers[4] (Increased healing and rapidly relieves pain; 45% of non-healing ulcers healed after 3 weeks and a further 25% reduced in size by over 60%)

5. HOCl is the key in promoting healing in variety of wound types[5]

NaOCl (Dakin's solution), povidone iodine, H_2O_2 and citric acid are cytotoxic and impede wound healing (Multiple references in journals)

1. Chong-Hou, Sam Hsein-Kun Lu. The Role of Hypochlorous Acid As One of the Reactive Oxygen Species in Periodontal Disease. *J Dent Sci* 2009; 4(2):45 -54
2. Mainnemare A, et al. Hypochlorous acid and taurine-N-monochloramine in periodontal diseases. J Dent Res 2004;83:823−31.
3. Grzesik WJ et al Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 2002;13:474−84.
4. Selkon Jb et al Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers. J Wound Care 2006;15:33−7.
5. Randell Varilla, (2012): Promoting Progressive Healing Hypochlorous acid is a very potent broad-spectrum antimicrobial agent. Nurses, May 23.

Certifications: HOCl

HOCl is GRAS (Generally Recognized As Safe) - US FDA (Food and Drug Administration)

Classified as Food Additive - USA FSIS (Food Safety Inspection Service)

Classified as disinfectant ---EPA (European Protection Agency)

Certified as disinfectant---SABS (South African Bureau of Standards)

- ISO 14001: 2004 Hypochlorous Acid Water Electrolysis Apparatus
- ISO 9001: 2008 Disinfectant Producing Apparatus
- ISO 9001: 2009 Disinfectant Producing Apparatus
- HOCl in Burns: 2015

HOCl in Wounds: 2016

Safety aspects of HOCl

1. Various Regulatory Authorities certifications as mentioned

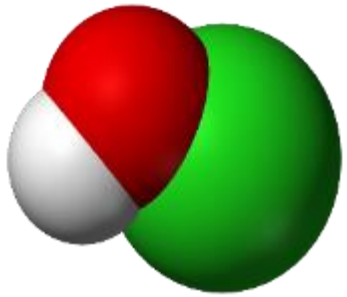
2. In vitro cytotoxicity profile in L929 cells and in vivo in various animal models also described (Skin, mucous membrane, eye sensitivity, repeat dose wound toxicity studies) = No Toxicity[1]

3. Own study:

- Excellent safety and tolerability profile in approximately 200 ophthalmic patients to date at a clinically optimised dose.
- Clinical dose-ranging and cross-over studies have identified the optimum dose based on ocular tolerability and efficacy in bacterial and viral conjunctivitis.

Positive efficacy outcomes in a range of ocular indications across ~ 200 patients and excellent safety and tolerability profile in severe wound-care patients (case studies)

1. Wang L, Bassiri M, Najafi R, et al. Hypochlorous Acid as a Potential Wound Care Agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds. 2007;6:65-79.



HOCl: General information

Whenever chlorine is used as a disinfectant, it is the free chlorine that acts as active ingredient.

Three free chlorine molecules:

1. HOCl
2. OCl^- (bleach)
3. $CL_{2\,(g)}$

The relationship between the 3 free chlorine molecules in water, is governed by the pH of the solution

HOCl is 80 – 100 more bactericidal than bleach [1, 2]

1. LeChevallier MW, Cawthon CD, Lee RG. Inactivation of biofilm bacteria. Appl Environ Microbiol. 1988, 54(10)
2. Morris JC. Future of chlorination. J Am Water Assoc. 1966(11), 58:1475-1482. :2492-2499.

HOCl: Summary of Qualities

1. Effectively penetrates and destroys biofilms

2. Very strong disinfectant: Time kill essay points to 3 – 6 mg/l being effective within 2 min against all bacteria, viruses and fungi

Two exceptions: *Aspergillus niger*:
* 86 mg/l, *Streptococcus pyogenes*
* 10 minutes

3. Strong anti-inflammatory: Reduces pain, swelling and redness.

4. Stimulates wound healing.

18 months shelf life when kept in a dark closed container

Presented in a strength of 360 mg/l, pH 5,0 - 6.0

Destroys Biofilm

1. A serious problem in chronic wound infections[1]
2. Biofilm a complex structure of microorganisms, create a protective shell[2]. It delays wound healing[3]
3. HOCl destroys these microorganisms as well as the Biofilm[3] (effective strength 3 ppm, 2 minutes)
4. When compared with povidone iodine (decreased cell migration in a wound healing essay), the HOCl showed an *increase* in cell migration[4]

