Hypochlorous Acid (HOCl)

Hypochlorous acid (HOCl) is a weak acid, naturally produced by the innate immune system. HOCl has known anti-microbial properties that also break down biofilm which helps cleanse the skin. It has also been shown to be anti-pruritic and anti-inflammatory, helping to provide Relief. By increasing oxygenation to wound sites, it helps restore the skin and improve healing.

HOCl is non-cytotoxic, non-irritating, and non-sensitizing.

HOCl is safe for ALL skin types & ALL ages. Non-Steroidal

HOCl can be safely used around the nose, mouth, and eyes. Safe as water.

There are No contraindications and will compliment your skin care routine / treatments.

HOCl is produced by the human immune system's phagocytic cells (Neutrophils) to fight infection. (Figure 1)

During the activation of neutrophils, respiratory bursts generate hydrogen peroxide (H_2O_2) and the activated granule enzyme myeloperoxidase converts H_2O_2 to hypochlorous acid (HOCl)

$2Cl^- + H_2O \rightarrow HOCl + HCl$

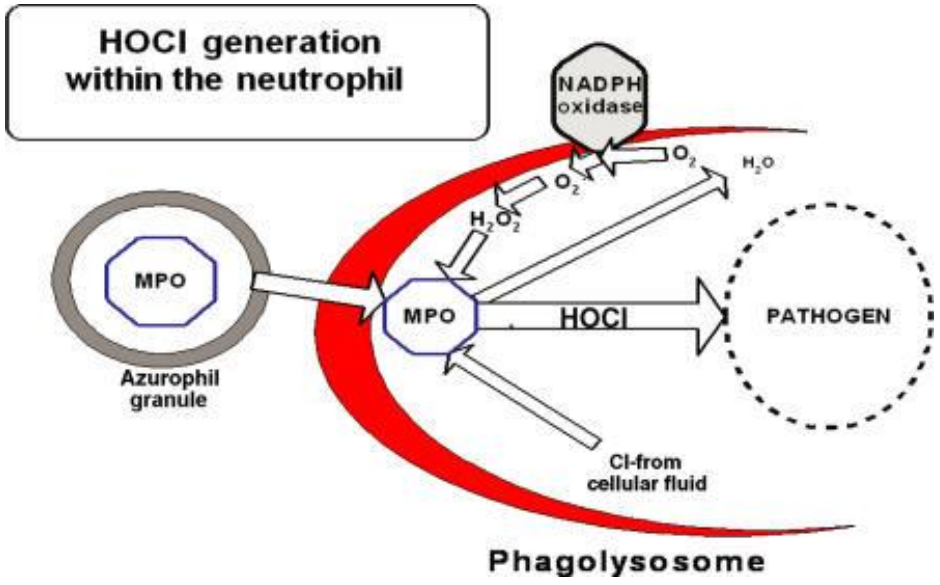
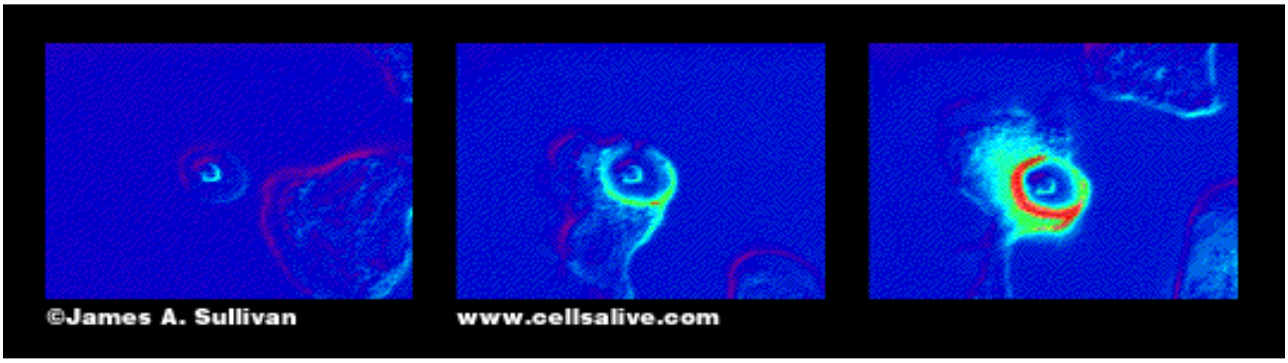


Figure 1.

