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Review Article

A Critical Assessment of Antimicrobial Efficacy of Hypochlorous Acid (HOCl) as a Safe Decontaminant Solution in Chronic Wounds

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Introduction

Hypochlorous acid (HOCl) is a weak acid that is produced naturally by our white blood cells as part of the immune response. It has been widely studied and used in wound care due to its antimicrobial properties and ability to promote wound healing while being safe on wounds. When applied to a wound, hypochlorous acid has antimicrobial effects against a wide spectrum of pathogens, including bacteria, fungi, and viruses without causing adverse reaction towards normal cells. It effectively kills a broad spectrum of microorganisms, including antibiotic-resistant strains such as methicillin-resistant Staphylococcus aureus (MRSA). Hypochlorous acid is generated by neutrophils, a type of white blood cell, through a process called the respiratory burst. Neutrophils release an enzyme called myeloperoxidase (MPO) into the surrounding environment, which converts chloride ions (Cl-) and hydrogen peroxide (H2O2) into hypochlorous acid (HOCl) and water (H_2O):

$$MPO + H_2O_2 + Cl^- \rightarrow HOCl + H_2O$$

Hypochlorous acid is highly reactive and acts as a potent oxidizing agent. It exerts its antimicrobial effects by disrupting the cellular structures and processes of pathogens [1].

The antimicrobial efficacy of hypochlorous acid is primarily attributed to its oxidative properties. It can penetrate the cell walls of microorganisms, disrupting essential enzymes and structures, and leading to cell death in a process known as osmotic shock. Osmotic shock is a phenomenon that occurs when a cell is exposed to a sudden change in osmotic pressure, leading to the rupture or damage of the cell membrane. When a cell is exposed to hypochlorous acid, the HOCl can penetrate the cell membrane and disrupt the balance of solutes inside the cell. This sudden change in osmotic pressure can cause water to rush into the cell or exit the cell rapidly, leading to cell swelling or shrinkage respectively. If the change in osmotic pressure is severe enough, it can result in the rupture of the cell membrane and the destruction of the cell, i.e., osmotic shock [2].

Prevalence Of Microbes On Chronic Wounds

Diabetic foot ulcers (DFUs), pressure ulcers (PU), venous leg ulcers (VLU), and surgical site infections (SSIs) are all general classes of chronic wounds. Utilization of HOCl in wound therapy as a cleansing agent can decrease the rate of bacterial accumulation and hence biofilm formation by killing off microorganisms that are frequently seen in in these polymicrobial wounds. After being cultured, studies [1] have shown that pathogens identified from these chronic wounds include gram-positive cocci, Staphylococcus aureus being the most common (Figure 1).

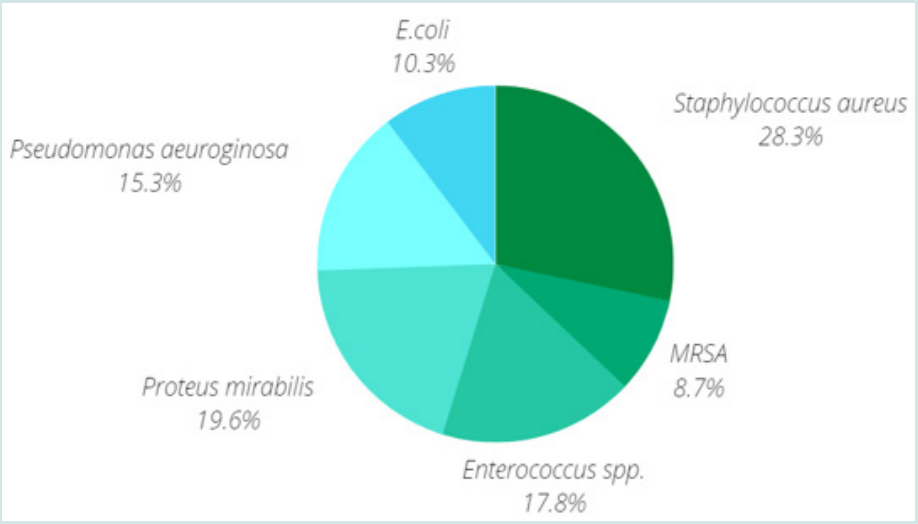

Figure 1:

Antimicrobial Studies

Time Kill Study (ASTM E2315)

Efficacy of hypochlorous acid can be evaluated in several ways. One of the method the ASTM time kill study, also known as an ASTM E2315 time kill study, which is a standardized test method developed by ASTM International (formerly known as the American Society for Testing and Materials) to evaluate the effectiveness of antimicrobial agents against microorganisms. In an ASTM time kill study, a specific concentration of the antimicrobial agent is applied to a suspension of microorganisms. Samples are taken at predetermined time intervals and plated onto growth media to determine the number of viable microorganisms remaining. The objective of the study is to determine the time required for the antimicrobial agent to kill or reduce the population of the microorganisms under test conditions [3].