1. Donlan RM, et al. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev.* 2002;15(2):167-193.
2. Hall-Stoodley L, et al. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*. 2004;2(2):95-108
3. Wolcott RD, Rhoads DD. A study of biofilm-based wound management in subjects with critical limb ischaemia. J Wound Care. 2008;17(4):145-148, 150-142, 154-155.
4. Serhan Sakarya, MD et al. Hypochlorous acid: An Ideal Wound Care Agent With Powerful Microbicidal, Antibiofilm, and Wound Healing Potency. *Wounds* 2014;26(12):342-350

Market needs

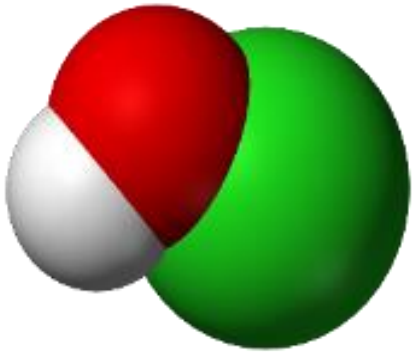
The continuous development of bacterial resistance to antibiotics as well as the current controversy regarding topical antiseptics in wound infection necessitate the development of new antimicrobial alternatives that are

- safe
- effective
- no toxicity
- low propensity for developing resistance.

HOCl answers these needs

Hypochlorous acid (HOCl) is presented as a viable
treatment modality in the management of burns.

Structure: HOCl



Burns: General Assumptions

1. Any traumatic wound, including burns can be considered infected[1]
2. Predisposed to infection due to de-vitalized tissue, bacterial presence[2]
3. Biofilm formation starts immediately and inhibits healing[3]
4. 95% of acute wounds are colonized by aerobic microorganisms[4]
5. Pain, odour due to bacterial adverse effects[5]

1. Riyat MS. & Quinton DN. (1997): Tap water as a wound cleansing agent in accident and emergency. J Accid Emerg Med 1997; 14(3): 165-6.
2. Muhammad NK. & Abul HN. (2006): Antiseptics, iodine, Povidine iodine and traumatic wound cleansing: Wound cleansing 16 (4), 6-10.
3. Wolcott RD. & Rhoads DD. (2008): A study of biofilm-based wound management in subjects with critical limb ischemia. J Wound Care 17:145-8, 150-2, 154-5.
4. Bowler PG. & Davies BJ. (1999): "The microbiology of acute and chronic wounds", Wounds 11: pp. 72–78 5. White RJ. (2009): Wound infection-associated pain. J Wound Care 18:245-9.

Burns: Principles of Wound Management

(Excluding general management e.g. fluids and nutrition)

1. Wound bed preparation: Treat infection[1]

2. Purpose: reduce bacterial counts to levels where the host immunity can cope[2]

3. Bacterial control will control odour and pain management[2]

4. Rapid wound bed preparation (removal of non-vital tissue and reduction in bacterial burden) will move the general situation from inflammation to proliferation[3]

5. Avoid NaOCl (Dakin's solution), citric acid, H_2O_2, povidone iodine: all have been proven to be toxic, exert negative chemotaxis (delay wound healing)

6. HOCl has been shown in multiple studies to reduce pain, bacterial burden, control odour and enhance healing[4]

1. Fleck CA. (2006): "Fighting infection in chronic wounds", Advances in Skin and Wound Care; 19(4): pp.184-188
2. Bowler PG. (2003): The bacterial growth guideline: reassessing its clinical relevance in wound healing. Ostomy Wound Manage; 49(1):44-53.
3. Dire DJ. & Welsh AP. (1990): A comparison of wound irrigation solutions used in the emergency department. Ann Emerg Med. 19(6): 704-8.
4. Sampson MN. & Muir AV. (2002): Not all super-oxidized waters are the same. J Hosp Infect, 52:228-9.