Figure 2 shows what occurs when a neutrophil encounters a spore cell in the body.

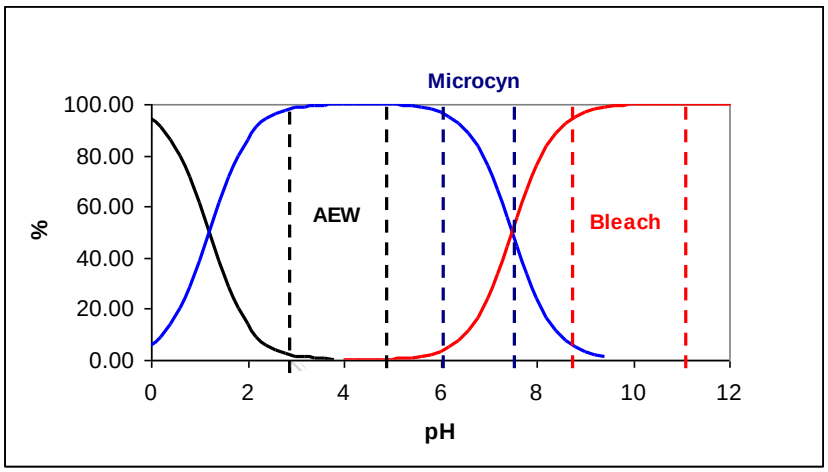
Figure 2.



Neutrophil encounters a spore cell (pathogen/bacteria) and sends out oxidative burst.	Oxychlorine compound, heavy on HOCl (which is caused by the oxidative burst), surrounds spore cell.	HOCl destroys the wall of the spore cell (pathogen/bacteria), killing it inside the body.

HOCl is a very reactive compound and needs to be stable to be most effective. Hypochlorous acid (HOCl) is a weak acid that is formed when chlorine dissolves in water. *Figure 3* shows the chlorine equilibrium. Maintaining a neutral pH is crucial to sustaining hypochlorous acid (HOCl). The solution's chemistry influences the product and activity. When the pH elevates > ~5.7, HOCl begins to ionize forming NaOCl (sodium hypochlorite) or bleach. Bleach (NaOCl) is antimicrobial at high concentrations (>500 ppm). At the concentrations in which NaOCl becomes active, the solutions are quite toxic. In contrast, HOCl is very potent at low concentrations. HOCl, also known as, super-oxidized solutions (SOS) has been successfully utilized worldwide for a wide range of applications.

Figure 3. Chlorine equilibrium



Hypochlorous acid (HOCl) Characteristics / Actions

Although Hypochlorous acid is known for its antimicrobial properties, it exhibits several other actions that are evidence based.

1. **HOCl** is a known potent **antimicrobial oxidant**

   HOCl has a broad antimicrobial spectrum. It has been shown to eradicate bacteria (including MRSA, VRE), viruses, fungus and spores to six log within 30 seconds in stability performance testing.

2. **HOCl** has been shown to **break down Biofilm.**

3. **HOCl** has displayed a **reduction of inflammation** via inhibition of histamine release in mast cells.

4. **HOCl** has demonstrated a **reduction of pain and itch**.

5. **HOCl** has exhibited **increased oxygenation** of wound sites.

1 HOCl Action: Eradicate Bacteria

- HOCl reacts with surface proteins, interfering with function of affected proteins.
  Eg: Transmembrane proteins transport nutrients; enzyme activity
- HOCl reacts with lipids in membrane, destabilizing cellular integrity leading to lysis.
- HOCl and reaction products penetrate cell wall and cell membrane, interfering with intracellular processes.

