

# Antimicrobial Activity Comparison of Pure Hypochlorous Acid (0.01%) with GP-55 Other Wound and Skin Cleansers at Non-Toxic Concentrations

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## Abstract

To compare the antibacterial activities of commercially available skin, wound, and skin/wound cleansers at cell safe (non-toxic) concentration saline and 19 other commercial cleansers were first evaluated for cytotoxicity on L929 mouse dermal fibroblasts. Cells were exposed to serial 10-fold dilutions of each cleanser until treatment-induced cytotoxicity was comparable to the baseline cytotoxicity of unexposed control fibroblasts.

Next, time-kill kinetics of these non-toxic concentrations of cleansers was tested against methicillin resistant *S. aureus* ATCC 33591. The experimental design allowed calculation of relative cytotoxicity indexes ranging from 0 to 100,000. Two poloxamer 188 solutions and saline were found to be the least toxic (toxicity index 0). Chlorhexidine gluconate solution (4.0% w/v), polyoxyrbutol 20 solution and povidone-iodine (7.5% most toxic (toxicity index 10,000). At non-cytotoxic concentrations pure hypochlorous acid (0.01%)<sup>\*</sup> was the most rapidly bactericidal, with 4 log reduction in CFU in less than 60 seconds. A mixture of hypochlorous acid / sodium hypochlorite (molar ratio 50/50) at pH 7.4 was next at 30 minutes, while most of the agents tested required > 24 hours. Wound healing depends on maintaining bacterial balance while not damaging the healing tissues. *In vitro* toxicity indexes provide helpful guidelines for subsequent *in vivo* evaluation and clinical applications. The study findings suggest that pure hypochlorous acid (0.01%)<sup>\*</sup>, in contrast with many commercially available wound cleansers, has rapid bactericidal activity at concentrations that are safe for human cells. <sup>\*</sup>NeuroPhase<sup>®</sup>

## Introduction

Chronic non-healing wounds, such as venous ulcers, and pressure ulcers cause tremendous patient suffering. Treatment of such wounds presents a serious unmet medical need. Strategies that optimize the wound healing have evolved with advances in understanding of the tissue repair process.

An ideal wound cleanser provides periodic reduction of bacterial contamination and removal of debris without adversely impacting cellular activities crucial to the wound healing process. Therefore it is important to evaluate wound care products and their potential cytotoxicity.

In this study, we determined the non-cytotoxic, safe concentration of all twenty important and widely used skin/wound cleansers and compared the microbiocidal activity of these cleansers at their non-cytotoxic concentration.

## Materials & Methods

Test Agents: Twenty commercial skin, wound, and skin/wound cleansers were evaluated. Cleansers were obtained from manufacturers or distributors (Table 1).

Cells and Testing: L929 mouse fibroblasts were obtained from ATCC. Cytotoxicity was evaluated by modified methods as described by Wilson et. al. (1). Cells were cultured in 96-well plates to approximately 70% confluency prior to exposing them to various cleansers. Cleansers were aspirated off the wells after exposure for 30 mins at 37°C. Cells were incubated in fresh media overnight prior to determining cell viability using CellTiter 96 cell proliferation assay. Cleansers were serially diluted 1:10 with PBS.

Time-Kill Bacterial Assay: Time-kill assay is a measure of how long it takes for an antimicrobial product to achieve an effective 4 log kill of a defined inoculum. The assay was conducted using modified CLSI methods. A standard inoculum of MRSA ATCC 33591 was grown, centrifuged, and re-suspended to approximately 10<sup>8</sup> CFU/mL before incubation with cleansers at their non-cytotoxic dilution for 1, 5, 15, 30, 60 min, 4 hr, and 24 hr. 10-fold serial dilutions of the cleansers containing the inoculum were plated on TSA, incubated overnight at 37°C and CFUs were counted.