The test method provides a quantitative measure of the antimicrobial activity and can be used to compare the efficacy of different antimicrobial agents or formulations. It is commonly used in the development and evaluation of disinfectants, antiseptics, and other antimicrobial products. A time-kill study data was evaluated on a commercially available hypochlorous acid wound care solution, ELECTROCYN soma. (Manufactured by V3bio Sdn. Bhd, Malaysia) to determine the time kill profile of various type of microbes. ELECTROCYN soma contains stabilized hypochlorous acid as the active ingredient that prevents proliferation of microbes. The results of bacteria up to 99.99% within 1 minute. Yeast/fungi such as Candida albicans and Aspergillus niger shows the 99% reduction is achieved at a slightly longer exposure duration at 3 minutes (Table 1).

Table 1 : Time kill study results of hypochlorous acid wound care solution.

List of Organism	Percentage of Reduction (%)		
	1 min	2 mins	3 mins
Enterococcus faecalis ATCC 29212	99.99	99.99	99.99
Escherichia coli ATCC 8739	99.99	99.99	99.99
Klebsiella pneumoniae ATCC 700603	99.99	99.99	99.99
Pseudomonas aeruginosa ATCC 9027	99.99	99.99	99.99
Methicillin resistant Staphylococcus aureus ATCC 33592	99.99	99.99	99.99
Staphylococcus aureus ATCC 6538	99.99	99.99	99.99
Bacillus subtilis ATCC 6633	99.99	99.99	99.99
Salmonella typhimurium ATCC 14028	99.99	99.99	99.99
Candida albicans ATCC 10231	84.15	94.99	99.00
Aspergillus niger ATCC 16404	80.05	93.69	99.00
Candida auris CDC B11903	99.99	99.99	99.99

The test results above indicate that hypochlorous acid can reduce microbial load (wound bioburden) that will help support wound healing and minimize risk of infection on wound [4].

Antimicrobial effectiveness testing (USP 51)

The objective of the USP 51 test is to evaluate the effectiveness of antimicrobial preservatives in pharmaceutical products, such as injectable drugs, oral solutions, and ophthalmic preparations, in preventing the growth of microorganisms. It assesses the ability of the preservative system to inhibit the growth of specified microorganisms that may be introduced into the product during its use or storage. The test involves challenging a commercially available hypochlorous acid containing wound care solution (ELECTROCYN soma) with microorganisms, including bacteria, yeast, and mold, which are known to be difficult to control. The microorganisms used in the test typically include Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, and Aspergillus niger. The USP 51 test evaluates the ability of the preservative system to reduce the microbial count over a specified period, up to 28 days. The number of surviving microorganisms is measured at 0, 14 and 28-days intervals during the test period. The acceptance criteria for passing the test are not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at

Test results show that all microorganisms have demonstrated log reduction of more than 5.5 log reduction that meets the requirements of USP 51 (more than 2.0 log reduction required as minimum) [12] (Table 2).

Table 2: Results of USP 51 test on hypochlorous acid wound care solution.

Test Organism	Inoculum spiked in (cfu/ml)	Day 0		Day 14		Day 14	
		Count	Log reduction	Count	Log reduction	Count	Log reduction
Escherichia coli	9.6 x 10^5	5.2 x 10^5	0.3	No growth	> 6.0	No growth	>6.0
Pseudomonas aeruginosa	6.7 x 10^5	3.6 x 10^5	0.3	No growth	>5.8	No growth	>5.8
Staphylococcus aureus	6.1 x 10^5	3.1 x 10^5	0.3	No growth	>5.8	No growth	>5.8
Candida albicans	4.8 x 10^5	3.2 x 10^5	0.2	No growth	>5.7	No growth	>5.7
Aspergillus niger	3.2 x 10^5	2.1 x 10^5	0.2	No growth	>5.5	No growth	>5.5

In another study done at outpatient plastic and vascular surgery clinic at Assiut university Hospital, Egypt [2], a comparative study was done using HOCl versus iodine and hydrogen peroxide on infected diabetic foot ulcer patients. Microbiological measurement was assessed by using a sterile swab that was pressed on the wound to express underlying fluid and exudates. It was taken for determining types of bacteria and for microorganism count at the start, and every 5 days until the end of the study [6].