Preservative time kill testing has been conducted for Microcyn solution

PRESERVATIVE TESTING

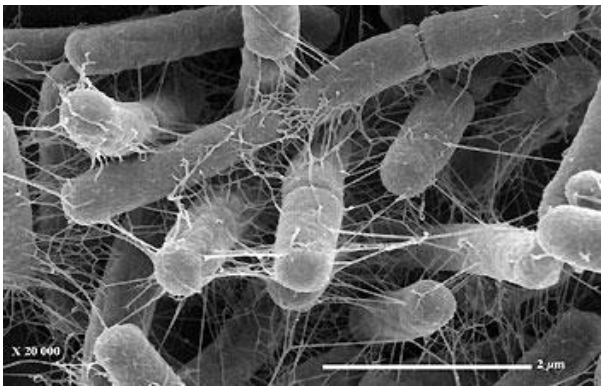
Successfully meets USP <51> Category 1 Criteria

Organisms	Result Day 7	Result Day 14	Result Day 28
P. aeruginosa *ATCC 9027*	>1 log reduction from the initial calculated count	>3.0 log reduction from the initial calculated count	No increase from Day 14
E. coli *ATCC 8739*			
S. aureus *ATCC 6538*			
C. albicans *ATCC 10321*	No increase from the initial calculated count	No increase from the initial calculated count	No increase from the initial calculated count
A. Niger *ATCC 16404*			

Stabilized Hypochlorous acid (HOCL) Preservative Time Kill Testing

Name of Organism	Log Reduction	Time to Kill	Percent Reduction
Acinetobacter baumannii	6.3692	30 seconds	99.9999%
Bacteroides fragilis	7.6435	30 seconds	99.9999%
Candida albicans	6.3345	30 seconds	99.9999%
Clostridium difficile (spores)	4.6475	30 seconds	99.9977%
Enterobacter aerogenes	6.0881	30 seconds	99.9999%
Enterococcus faecalis-VRE	6.3646	30 seconds	99.9999%
Enterococcus faecium-VRE MDR	6.5119	30 seconds	99.9999%
Escherichia coli	5.6990	30 seconds	99.9998%
Haemophilus influenzae	5.1775	30 seconds	99.9993%
Klebsiella oxytoca-MDR	6.0492	30 seconds	99.9999%
Klebsiella pneumoniae	6.1430	30 seconds	99.9999%
Micrococcus luteus	5.8420	30 seconds	99.9999%
Proteus mirabilis	6.2028	30 seconds	99.9999%
Pseudomonas aeruginosa	5.8096	30 seconds	99.9998%
Serratia marcescens	5.9978	30 seconds	99.9999%
Staphylococcus aureus-MRSA	6.3454	30 seconds	99.9999%
Staphylococcus aureus	6.2266	30 seconds	99.9999%
Staphylococcus epidermidis	6.0233	30 seconds	99.9999%
Staphylococcus haemolyticus	5.9112	30 seconds	99.9999%
Staphylococcus hominis	5.4456	30 seconds	99.9996%
Staphylococcus saprophyticus	5.9590	30 seconds	99.9999%
Staphylococcus pyogenes	6.7160	30 seconds	99.9999%