Burns: Avoid NaOCl (Dakin's solution), H_2O_2 citric acid and povidone iodine[1, 2, 3, 4, 5]

Multiple studies

1. Cooper ML, Boyce ST, Hansbrough JF, Foreman TJ. & Frank DH. (1990): Cytotoxicity to cultured human keratinocytes of topical antimicrobial agents. J Surg Res; 48:190-5.
2. Wilson JR, Mills JG, Prather ID. & Dimitrijevich SD. (2005): A toxicity index of skin and wound cleansers used on in vitro fibroblasts and keratinocytes. Adv Skin Wound Care; 18:373-8.
3. Zapata-Sirvent RL. & Hansbrough JF. (1993): Cytotoxicity to human leukocytes by topical antimicrobial agents used for burn care. J Burn Care Rehabil; 14(2):132-40.
4. Warriner R. (2005): "Infection and the chronic wound, a focus on silver", Advances in Skin and Wound Care, 18(1): pp. 1S–12S.
5. Wang L, Bassiri M. & Najafi R. (2007): Hypochlorous acid as a potential wound care agent:part I. Stabilized hypochlorous acid :a component of the inorganic armamentarium of innate immunity. J Burn Wounds; 6-7.

HOCl in Burns: 2015

HOCl in Wounds: 2016

treatment modality in the management of wounds.

1. Effective biofilm and infection control.
2. Reduction in inflammation (less pain, redness and swelling).
3. Enhanced healing.

HOCl: Effect on Chronic Infected Wounds

HOCl daily washes 3-5 minutes
Povidone iodine daily dressings

	HOCl	Betadine
Pain free	100% after 7 days	20% pain free after 14 days
Odor	100% gone at 7 days	30% gone at 7 days 50% gone at 14 days
14 days ready for reconstruction	100%	0%
Time to reconstruction	100% 14 days	97% after > 28 days
Bacterial counts	Eradicated	Continuous presence of Klebsiella, Proteus, MRSA, Pseudomonas

Mekkaway, M et al. A Randomized Clinical trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wounds. Journal of Education and Practice: Vol. 5, No.16, 2014

Clinical Studies-Wound Care

CASE STUDY—Septic left cubital fossa wound[1]

7 days of daily HOCl followed by skin graft

15 days

1. Mekkaway, M et al. A Randomized Clinical trial: The Efficacy of Hypochlorous Acid on Septic Traumatic Wounds. Journal of Education and Practice: Vol. 5, No.16, 2014

Clinical Studies-Wound Care
Clinical Studies - Efficacy

CASE STUDY—Abrasion Wound

5 Days treatment given twice per day (10 treatments) with 100mg/l (spray)
HOCl
No dressings

Clinical Studies-Wound Care

Clinical Studies - Efficacy

CASE STUDY— pressure ulcer

84 y old female pressure ulcer, right leg.
Wound age: 2 months
Swab +++ Staph Aureus and +++ E. Coli
Offensive odour
Soak with Hypochlorous Solution 350ppm for 10-15min twice a week and apply foam dressing
No pain verbalized

Two weeks post initialising of Hypochlorous applications
No necrotic tissue
No Odour
Bioload reduced. No cultures
Wound bed healthy and granulating.
Oedema and surrounding erythema reduced

Clinical Studies-Wound Care
Clinical Studies - Efficacy

CASE STUDY— lower leg ulcer

77 year old female, lower leg ulcer
Wound age: 8 months
Wound swab +++ E.coli
Surrounding Erythema
Pain 8/10
Offensive odour
Soak with Hypochlorous solution 350ppm for 20min twice a week. Apply foam dressing and compression.

Three weeks from initializing Hypochlorous applications.
Healthy granulation tissue, erythema and oedema reduced. Exudate reduced. No odour.
Pain has reduced 3/10

Clinical Studies-Wound Care
Clinical Studies - Efficacy

CASE STUDY— pressure ulcer

40year old paraplegic patient, stage 4 pressure ulcer over right buttock area.
Wound age: 4 months
Offensive odour, high exudate level. Bone exposed.
Wound irrigated with Hypochlorous solution 400ppm for 15-20 min every second day.
Negative pressure dressing applied.

Two weeks after initializing treatment with Hypochlorous solution, 60% health granulation tissue, Wound edges viable.
Odour reduced drastically
Bone covered with granulation tissue
Surrounding erythema and oedema reduced

Summary

Hypochlorous acid (HOCl) is an effective; easy to perform, comfortable, inexpensive and safe in cleansing (treating) infected wounds and allows for earlier surgical closure and hospital discharge.

HOCl controls the tissue bacterial bioburden without inhibiting the wound healing process rapidly relieves pain, controls odor and the area becomes well prepared for skin graft.

Thank you

Summary Benefits of HOCL

-Anti-Bacterial (80-100x more potent than sodium hypochlorite)

- Antifungal
  - Antiviral
  - Anti-inflammatory
  - Sporacidal
  - Non-Toxic
  - Hypoallergenic
  - Purity (99.9% pure stabalised HOCL)
  - Natural
  - No side effects