## Results

Agents Tested	Use	Manufacturer	Non-toxic dilution	Toxicity Index	Time to 4 Log <sub>10</sub> Kill
Saline (0.9% sodium chloride)	Wound	Reflexion Pharmaceuticals, Libertyville, IL	10 <sup>8</sup>	1	>24 hrs
Shur-Cleans <sup>®</sup>	Wound	ConnaFac, Skillman, NJ	10 <sup>8</sup>	1	>24 hrs
NeuroPhase <sup>®</sup> OTC	Wound	Chlorine Innovations Sciences for Research, Inc., Bialla, CA	10 <sup>8</sup>	1	30 min
WoundClenz OTC Wound Cleanser	Wound	Catality Pharmaceuticals, Inc., Indianapolis, IN	10 <sup>8</sup>	10	>24 hrs
3M <sup>™</sup> Wound Cleanser	Wound	3M Health Care, St. Paul, MN	10 <sup>8</sup>	10	>24 hrs
Demargan <sup>®</sup> Wound Cleanser	Wound	Dermo Sciences Inc., Princeton, NJ	10 <sup>8</sup>	10	>24 hrs
NeuroPhase <sup>®</sup>	Wound	Neobay Pharmaceuticals, Inc., Emeryville, CA	10 <sup>8</sup>	10	>1 hr
Beal's <sup>™</sup> Wound Cleanser	Wound	J.R. Beal Inc., Conington, GA	10 <sup>8</sup>	10	>24 hrs
Carroll's <sup>™</sup> Dermal Wound Cleanser	Wound	Carroll's Lab, Irving, TX	10 <sup>8</sup>	10	>24 hrs
SAF-Cleans <sup>™</sup> AF	Wound	ConnaFac, Skillman, NJ	10 <sup>8</sup>	100	24 hrs
Restatone <sup>™</sup> Wound Irrigation Solution	Wound	B. Braun Medical Inc., Bethlehem, PA	10 <sup>8</sup>	100	>24 hrs
Allclenz <sup>™</sup> Wound Cleanser	Wound	Healthpoint, San Antonio, TX	10 <sup>8</sup>	100	24 hrs
Hydrogen peroxide (3%)	Wound	Hydro Laboratories, Elgin, IL	10 <sup>8</sup>	1,000	>24 hrs
Eltar <sup>®</sup> Perineal Wash	Wound	Smith & Nephew, Largo, FL	10 <sup>8</sup>	1,000	>24 hrs
Dermal Wound Cleanser	Wound/Skin	Hydrexia, Norcross, GA	10 <sup>8</sup>	1,000	>24 hrs
Johnson & Johnson <sup>®</sup> Baby Shampoo	Skin	Johnson & Johnson, Skillman, NJ	10 <sup>8</sup>	1,000	>24 hrs
Hibiclens <sup>®</sup> Chlorhexidine gluconate solution (4.0% w/v)	Skin	Reflexion Pharmaceuticals, Libertyville, IL	10 <sup>8</sup>	10,000	>24 hrs
Restatone Skin Cleanser	Skin	Parkes Product L.P., Stamford, CT	10 <sup>8</sup>	10,000	>24 hrs
Betadine <sup>®</sup> Surgical Scrub (Povidone-iodine, 7.5%)	Skin	Bayer American Products, Inc., Dallas, TX	10 <sup>8</sup>	10,000	>24 hrs

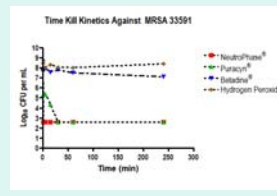
**Table 1. Toxicity index and antibacterial time kill kinetics of cleansers.** Each cleanser was serially diluted 1:10 with PBS (from 10 to 10,000 fold dilutions) and each dilution was tested for cytotoxicity until the results of the cells exposed to the diluted test solutions were similar to those cells exposed to PBS alone.

Shur-Cleans<sup>®</sup>, Restore Wound Cleanser and saline were found to be the least toxic to fibroblasts, requiring no dilution to maintain viable cells (a toxicity index of zero). Several agents (NeuroPhase<sup>®</sup>, Puracyn<sup>®</sup>, WoundClenz OTC, Bioclex<sup>™</sup>, CaraKlenz<sup>™</sup>, 3M<sup>™</sup> Wound Cleanser, and Demargan<sup>®</sup>) required only one "10-fold" dilution (a toxicity index of 10). SAF-Cleans<sup>™</sup> AF, Protosan<sup>™</sup> and Allclenz<sup>™</sup> each had a toxicity index of 100. Dermal Wound Cleanser, Johnson & Johnson's<sup>®</sup> Baby Shampoo, Eltar<sup>®</sup> Perineal Wash and hydrogen peroxide had indices of 1000 while the toxicity index of Betadine<sup>®</sup> Surgical Scrub, Hibiclens<sup>®</sup>, and Restore Skin Cleanser was 10,000.

The time to kill at the non-toxic dilution of NeuroPhase<sup>®</sup> (10-fold dilution) was less than 1 min, followed by Puracyn<sup>®</sup> (10-fold dilution) at 30 minutes. The time to kill at non-toxic dilutions of all other commercially available wound cleansers were greater than or equal to 24 hours.