2 HOCl Action : Breaks Down Biofilm



Microcyn® solutions destroy Pseudomonas and E coli biofilms in vitro

Neutral super-oxidised solutions are effective in killing P. aeruginosa biofilms

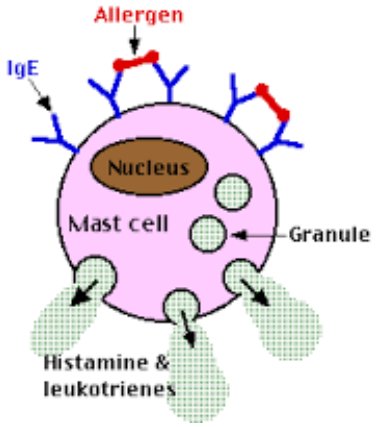
Karin Sauer[a]*, Eileen Thatcher[b], Robert Northey[b] and Andres A. Gutierrez[b]
[a]Department of Biological Sciences, Binghamton University, SUNY at Binghamton, Binghamton, NY 13902, USA; [b]Oculus Innovative Sciences, Petaluma, CA 94954, USA
(Received 24 June 2008; final version received 28 August 2008)
Bacteria growing in biofilms can become up to 1000-fold more resistant to antibiotics and biocides as compared to their planktonic counterparts. As a result of this increased resistance, biofilms and biofilm-related infections cannot be effectively treated with conventional antibiotic therapy. The goal of this study was to determine the efficacy of three neutral pH, super-oxidised solutions (nSOSs, OIS-80, OIS-125, OIS-200, Microcyn Technology) varying in oxychlorine concentration (80, 125 and 200 ppm) against P. aeruginosa grown planktonically and as biofilms. Exposure for 20 s of exponential phase cells to any of the three solutions was sufficient to reduce viability by more than five logs. However, only exposure for 10 min to OIS-125 and OIS-200 for 10 min was sufficient to eradicate stationary phase P. aeruginosa cells. The efficacy of nSOSs on P. aeruginosa biofilms, grown to maturity in continuous flow tube reactors, was determined upon treatment up to 60 min. Viability pre- and post-treatment was determined by CFU counts. The effect of these solutions on P. aeruginosa biofilms and biofilm architecture was further visualised by confocal scanning laser microscopy and quantitatively analysed by COMSTAT. Under these experimental conditions, only OIS-125 and OIS-200 achieved a 4̃3-log reduction and biofilm disaggregation within 30 min of exposure. Because OIS-125 and OIS-200 enhance the disaggregation of biofilms, their use in the treatment of surface-related biofilm infections deserves further investigation.

Although products contain Hypochlorous acid (HOCl), claims cannot be made that are not supported by the FDA cleared package insert.

3 HOCl Action : Anti-Inflammatory

Inhibition of Mast Cell Degranulation

It does not eliminate it – but reduces it
Resulting in less inflammation and less redness



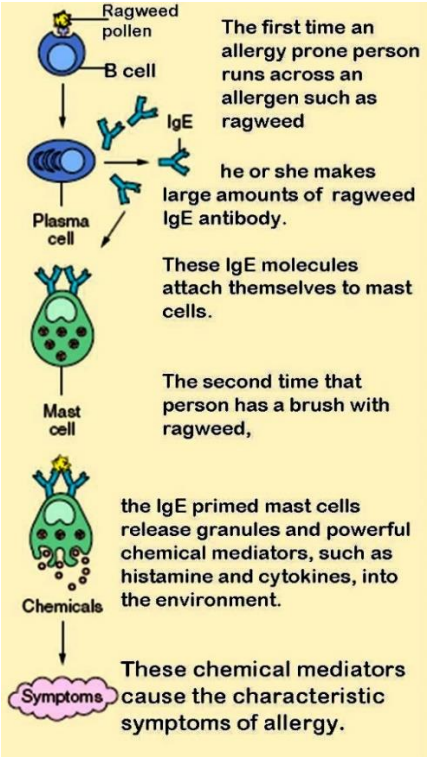
What are Mast Cells?

Mast cells are cells found throughout the body as part of our immune system.