In the study group, the results show a significant reduction in bacterial count over period of follow up of 30 days with intervals of 5 days evaluation of microbial presence on wound (Figure 2) (Table 3).

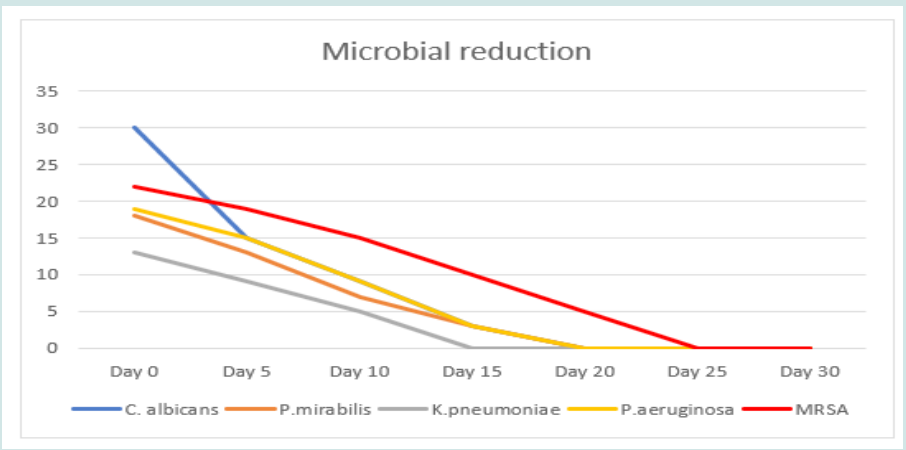

Figure 2:

Table 3 : Microbial presence on wound over period of 30 days

Microbes	Bacterial presence							Reduction % within 15 days
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	
C. albicans	30	15	9	3	0	0	0	90%
P.mirabilis	18	13	7	3	0	0	0	83%
K.pneumoniae	13	9	5	0	0	0	0	100%
P.aeruginosa	19	15	9	3	0	0	0	84%
MRSA	22	19	15	10	5	0	0	55%

Antibiofilm performance of hypochlorous acid

The biofilm is formed when a group of microorganisms stick to each other and become embedded within a self-produced matrix of extracellular polymeric substance composed of extracellular DNA, polysaccharides, and proteins. Among the most common biofilm-forming bacteria are Staphylococcus aureus and Pseudomonas aeruginosa. To disrupt biofilm within a wound, an agent must kill the bacteria and decrease the polysaccharides and proteins in the extracellular matrix of the biofilm. Following studies demonstrate that hypochlorous acid solution is effective in reducing biofilm [7].

Test Procedure

A sample of a commercially available hypochlorous acid wound care solution (ELECTROCYN soma) was tested against a Pseudomonas aeruginosa biofilm. The biofilm was established on pegs in a 96 well plate using the MBEC assay. The biofilm was exposed to the product at one contact time and at room temperature, and the number of surviving cells in the biofilm were quantified by cultivation and OD measurements. The number of surviving microorganisms were compared with a control sample in which the product was replaced with buffered water [8].

a. **Test organisms:** Pseudomonas aeruginosa ATCC 15442

b. **Contact times:** 15 min.

c. **Test temperature:** Room temperature (20-25°C)

d. **Incubation:** (35 ±2) °C for 18-24 hours

e. **Product concentrations:** 100% (undiluted)

Results show that the hypochlorous acid solution can achieve log reduction of 2.3 within 15 minutes of exposure time. The obtained log reduction corresponds to a reduction of 99.4 %. This further confirms the capability of HOCl is eradicating biofilm [12].

In another study[3] of a similar hypochlorous acid solution shows that Staphylococcus aureus bacterial numbers were reduced by > log 5 CFU/cm following a 1-minute exposure to hypochlorous acid. A reduction of > log 6 CFU/cm was observed after 3, 5, 7, and 10 minutes exposure to hypochlorous acid [3].