Antibacterial Activity	Low Cytotoxicity (toxicity index 1-100)	High Cytotoxicity (toxicity index 1,000-10,000)
Antibacterial activity at 'cell safe' concentration <10 minutes	NeuroPhase <sup>®</sup>	none
Antibacterial activity at 'cell safe' concentration >10 minutes	Puracyn <sup>®</sup> OTC SAF-Cleans <sup>™</sup> AF	none
	Allclenz <sup>™</sup> Wound Cleanser	
Non-antibacterial in 24 hours at 'cell safe' concentration	Restore Wound Cleanser Saline (0.9% sodium chloride) Shur-Cleans <sup>®</sup> WoundClenz OTC Wound Cleanser 3M <sup>™</sup> Wound Cleanser Hibiclens <sup>®</sup> (Chlorhexidine gluconate solution 4.0% w/v) Demargan <sup>®</sup> Wound Cleanser Bioclex <sup>™</sup> Wound Cleanser CaraKlenz <sup>™</sup> Dermal Wound Cleanser Protosan <sup>™</sup> Wound Irrigation Solution	Hydrogen peroxide (3%) Eltar <sup>®</sup> Perineal Wash Dermal Wound Cleanser Johnson & Johnson's <sup>®</sup> Baby Shampoo Restore Skin Cleanser Betadine <sup>®</sup> Surgical Scrub (Povidone-iodine, 7.5%)

**Table 2. Cytotoxicity and antibacterial activity of skin and wound cleansers.** Potent wound cleansers with a high toxicity index (for example: Betadine, Hibiclens<sup>®</sup>, hydrogen peroxide) will likely have deleterious effects to living tissue. At the same time a non-toxic wound cleanser (examples: Saline, Shur-Cleans<sup>®</sup>, Restore Wound Cleanser) without antimicrobial activity, will likely provide minimal reduction in bacterial burden.



**Figure 1.** The time to kill at the non-toxic dilution of NeuroPhase<sup>®</sup> (10-fold dilution) was less than 1 min, followed by Puracyn<sup>®</sup> (10-fold dilution) at 30 minutes. Betadine<sup>®</sup> and hydrogen peroxide showed no log reduction at 24 hours.

## Discussion

An ideal wound cleanser should have minimal cytotoxicity together with potent and rapid antimicrobial activity. In this study *in vitro* methods were used to evaluate the potential deleterious effects of cleansers on wound healing and the likely antimicrobial activities of cleansers. The *in vitro* findings correlated with many *in vivo* studies and with clinical advice. The antimicrobial activity of two of the nineteen agents studied, NeuroPhase<sup>®</sup> and Puracyn<sup>®</sup> stood out. Both cleansers contain hypochlorous acid, a particularly rapidly acting antimicrobial produced endogenously as part of the body's innate immune system. These studies should prove useful to clinicians developing wound care strategies and to those wishing to develop and expand *in vitro* methods to evaluate the potential effects of agents used for wound care.

This *in vitro* study demonstrates that many wound and skin cleansers may be toxic to fibroblasts, one of the key cells in wound repair, and suggests that these cleansers might also be toxic to other cells. When diluted to "cell safe" concentrations, most of the cleansers lost antibacterial activity, as reflected by the length of time needed to reduce the growth of *S. aureus*.

The results of these studies offer some guidance for wound care in the complex circumstances encountered in most wounds. Several of the cleansers studied are not toxic to cells even undiluted, while a single 10-fold dilution is sufficient to render another group non-toxic.

## Conclusions

•Wound healing depends on maintaining bacterial balance while not damaging the healing tissues. *In vitro* toxicity indexes provide helpful guidelines for subsequent *in vivo* evaluation and clinical applications.

•This study demonstrates that many wound and skin cleansers may be toxic to fibroblasts

•When diluted to 'cell safe' concentrations, most of the cleansers lost antibacterial activity when tested against MRSA in a time-kill kinetic study

•The study findings suggest that pure hypochlorous acid (0.01%)<sup>\*</sup>, in contrast with many commercially available wound cleansers, has rapid bactericidal activity at concentrations that are safe for human cells. <sup>\*</sup>NeuroPhase<sup>®</sup>

Disclaimer: NeuroPhase is a 510k cleared product for wound cleansing only and we are claiming only antibacterial activity in solution

## References

- Wilson JR, Mills JG, Prather ID, Dimitrijevic SD. A toxicity index of skin and wound cleansers used on *in vitro* fibroblasts and keratinocytes. *Advances In Skin & Wound Care* 2005; 18:373 – 378