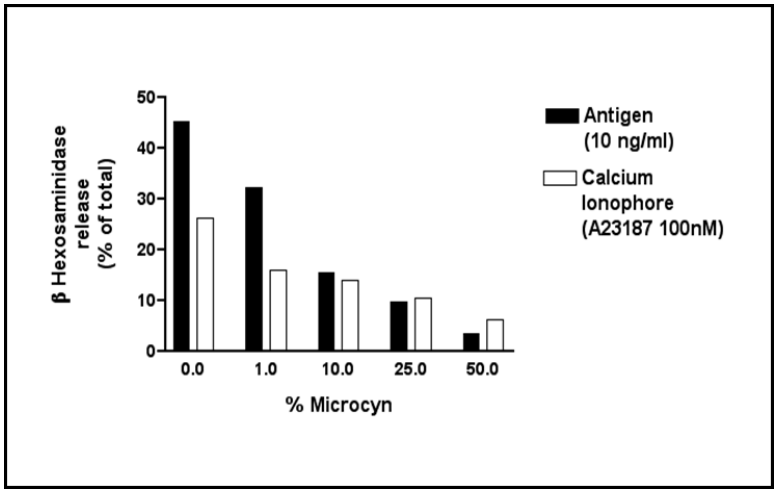
Mast cells are particularly prominent in tissues of our bodies that interact with our external world.

Mast cells play many important roles in the body, including:

- Setting off a rapid inflammatory response to outside invaders, such as germs, viruses, and parasites.
- Activation of the allergic response, with the release of the chemical histamine.
- Intimately involved in wound healing, angiogenesis.



Microcyn prevents allergen- and calcium-induced mast cell degranulation for up to eight hours after a single exposure. This reduces wound inflammation that inhibits healing.



Medina-Tamayo, *et.al.*, Nat'l Inst. of Rehab., Mexico City, 2005

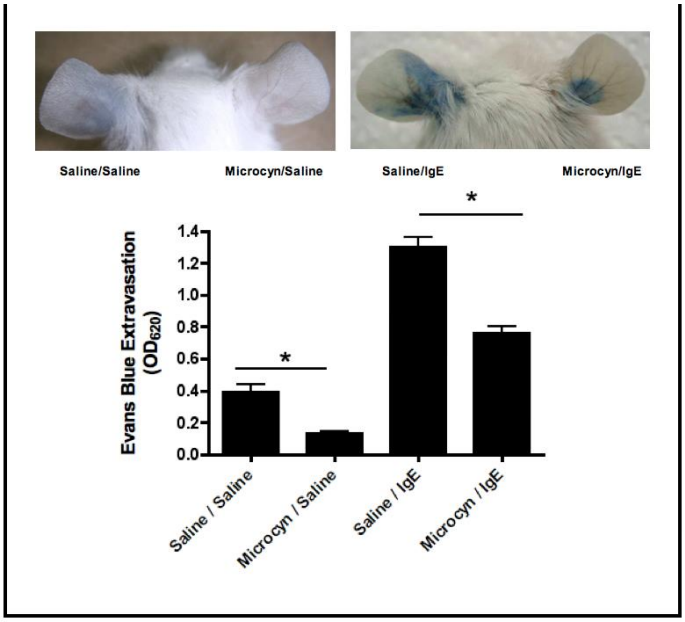
Super-oxidized solution inhibits IgE-antigen-induced degranulation and cytokine release in mast cells☆

J. Medina-Tamayo [a], E. Sánchez-Miranda [a], H. Balleza-Tapia [a], X. Ambriz [a], M.E. Cid [a], D. González-Espinosa [b], A.A. Gutiérrez [b], C. González-Espinosa [a]

Abstract

Activation of the high affinity IgE receptor (FcεRI) through IgE-antigen complexes induces mast cell degranulation, synthesis of lipid mediators and cytokine production. These effects are involved in Type I hypersensitivity reactions and controlling them has been the main objective of many anti-allergic therapies. Here we report that pretreatment of murine bone marrow derived mast cells (BMMC) with super-oxidized solution (SOS) inhibits FcεRI dependent-β hexosaminidase and cytokine release. This effect is exerted without altering total protein tyrosine phosphorylation, MAPK activation, cytokine mRNA accumulation or calcium mobilization after FcεRI triggering. Our data suggest that this neutral pH-SOS acts like a mast cell-membrane stabilizer inhibiting the cell machinery for granule secretion without altering the signal transduction pathways induced by IgE-antigen receptor crosslinking.

Animal model study showing Microcyn reducing the anaphylaxis reaction.