This log reduction represented complete removal of the Staphylococcus aureus biofilm. Furthermore, approximately 70% of biofilm polysaccharide and > 90% of biofilm protein was removed after 5, 7, and 10 minutes of contact time [10].

Safety Of Hypochlorous Acid – Antimicrobial Resistance (AMR) Perspective

Studies4 have shown that there is no risk of hypochlorous acid causing antimicrobial resistance. This HOCl molecule, being a similar substance found in human neutrophils, has no risk of creating any resistant microbes as compared to other chemical based substances or antibiotics [4]. The study further reveals following outcome.

Based on the evaluation of 7 types of active substances in wound care solution, silver and chlorohexidine are known to create risk of antimicrobial resistance, while solution that contains hypochlorous acid has no such risk. This further supports the safe use of hypochlorous acid in chronic wounds that usually require long term care (Table 4) [4].

Table 4: Risk of antimicrobial resistance development among various types of wound care solution.

Wound care solution	Development of resistance
Silver	Yes
Chlorohexidine	Yes
Acetic acid	No
Hypochlorous acid	No
OCT	No
PHMB	No
PVP-iodine	No

Conclusion

Based on the Time-Kill study and biofilm reduction studies, HOCl is found to be effective against wide range of microbes including commonly found microbes on wound. The advantages of HOCl as a wound cleansing solution not only lies on its efficacy, but also the safety nature of the HOCl:

A. Antimicrobial activity: Hypochlorous acid acts as a potent antimicrobial agent, helping to reduce the risk of infection in wounds.

B. Non-toxic and tissue-friendly: Hypochlorous acid is safe to use on the skin and does not cause tissue damage or irritation. It is non-cytotoxic and does not interfere with the wound healing process.

C. Promotes wound healing: Hypochlorous acid has been shown to accelerate wound healing by reducing inflammation and promoting angiogenesis (formation of new blood vessels). It also helps in the removal of necrotic tissue and promotes the growth of healthy granulation tissue.

D. Development of resistance to hypochlorous acid is minimal. This makes it a valuable option in combating antibiotic-resistant bacteria.

References

1. Rahim K, Saleha S, Zhu X, Huo L, Basit A, et al. (2017) Bacterial contribution in chronicity of wounds. Microb Ecol 73(3): 710-721.
2. Ragab II, Kamal A. (2017). The effectiveness of hypochlorous acid solution on healing of infected diabetic foot ulcers. J Education and Practice 8(8): 58-71.
3. Robson MC (2014) Treating chronic wounds with hypochlorous acid disrupts biofilm. Today's Wound Clinic.
4. Axel Kramer 1, Joachim Dissemond, Simon Kim, Christian Willy, Dieter Mayer, et al. (2018) Consensus of wound antisepsis: Update 2018. Skin Pharmacol Physiol 31(1): 28-58.
5. Wilkins RG, Unverdorben M (2013) Wound cleaning and wound healing: A concise review. Adv Skin Wound Care 26(4): 160 -163.
6. Schultz G, Bjarnsholt T, James GA, Matthew Malone, Paul Stoodley et al. (2017) Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds. Wound Repair Regen 25(5): 744 -757.
7. Joachim D (2020) Wound cleansing: benefits of hypochlorous acid. J Wound Care 29(Sup10a): S4 -S8.
8. Armstrong DG, Bohn G, Glat P, Robert Kirsner, Robert Snyder, et al. (2015) Expert recommendations for the use of hypochlorous solution: science and clinical application. Ostomy Wound Manage 61(5): S2 -S19.
9. Sakarya S, Gunay N, Karakulak M, Ozturk B, Ertugrul B (2014) Hypochlorous Acid: An ideal wound care agent with powerful microbicidal, antibiofilm, and wound healing potency. Wounds 26(12): 342 -350.
10. Block MS, Rowan BG (2020) Hypochlorous acid: a review. J Oral Maxillofac Surg 78(9): 1461-1466.
11. Ono T, Yamashita K, Murayama T, Sato T (2012) Microbicidal effect of weak acid hypochlorous solution on various microorganisms. Biocontrol Sci 17(3): 129-133.
12. Antimicrobial Efficiency report, V3bio Sdn. Bhd (Report No: RRD-007-22).