Indicates that Microcyn™ diminishes the non-specific and the IgE-antigen-specific inflammation in a murine model of atopic eczema.

Elizabeth Sanchez- Miranda

In vivo characterization of Microcyn™ effects on early phase of allergic reactions using a murine model of passive cutaneous anaphylaxis
April 9th 2008

Elizabeth Sanchez- Miranda[1], Gonzalez, D[2]., Gutierrez, AA[3]., and Gonzalez-Espinosa, C.[1]*

Results

A single application of Microcyn™ is able to diminish passive anaphylaxis in mice. In order to test if Microcyn topical administration can diminish the intensity of PCA reaction, ears from different mice were sensitized with an intradermal injection of saline solution or monoclonal anti DNP IgE. Eighteen hours after the sensitization, a single application of Microcyn was performed in corresponding ears 30 minutes before antigen intravenous challenge. It was possible to observe that a single topical application of Microcyn™ 30 minutes prior to antigen addition was able to block almost 35% of PCA reaction (Figure 1). Interestingly, saline solution application, during the sensitization phase or applied only 30 minutes before Evans blue addition, was able to induce some Evans blue extravasation. This unspecific inflammatory state was also diminished by the application of Microcyn™.

4 HOCl Action : Pain and Itch

“Evaluation of a gel formulation of Hypochlorous Acid and Sodium Hypochlorite to reduce pruritus in mild to moderate Atopic Dermatitis” – Z Draelos, MD

Study results

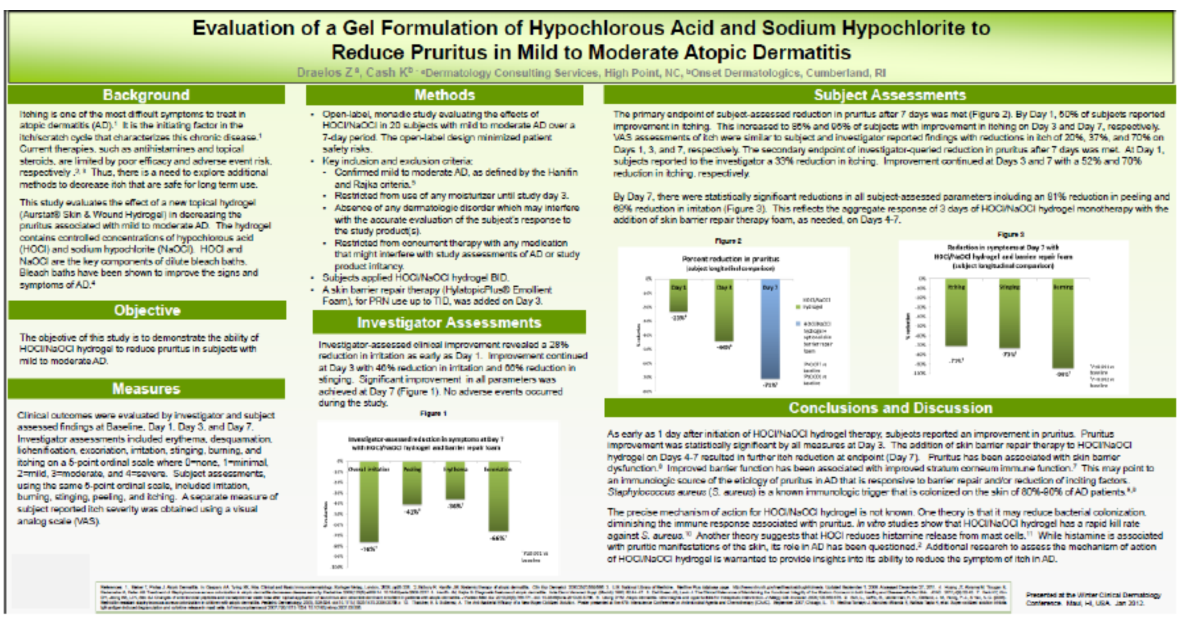
Investigator-assessed clinical improvement revealed a 28% reduction in irritation as early as day 1

Improvement continued at day 3 to 46% reduction in irritation

By <u>Day 1</u>, 50% of subjects reported improvement in itching

By <u>Day 3</u>, 85% of subjects reported improvement in itching

The addition of the barrier repair therapy to HOCl/NaOCl hydrogel on days 4-7 resulted in further itch reduction at endpoint (day 7)



‘Investigator Blinded Randomized Study Evaluating HOCl in the Treatment of Atopic Dermatitis-Associated Pruritus’

- Brian Berman, MD, PhD, Mark Nestor, MD, PhD

3-day study designed to evaluate the effect of HOCl on pruritus in patients with AD.

- Investigator blinded randomized phase 2 72-hour study investigated the antipruritic effect of HOCl with patients diagnosed with AD.
- Subjects were enrolled into the study if they had AD as defined by the Hanifin criteria and had a score of >2 on an itch severity scale (0-4).
- 30 subjects were enrolled over the course of the study, with 20 randomized to the treatment group (HOCl), and 10 randomized to the untreated control group.
- Subjects who were randomized to the treatment group were instructed to use HOCl BID or PRN for 72-hours
- The 3 primary measures used in this study were the Participant Global Assessment (PGA), Investigator Global Assessment (IGA), and VAS itch score.

Study Group	n	Mean (μ) IGA Itch Score at Baseline	Standard Deviation (σ) of mean
TREATED	20	**1.55**	0.826
UNTREATED	10	**1.70**	0.483

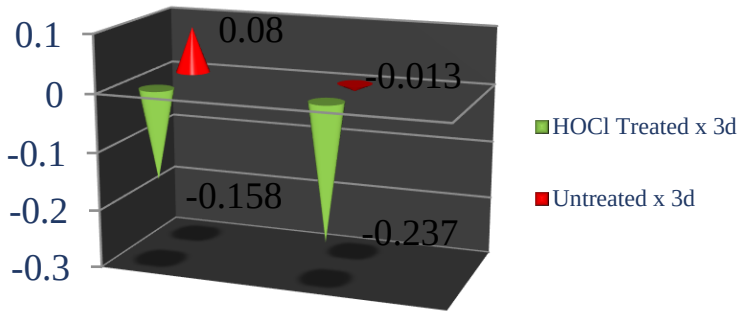
Statistical Test	p-value (α=0.05; β=0.20)	95% Confidence Interval
Independent Samples t-test	**0.601**	-0.732 - 0.432

The mean VAS itch score between the 2 groups were similar at baseline (Table 1)

Results

29 subjects were included in the final analysis.

Mean change in PGA and IGA between baseline and 72-hours were both shown to be significantly different, with a decrease (improvement) in favor of the treatment group (PGA: p-value=0.128; IGA: p-value=0.012) (Figure 1).



The mean itch VAS scores were significantly lower in the treated group (Figure 2).

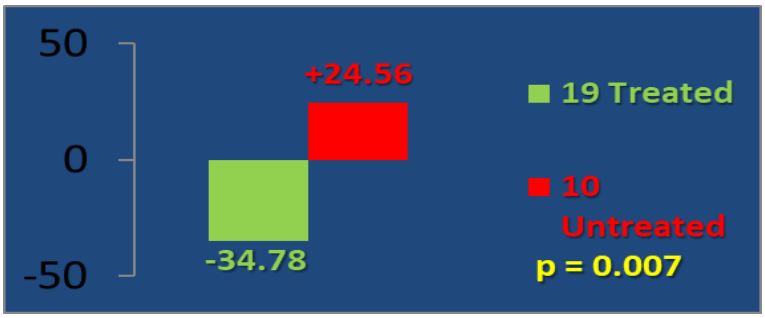


Figure 2. Mean % Change in Itch VAS With and Without Treatment with HOCl Gel x 3 days

The analysis showed 73.7% of the subjects in the treated and 30.0% of the subjects in the untreated group experienced a reduction in itching between baseline and 72 hours post application.
There were no treatment related discontinuations or SAEs.

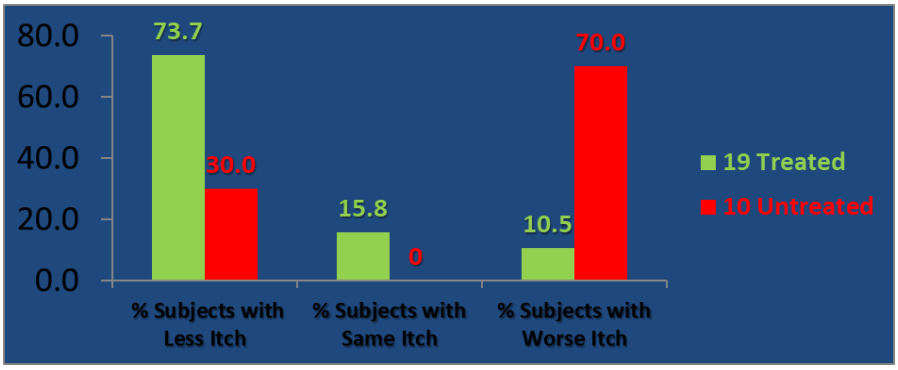
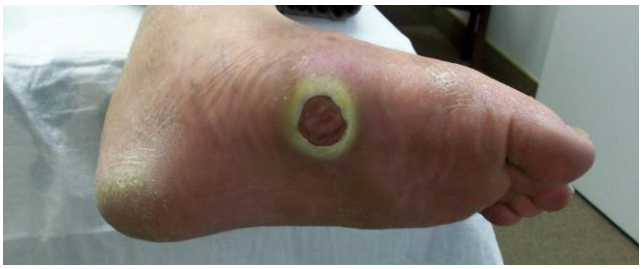


Figure 3. Effect of with HOCl Gel Treatment on Itch in Atopic Dermatitis - day 3

5 HOCl Action : Increases Oxygenation of wound sites to improve healing -TcpO2 (Transcutaneous Oxygen Pressure)

HOCl solution provided higher levels of oxygen at a wound site after application. This elevated blood and oxygen flow delivers increased wound-healing nutrients.



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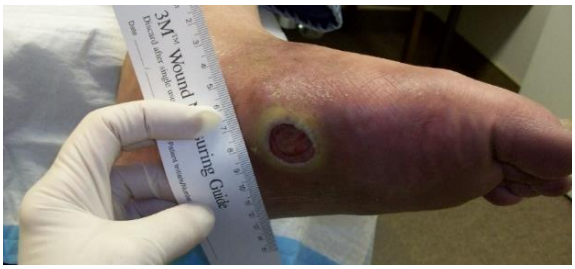

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Persistent Improvement of Diabetic Foot Ulcer Perfusion Results From Application of Superoxidized Water Cheryl M. Bongiovanni, PhD,RVT,CWS Lake District Hospital, Lakeview, OR

Persistent Improvement of Diabetic Foot Ulcer Perfusion Results From application of Superoxidized Water

*Cheryl M. Bongiovanni, PhD,RVT,CWSLake District Hospital, Lakeview, OR

- 191patients w/ 131 diabetic plantar ulcers
- 27-83 y/o; 63 men, 56 women
- 116 Type 2; 3 Type 1
- 17 Type 2 (11 men, 6 women) active smokers (>10 cigarettes/day)
- Tcp02 measurements made via Radiometer TCM4 with sensors @ mid-sternum, mid-calf and 2-3mm from wound bed; before-, immediately after & 36 hours s/p treatment with superoxidized water (wound flushed with 20 ml superoxidized water over 15 seconds)

Conclusion

Persistent improvement (to 36 hours) of local blood flow and tissue oxygenation occurs in response to wound treatment with superoxidized water.

Improved perfusion can persist to 36 hours post-treatment in both Type 1 and Type 2 diabetics.